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ACUTE TOXICITY STUDIES ON APHRODISIACS OF HERBAL ORIGIN IN PHARMACIES WITHIN GREATER ACCRA METROPOLIS.

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FULFILLMENT OF THE AWARD OF A MASTER OF PHILOSOPHY DEGREE IN PHARMACOLOGY

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DECLARATION

DECLARATION

DECLARATION BY THE CANDIDATE

I hereby declare that except for cited references duly acknowledged, this thesis is a true representation of my own work and has not been presented for a degree elsewhere.

Dennis Amankwah **15th August, 2022**

(10803979) Signature Date

DECLARATION BY THE SUPERVISORS

We declare that we supervised the principal work and presentation of the thesis per guidelines on supervision of the thesis laid down by the University of Ghana.

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ABSTRACT

Background: Erectile dysfunction (ED) is the inability of a man to attain and sustain adequate erection for satisfactory coitus. Herbal medicines are widely promoted as remedies for ED. The safety profile of these products are largely unknown. In this study, five products of herbal origin, sold as aphrodisiacs in pharmacies within the Greater Accra region, are screened for activity across three functional domains: autonomic, neuromuscular/motor, and CNS using the modified Irwin test.

Method: Groups of male Sprague-Dawley rats (150 - 200 g, n = 5) were used. Each candidate herbal product was administered *p.o.* at three dose levels to different groups of rats. Chlorpromazine (30 mg/Kg, p. o) and caffeine (25 mg/Kg, p. o.) were used as positive controls. The vehicle-treated controls were given normal saline (1 ml/kg, p.o.). Following drug administration, animals were monitored for changes in behaviours indicating alterations in autonomic, neuromuscular/motor, and CNS activity at 0-15 mins, 30 mins, 1 hr., 2 hr., 4 hr., 8 hr., 24 hr. and 48 hr. Hematological, biochemical, and histopathological analyses were performed on blood and isolated organs of the experimental animals 14 days' post drug administration.

Results: No mortality was recorded after 48 h in all groups. Except for sedation, there were no clinical signs of interference with any system such as autonomic, CNS and motor activity for all the five herbal aphrodisiac products. Products 1, 2 and 4 caused significant ($p<0.05$) increases in the weights of the liver, heart, and spleen at 1200, 150 and 400 mg/kg, p.o. respectively. Products 1 and 2 caused elevated levels of only ALP at low doses while higher doses produced no change in enzyme levels. From the biochemical indices, there was therefore no definite evidence of toxicity from the Products to the liver. Haematological parameters of animals were not adversely affected after 14 days of administration of the Products. Histopathological studies did not also provide any equivocal evidence of toxicity to any organ.

Conclusion: The extracts produced no evidence of significant toxicity to major organs of the rats. Transient behavioural alterations, lasting less than 48 hours, suggesting involvement of peripheral and central nervous systems were observed. Caution should be exercised in the use of these Products while further tests are carried out to elucidate the safety profile of the commercial herbal aphrodisiacs.

DEDICATION

This work is lovingly dedicated to my wife and children, who have been there for me at all times.

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At last, the journey has come to an end after 2 years of intensive training and research in pharmacology and toxicology. Reflecting on this achievement, it is without doubt that I have come this far by grace and the inspiration of great individuals. I want to thank my uncle, Mr. Michael Appiah, for believing in me and investing in my education for many years. I am indebted to you eternally. I also want to thank my wife and children for their unrelenting support during these 2 years.

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BACKGROUND

Erectile dysfunction (ED) is the inability of a man to attain and sustain adequate erection for satisfactory sexual coition (Kalsi and Muneer, 2013). Estimated data from US, Australia and UK studies revealed the same complete ED prevalence rate of about 5 % ,10 %, 15 % and 30-40 % among 40, 60, 70 and 80-year-olds men respectively (Saadat, 2015). By 2025, ED is estimated to affect about 320 million adult males globally (Ahmed et al., 2011). Clearly, ED is regarded as a major health problem for the increasingly healthy ageing population (Chew, 2004).

Erectile dysfunction is mostly caused by penile vascular disorders involving several pathophysiological mechanisms such as impaired arterial inflow, increased cavernosal smooth muscle contraction induced by chronic ischemia, impaired smooth-muscle cavernosal relaxation, veno-occlusive dysfunction and cavernosal fibrosis and chronic or episodic hypoxemia. Risk factors associated with vascular disorders are age, lack of exercise, hypertension, diabetes, abnormal lipid profile, smoking and obesity (Saadat, 2015). Another known cause of ED is the loss of functional integrity of the endothelium of arterioles to relax known as endothelial dysfunction (Saenz de Tejada *et al*.,1989). Thus, any condition that damages the endothelial function can result in ED in general. Neurological, psychological (depression) and endocrine disorders such as hormonal deficiency, hyperprolactinemia, testosterone deficiency and raised sex hormone-binding globulin are other factors that can affect ED.

A variety of plant, animal or mineral based supplements are known to possess potential sexual benefits, confirming older believes and inspiring newer hopes for male vitality and management of sexual dysfunctions. These sex enhancing substances are called Aphrodisiacs. Aphrodisiacs are either food or drugs sourced from plants, animals or minerals that arouse the sexual instinct, desire and performance (Kotta *et al*., 2013 and Malviya *et al*., 2011).

In Africa, cultural values define masculinity based on sexual prowess and fertility placing a high premium on sex (Tabil, 2011). Thus, societal insensitivity and stigma associated with ED causes serious distress with noticeable effects on the self-esteem and the associations of men

experiencing it (Tabil, 2011). Therefore, aphrodisiacs are patronized for both recreational and therapeutic purposes. Ayurveda's classify aphrodisiacs therapeutically as (i) drugs which boost or stimulate semen production for example. Asparagus racemosus, Microstylis wallichii, Mucuna pruriens, Roscoea procera and Polygonatum verticillatum. (ii) drugs which enhance the quality of semen and purifies it namely, Sesamum indicum, Myrica nagi, Anthocephalus cadamba, Vetiveria zizanioides, and Saussurea lappa. (iii) drugs which enhance ejaculatory functions namely, Cannabis sativa, Strychnos nux vomica Myristica fragrans, and Cassia occidentalis. (iv) drugs which prolong ejaculation and performance for example, Cannabis sativum, Asparagus racemosus, Mucuna pruriens, Anacyclus pyrethrum, Sida cordifolia, and Cinnamomum tamala. (v) drugs arousing desire to have sex, namely, Withania somnifera, Opium, Datura stramonium, Hibiscus abelmoschus, Anacyclus pyrethrum and Asparagus racemosus (Chauhan *et al*., 2014).

Aphrodisiacs can also be classified based on their formulations or constituents, mechanism of action and origin. Classification based on formulation are firstly; psychophysiological stimulants involving visual, olfactory, tactile and aural stimulations that improve sexual drive, gratification and erection through hormonal alteration and increase blood flow in spongy tissues (Yidana *et al*., 2019). Secondly, 'internal' preparations that involves love portions, alcoholic drinks and food (Malviya *et al*., 2011).

Classification based on the mechanism of action is in 3 groups: libido enhancers, effectiveness of erection and sexual delight. Generally, mechanism of action of aphrodisiac is through dilating blood vessels of corpus cavernoseum leading to the influx of blood into penile tissues hence erection (Mahima *et al*., 2017, Brunetti *et al*., 2020 and Kotta *et al*., 2013).

Classification based on origin are natural or unnatural (synthetic). The natural aphrodisiacs are those from natural substances whether plant or animal based. Synthetic aphrodisiacs are manufactured to mimic the natural substance. Commonly found synthetic aphrodisiacs include Sildenafil (Viagra®), Vardenafil (Levitra®) and Tadalafil (Cialis®).

Limitations with the use of these synthetic aphrodisiacs include limited efficacy, contraindications in certain disease conditions and unwanted side effects notably, dyspepsia,

headache, flushing and nasal congestion (Mathur, 2012 and Kotta *et al*., 2013 and Hammoud *et al*., 2017).

Thus, plant-based remedies are and will keep being a common alternative for men with unimproved sexual life in spite of the increasing availability of effective synthetic medical treatment (Kotta *et al*., 2013). In Ghana, there are a number of these herbal aphrodisiacs, some of which are summarized as in table 1.0

Table 1: list of herbal aphrodisiacs and their respective ingredients.

PRODUCT	INGREDIENTS
Mr. Q	*Rehmanius* root, *Achyrmthes* root, fruit of *Puncturevine*, *Cnidium* fruit, herb of Korean *Epimedium*, fruit of *Magnoliavine*, *Songaria cynomorium* herb, *Red ginseng*, *Astragalus* root, *Rhodiola* root, *Aucklandia* root.
Tentex Royal	*Tribulus terrestis* 100mg, *Blepharis edulis* 115mg, *Crocus sativus* 14 mg, *Asteracantha longifolia* 145mg, *Prunus amygdalus* 126mg.
New Kingdom Ginseng Power capsule	*Radix ginseng* 100%, *Ginko biloba*, *Daminana* ,
Givers P Capsules	*Penianthus zenkeri*, *Clausina anista*
Ziipman Herbal Capsules	*Paulina pinnata*
Vigomax Forte	*Withania somnifera* 500mg, *Mucuna pruriens* 200mg, *Ricinus communis* 150mg, *Chlorophytum arundinaceum* 100mg
Pa-Kum Capsules	*Cissus popunea*, *Cyperus esculentus*, *Musa paradisiaca*, *Epimedium grandiflorum /Horney goat weed extract*
Extra Strength Adams Secret	*Epimedium sagitatum*, *Coleus froshkohlii*, *Maca extract*, *Cnidium monnieri*, *Catuaba*, *Xanthoparmelia scabrosa*, *Tongkat ali*, *Horny Goat Weed Extract*, *Damiana* extract-50mg, L-Arginine-100mg, Vitamin B6-20mg , Vitamin B2- 20mg, Vitamin E- 50 iu , Vitamin B1-20mg , Saw Palmetto Extract -

	50mg, Rhodiola rosea 150mg, calcium sulphate, magnesium stearate.
Today Man Capsule	*Penianthus zenkeri, Clausina anista*
Gidi Powa Herbal	*Capparis erythrocarpus, Khaya ivorensis, Clausina anista, Paulina pinnata*

Factors such as age, educational status, peer pressure, presence of sexual problems, continual advertisement and knowledge of unwanted effects of orthodox medicines are reported to influence the use of herbal aphrodisiacs (Manortey *et al*., 2018). A study conducted in Ashaiman municipality on use of herbal aphrodisiac revealed 52.6 % of 352 men interviewed, reportedly used aphrodisiacs at some point in time with majority (68 %) between the ages of 18 - 25 years. Approximately 50 % of aphrodisiac users had no sexual problems indicating recreational use only or just for the fun of it (Manortey *et al*., 2018).

A similar study at Agbogbloshie, a suburb in Accra metropolis, by Atuobi-Bediako, 2019, revealed that out of 383 men interviewed, 42 % reportedly used aphrodisiacs at certain points in their lives; 82 % of the respondents were below 40 years. Advertisement was a major determinant of the use of aphrodisiac.

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PROBLEM STATEMENT

Globally, male infertility is increasing with varying consequences (Mathur, 2012 and Kotta *et al*, 2013). It is reported that about 10 million people living with erectile dysfunction globally do not opt for treatment (Lim, 2017). Due to societal sensitivity and stigma, many sufferers of erectile dysfunction resort to herbal aphrodisiacs (Dike *et al*., 2020). It is expected that majority of people who patronize aphrodisiacs should be the elderly since age affects male sexual function and diminishes their libido (Chung, 2019). To complicate issues, ED sufferers also have underlying conditions such as atherosclerosis, hypertension, diabetes mellitus, depression or other medications that are constantly been taken (Frimpong-Manso *et al*., 2016). Also, many investigators have reported a high degree of use among the youth with normal sexual function.

Reasons adduced for usage of aphrodisiacs include recreational purposes, increasing erectile rigidity, maintaining an erection, improving sensation, prolong sexual intercourse, impress the other partner, satisfy ego, curiosity, increasing sex drive (Makwana *et al*., 2013 and Atuobi-Bediako, 2019).

Consumption of these products by especially the elderly exposes them to various risks like increased susceptibility to cerebrovascular accidents and priapism (Atuobi-Bediako, 2019)

Little is known about the toxicity of herbal aphrodisiacs as there are no comprehensive precautionary labels on the products. Additionally, there is general misconception that medicines of herbal origin are safe. It is therefore imperative that additional investigations on the potential and actual toxicity of herbal products be done to supplement Food and Drug Authority (FDA) pre-registration toxicity requirements.

The Irwin test, a widely used model for quick and broad-spectrum screening compounds/products will be employed in this study to screen aphrodisiacs of herbal origin for safety.

JUSTIFICATION OF STUDY

Cultural values define masculinity based on sexual prowess and fertility placing a high premium on sex. Thus, societal insensitivity and stigma associated with ED causes serious distress with noticeable effects on the self-pride (Wattanathorn *et al*., 2019). Sufferers of ED and adventurous young men patronize herbal aphrodisiacs for both therapeutic and recreational purposes. Despite their therapeutic benefits, some components of the product are potentially harmful and pose health risks. Most herbal aphrodisiac are poly-herbals increasing the risk of possible interactions with either concomitant diseases or medicines.

Vulnerable group for erectile dysfunction is mostly the aged (Gareri *et al*., 2014). The continuous use of aphrodisiacs exposes them to a higher risk of developing adverse effects due to: compromised organ functions, diminished ability to excrete drugs and multiple comorbidities (Nobili *et al*., 2011). This category of patients may be on other medications

which may also increase the risk of drug-drug interactions with any of their poly-herbal constituents (Gareri *et al*., 2014). Additionally, Vardenafil, a synthetic medication used to treat erectile dysfunction has been reported to be a major adulterant in many herbal aphrodisiacs (Atuobi-Bediako, 2019). Use of these products have been associated with heart attacks, kidney failures, cardiac arrest and even impotence (Ocloo, 2015).

As part of the continuing efforts to ensure safety of herbal products, this study investigated the acute toxicity profiles of herbal aphrodisiacs.

RESEARCH HYPOTHESIS

Herbal aphrodisiacs could possess adverse effects.

AIM OF STUDY

To carry out acute toxicity screening of five herbal aphrodisiacs in pharmacies within the Accra metropolis.

SPECIFIC OBJECTIVES

- To determine the acute toxicity profiles of five herbal aphrodisiacs in Sprague Dawley rats using the modified Irwin test.
- To perform histopathological studies on brain, livers, hearts, spleen and kidneys of the experimental animals.
- To determine effects of the herbal products on hematological and biochemical parameters of the experimental animals.

Chapter 2

LITERATURE REVIEW

INTRODUCTION

At the commencement of human existence, natural products have been used in various ways from generation to generations by man (Shakya, 2016). These natural products were used for prophylaxis and therapeutic purposes and came in the form of microorganisms, animals, marine organisms and plants (Yuan *et al*., 2016).

The knowledge about these products being edible or medicinal developed as man went out searching for food and ended up taking poisonous substances that resulted in vomiting, stomach upsets, coma and death (Yuan *et al*., 2016). Such experiences brought distinguishing knowledge of what is food and what is not food. These natural products that were recognized and separated as medicines were called traditional medicines and their knowledge was then transferred from generation to generation in a more refined and acceptable way. Traditional medicine has therefore, been a kingpin in treating diseases since ages due to their inherent pharmacological effects like anti-infectives, anti-hyperlipidemia and anti-inflammatory etc. (Ezekwesili and Nwodo, 2013). World Health Organization (WHO) reports of more than 80 % of the population in the world rely on them for their basic healthcare needs currently (Vedhanarayanan *et al.*, 2013). The dependency is more notable in developing countries. It has gained more acceptability due to its easy accessibility, affordability, personal believes and perception of harmlessness that has been linked to it (Amoah *et al*., 2014). Despite the above advantages, imprecise dosage regimen, impure and sometimes unhygienic nature had been linked to preparations and usage over the years (WHO, 2004).

With the onset of technology, traditional medicines usage and acceptability has become more acceptable among the elite with the knowledge that products have undergone an immense trans-formational treatment such as extraction, distillation, expression, fractionation, purification and concentration to become more effective and safer. Traditional medicines that basically use medicinal plants are called herbal drugs or medicine. Popularity of herbal

medicines as a natural or alternative way of treatment and prevention of various conditions has increased with time and has been widely accepted.

Herbal medicines use various parts of the plant and their extracts for treatment and prevention of illnesses (Patrick-Iwuanyanwu and Nkpaa, 2015). A typical example is herbal aphrodisiacs. Traditionally, these drugs are used for treating erectile dysfunction (ED) among the aged (Dike *et al*., 2020). They have been formulated into capsules, solutions, suspensions and tablets and packaged to not only be hygienically presentable but also appeal to all classes of people. Herbal aphrodisiacs are widely accepted and patronized by the young despite purposely being made for the aged (Manortey *et al*., 2018).

The claims of these products been natural and therefore safe compared to the synthetic aphrodisiacs have made them popular products in Ghanaian pharmacies. But this claim of safety on human health should be continually be monitored since several reports on systemic toxicity and potential unwanted effects of several natural products have been made (Lüde, 2016). There is a possibility for some natural products to possess 'silent' harmful effect that is not evidential within a short time after preparation (Patrick-Iwuanyanwu and Nkpaa, 2015). Natural products have different damaging effect on the different systems of an individual's body especially central nervous system, respiratory system and cardiovascular system. The vulnerability of these systems in the body to these products are as a result of high vascularization of these organs making them easily accessible to natural products at high concentrations. In addition, when products reach some organs (heart, lungs, brain) notably known to regulate more sensitive bodily functions in high concentrations, they bring about significant changes that affect homeostasis (Gordan, 2015). Past generations have overlooked chronic and subtle forms of toxicity like hepatotoxicity, carcinogenicity and mutagenicity in relation to some herbal medicine's toxicity. Some herbal medicines have been associated with serious liver toxicity (Patrick-Iwuanyanwu and Nkpaa, 2015).

HEPATOTOXICITY

The main target for toxic compounds is the liver due to its prior exposure to intestinally absorbed foreign substances before they enter into blood circulation (Alelign *et al*., 2020). The

liver detoxifies toxins even though these toxins may harm it (Ravikumar and Gnanadesigan, 2012). Thus making liver function test for experimental animals very important. This test increases understanding of the toxic effects, which can be generalized for safety use in humans. Liver cells produce enzymes, these enzymes include alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Alanine aminotransferase (ALT) produced in the hepatocytes is a very specific marker for liver damage compared to AST due to it lower concentrations in other tissues. It may go up with certain drugs. Aspartate aminotransferase (AST) which occurs in two isoenzymes namely mitochondria produced in hepatocytes and response to stress in membranes similar to alanine aminotransferase (ALT) and the cytosolic which is non-hepatically produced in the kidney tissue, heart muscle and skeletal muscle. ALT and AST high levels are used in conjunction for hepatocellular damage. ALP is produced bile cells lining canaliculi and bile ducts. Also produced in the intestine, kidney, WBC, placenta and the bone. Therefore, ALP levels may be raised even in the presence of healthy liver, with bone disease been the most common cause (Hall and Cash, 2012, Levick, 2017).

Test performed to ascertain the levels of these enzymes is called liver functioning test (LFT). The liver functioning test seeks for these enzymes (parameters) which are present in the serum at low concentrations under normal physiological conditions but becomes elevated in the serum when there are lesions (Agbodjento *et al*., 2020).

Fairly specific liver enzymes are ALT and AST however, ALT or AST raised levels exceeding upper normal limit than ALP, indicates hepatocellular pattern i.e., drug or alcohol-induced hepatitis, metabolic liver disease, viral hepatitis and auto-immune hepatitis. In the same way, raised ALP levels more than the upper normal limit, than either AST or ALT indicates a cholestatic pattern suggesting a biliary pathology i.e., bile cholestatic drug-induced liver injury (new causative medication) in acute settings. These liver enzymes abnormalities can be classified as mild if <5 times, moderate if 5-10 times or marked if >10 times the upper normal limit respectively (Levick, 2017). Many herbal medicines have been linked to hepatotoxicity and is from mild hepatitis to acute liver failure (Fatima and Nayeem, 2016).

RENAL TOXICITY

Also, the kidney is a vital organ whose function can be analyzed to ascertain the toxic effect of a drug taken. i.e., functional analyses of the kidneys are another index of ascertaining toxic effects of plant extracts (Zainal *et al.,* 2020). The integrity of the kidney is and not limited to excretion of toxins such as creatinine, urea and uric acid from the blood. Urea is a nitrogenous based compound formed by the liver and 85 % excreted by the kidneys and 15 % by the digestive tract. Urea levels in serum can be attributed to either decrease renal clearance in the case of acute and chronic renal impairment or digestive tract disorders like upper gut bleeding, dehydration, high protein diets and catabolic states. Decrease in urea levels can be as a result of severe liver damage, severe hunger or low protein diet (Gounden *et al.*, 2020). Hence urea levels help in renal function analyses to some extent but not a sure marker as creatinine. Creatinine is a more accurate indicator of the kidney glomerulus, produced constantly as a byproduct of muscle's creatinine phosphate and excreted solely by the kidneys. High creatine levels show low blood cleansing function of the kidneys and vice versa making it more accurate indicator of renal function (Seriana *et al.*, 2021). Damage to the kidney as a result of herbal medicines may be attributed to presence of heavy metals, adulterations and careless way of preparing them (Fatima and Nayeem, 2016).

BRAIN TOXICTY

Another vital organ whose functionality can help determine the toxic effect of a drug is the brain which is part of the central nervous system (CNS). Drugs that can penetrate the Central nervous system could have both pharmacological and detrimental effect on the brain. Toxicity to the brain in the addition to the spinal cord is the most dangerous since it controls other systems like endocrine, gastrointestinal, cardiovascular and respiratory which are sometimes exhibited as urine and stool retention, impotence and loss of activity (Fatima and Nayeem, 2016).

HEMATOLOGICAL STUDIES

To prevent toxic effects on vital organs, a higher predictive value like hematological parameters is employed to indicate potential health hazards on humans when results are translated from animal studies (Patrick-Iwuanyanwu and Nkpaa, 2015, Arsad *et al*., 2013 and Ghadirkhomi *et al.,* 2016) or help determine the toxic effect in relation to dose and time response (Alelign *et al*., 2020).

Some blood parameters like mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular volume (MCV) are used for diagnosis of anemia (Amna *et al.,* 2013). MCV reflects red blood cell's size; whilst mean corpuscular hemoglobin (MCH) and MCHC are used to define hemoglobin concentration mathematically (Mahmoud, 2013). Animal's percentage of total red blood cells in whole blood are associated with hemoglobin (HB) and Parked cell volume (PCV) and are also anemic determinant (Alelign *et al*., 2020). Low levels of PCV, Hb, RBC and platelets suggest direct or indirect or both destruction of RBCs and low hemoglobin concentration. Oxidative damage is the indirect destruction of RBC. Hb, RBC and PCV are associated with the total population of red blood cells whilst MCV and MCH are associated to individual RBCs. Production of RBCs is called erythropoiesis which is stimulated by a circulating hormone called erythropoietin (a glycoprotein). The higher the levels of this hormone the higher the production of RBC and vice versa which can lead to normochromic, normocytic anemia. The size of the RBC which is MCV also determines the category of anemia i.e. MCVs below normal indicates RBCs smaller than normal and is termed as microcytic anemia whilst MCVs elevated than normally indicates RBC larger than normal and is described as macrocytic but normal size RBCs are termed normocytic (Patrick-Iwuanyanwu and Nkpaa, 2015).

In addition, platelet distribution width (PDW), platelet large cell ratio (P-LCR) and mean platelet volume (MPV), and are also needed for toxicity screening (Elsewefy *et al*., 2014). Clotting or bleeding abnormalities are indicative of platelets rise or fall respectively (Alelign *et al*., 2020). All the above information makes studies that evaluate pharmacological safety and toxicity of new natural products very important (Aydın *et al.*, 2016).

In the development of new plant-based medicines, safety pharmacological studies are very important (Tornatore *et al.*, 2019). This study aims at investigating the probable unwanted drug

effects on physiological functions, using doses that falls within and above therapeutic ranges (Bass *et al*., 2004).

Safety Pharmacology predicts the safety of a new compound during the preclinical and clinical stages of its development using basic principles of pharmacology in a well regulatory-driven process.

Different methods are employed in safety pharmacology, notable amongst them is the modified Irwin test.

MODIFIED IRWIN TEST

The Irwin test was developed originally to detect psychologically active compounds in mice (Irwin, 1968), hence use of the term 'modified Irwin test' when applied to rats. The Irwin pharmacological test is used to evaluate both the therapeutic and toxicity effects of a new compound or substance. It is an observational test that studies behavior and physiological function of a new compound, from the first observable effective dose up to doses that induce clear behavioral toxicity. This is carefully carried out by trained observers and can be used in providing minimum dose lethal for a test substance. It gives initial estimate of a compound's duration of action on different end point (Castagne´ *et al.*, 2014, Jackson *et al*.,2019, Gauvin *et al*., 2016).

The Irwin test is a powerful tool to detect potential issues of safety, including motor impairment, sedative problems and convulsive potentials. It is also useful in determining qualitative effects of compounds on physiological and behavioral functions thus a broad spectrum of activity a product can produce. Irwin test is preferred to other safety pharmacological studies like functional observational battery due to fewer animals required making it less costly and less time consuming (Mathiasen and Moses, 2018). It gives us the initial signal that we can investigate with further specific tests to confirm these findings. Ahmed and Aslam, 2018 have used Irwin test to screen many aphrodisiacs of herbal base giving reproducible results.

Generally, experimental animals are used in modified Irwin test with the reason of close correlation to human toxicity. When test substances are administered by the same route as intended, the systems of animals incorporate similar pharmacokinetic (absorption, distribution, metabolism) as well as taking into consideration, other physiological events that can influence toxicity.

Potential toxicity is well predicted with cell-based assays but the added advantage with whole animal experiment is that it critically measures the toxicity of a test substance that manifest with increasing doses of the test substance gradually. Nevertheless, there are some drawbacks with the use of whole animal experiment namely; high costs of the animals used, slight variations within species may affect the desired outcome to be observed and difficulty of arranging long term experiments if need be (Ifeoma and Oluwakanyinsola, 2013).

Animal wellbeing is a major issue in safety and toxicity testing. Pain and suffering of these animals in herbal research are of much concern to animal ethical committees. In other to minimize pain and suffering when using animal-based toxicity testing, it is important to reduce the number of experimental animals because increasing number of animals will correlate to inflicting pain on more animals. Also, the tests methods can be improved and where possible, replace animal tests with other alternatives that are valid like the human cells (Ifeoma and Oluwakanyinsola, 2013). These conditions must be considered before conducting animal experiment.

Test animals used to perform Irwin test were initially mice but currently, rats are also being used as test subjects in what is termed as Modified Irwin test. The advantages of using mice are namely; more economical, both in terms of cost and the quantity of test substance needed for conducting the experiments and is the preferred animal for transgenic studies. Nevertheless, rats are considerably an acceptable option for studies of substances with probable effects on the CNS. Rats' behavioral repertoire is considered as richer making it useful in acquiring other data in the species. It is also the desired species for chronic treatment, biochemistry, pharmacokinetics, toxicology and therefore often preferred for core CNS safety batteries (Mathiasen and Moses, 2018 and Yamada, 2015).

The subject of exact relationship between ages of rats to that of human is still a matter of discussion and controversy. Rats' development is rapid during childhood, reach sexual and

social maturity at about six weeks old and five to six months later respectively. But in adulthood, every two and half years of human is approximately equivalent to every month of rat's adult life. This has been confirmed by several experimental studies that has estimated 30 days of human life to be equal to a day's life of the animal (Andreollo *et al*., 2012).

Based on the aim of the experiment, the test animals are sometimes pre-exposed to certain environmental conditions that will acclimatize them for some days prior to the experiment. Example is the exposure of the animals to dim light in the laboratory for a stipulated daily testing time for 6 days prior to an experiment that will be carried out in dim light (Owiredu *et al.*, 2007)

The route of administration in this test subjects are similar to that of humans as suggested by The ICH S7A guidelines in safety pharmacology. The per oral route used frequently in CNS core battery studies is not easy to predict in terms of similarity between rats and human absorption and metabolism of the new compound. Example is the poor absorption of some neuroleptics like haloperidol or sulpiride in rats after oral administration compared to that of humans with the same route of administration. More effective routes such as subcutaneous or intraperitoneal for safety pharmacological screening is therefore more justifiable (Castagne´ *et al.*, 2011). Despite of the above advantages of these routes and the limitations of the oral route, the latter was used in this study because of the nature of the products where most were crude plant products, making them unsuitable for any parenteral route.

Unlike traditional LD50 acute toxicity test that requires large numbers of test animals, the use of Irwin test in safety pharmacology generally limit the number of test subjects whilst ensuring that reliable results are obtained for making good regulatory decisions.

The substance to be tested is usually evaluated at three or four different doses given immediately before the test. For safety pharmacological studies, the lowest dose is the closes therapeutically estimated dose from preclinical tests and the highest dose is 100 or 300 times this initial dose if possible, physically. The doses of both the test substance and control vehicle are given based on the calculated weight of the test animals. Up to 48h observations are usually performed post administratively thus continuously from 0 to 15 min post-dose, and separately at 15, 30, 1h, 2h, 4h, 24h and 48h post dose. Measurements at 48 post administration are for

agents with prolong action duration or for further safety evaluation can be added (Owiredu *et al*., 2007).

Every safety pharmacology study aims at detecting risk. False negatives (type 2 errors) must decrease even if false positives (type 1 error) increases. To minimize type 2 errors, Irwin test incorporate controls without the test substance for comparisons (Castagne´ *et al*, 2011).
Physiological saline or water for injection solutions are mostly the control vehicles used. In cases that involve the comparison of the test substance with a particular class of substance, a right reference substance can be added in the evaluation.
Although it is essential to blind in most neurobehavioral testing and specifically during more subjective assays like the modified Irwin test, a non-blinded vehicle group is availably made to the experimenter, serving as a visual comparator for "normal" animal behavior subjected to the same testing conditions and at the same moment in time, in modified Irwin test (Lynch III *et al.*, 2011).
Specific items on modifications of behavior, neurotoxicity and physiological symptoms from Irwin test are recorded accordingly in a standardized observation grid (Porsolt, 2007). This grid is made up of the following parameters: loss of grasping, altered activity, increase or decrease fear/startle response, jumping, altered reactivity to touch, straub tail, aggression, fore-paw treading, head twitches, convulsions, tremor, head movements, chewing, sniffing, scratching, catalepsy, akinesia, abnormal gait (rolling and tip-toe), motor incoordination, loss of traction, writhing, loss of righting reflex, analgesia, loss of corneal reflex, ptosis, exophthalmia, altered respiration, loss of balance, piloerection, salivation, defecation/diarrhea, pupil diameter, lacrimation ,altered rectal temperature and even death (Lynch III *et al*., 2011). Some of these parameters can be broadly grouped, namely: excitation, sedation, stereotypy, autonomic, motor and others.

Excitation

Some parameters that fall under excitation are straub tail, increased reactivity, increase reactivity to touch, increase fear/startle, aggression, convulsions and tremors.

- The presence of straub tail phenomenon is noted when in the course of the experiment, the tail of the animal is consistently raised to 45° angle from bedding or standing upright or tail arched over the back of the animal in comparison to the normal which is flattened or elevated (very slightly) tail when walking.
- Generally, animals move freely in their cages, the presence of increase reactivity to touch is noted in comparison to controls when the animals are slightly more, rapidly or very rapidly active with or without pauses. Rats in nature are typically passive when touched or handled. Increase reactivity to touch is the flight reactions when little finger pressure is exerted on the hind quarters or irritability when handling or vocalization and sometimes increased urination/defecation (non-formed or liquid stool or increased number of fecal boli presence) and jumping.
- Fear/startle increase is noted and marked as presence when fingers are snapped above cage or a loud, sharp noise is made and immediately the animals respond by flinching, freezing, jumping slightly etc. compared with the control.
- The animals can be aggressive compared with the control and this is observed by the biting response of the animals to a poke towards their head using a ball-point pen. Very similar to reactivity to touch (Roux *et al*., 2004 and Mathiasen and Moser, 2018)

Sedation

Some parameters that fall under sedation are decreased fear/startle, decreased activity and decreased reactivity to touch.

- Unlike excitation, and under the same condition of removing the cover of the cage and manipulation of animals, if the animals move more slowly, very slowly or no movement at all compared to the control then it is recorded as decreased activity.
- If the fingers are snapped above cage or a loud, sharp noise is made and the animal does not respond by flinching, freezing, jumping slightly etc. compared with the control it will be noted as decreased fear/startle (Roux *et al*, 2004 and Mathiasen and Moser, 2018)

Stereotypy

Stereotypic behavior is repeated movement that are abnormal to that of the control and some parameters that fall under this are head twitches, scratching, fore paw treading, chewing, sniffing and head movements.

- Head twitches are noted with flicking or sudden jerking of the head only whilst twitching in general is more than head flicks and often leads to convulsive behavior.
- Scratching of the animals takes place using fore-or-hind paws on any part of the body. It marked as present if it is observed in comparison with the control.
- Presence of fore-paw treading is noted by the no displacement of the fore-paws despite repeated stamping around compared to the control.
- It is normal for the rats to sniff around cages and in an open space but excessive sniffing compared to the control is marked as stereotypically present (Roux et *al*., 2004 and Mathiasen and Moser, 2018).

Motor

Some parameters that fall under motor activity are abnormal gait (tip-toe), akinesia, abnormal gait (rolling), loss of grasping, catalepsy, loss of balance, loss of corneal reflex.
and righting reflex.

Corneal reflex (eyeblink) is involuntary blinking of the eyelid in response to a nearby object or a ball-point pen drawn closer to the eyes of the rats. Failure to completely close or blink eye is termed as loss of corneal reflex

- loss of righting reflex is the inability to resume optimal position when the animal is taking from its normal upright position.
- Presence of abnormal gait is noted with tip-toe or rolling movement of the animals in their cages compared to the control whilst loss of balance is noted when animals falls on its side during movement.
- Motionless in the form of temporary paralysis is termed as akinesia whilst catalepsy is the animal being in the state of trance and as a result it is unable to respond to stimuli (Roux *et al*., 2004 and Mathiasen and Moser, 2018).

- When the animal is held gently by the tail and placed on a wire mesh with the held tail horizontally pulled slowly and drawn backwards (1 second) over the wire mesh and there is no resistance to pull despite being repeated three times, it is scored as loss of grasping. Some resistance will be provided by the control group. Loss of traction is measured by assessing the inability of the rats to pull themselves up onto a wire mesh frame made of stainless-steel placed in a horizontal position approximately 25 cm above a top of a table (Redfern *et al.*, 2019).

Autonomy

Some parameters that fall under autonomy are ptosis, defecation/diarrhea, urination,

piloerection, salivation and lacrimation.

- Ptosis. Palpebral (eyelid) closure whilst exophthalmos is protrusion of the eyeball or eyeballs (bulging eyes).
- Lacrimation is the visible dampness around eyes. Lacrimal secretion appearing reddish is called chromodacyrrea.
- The region around the mouth of the rats are usually dry. If it has wet margins on entire submaxillary region or there is visible dampness around mouth then there is salivation.
- Mostly, the furs of the animals do not stand on end, the presence of fur standing on end is termed as piloerection. This erection of fur can either be ruffled or puff-ball in appearance
- It is usual for rats to defecate or urinate when handled but increased urination or defecation (number of fecal boli) is marked as increase urination and diarrhea respectively in comparison to the control (Roux *et al.*, 2004 and Mathiasen and Moser, 2018).

Other measures

- Analgesia/ Tail pinch response is inability of the animal to respond to pain (analgesia) when the tail is pinched at the base . It is scored as absence of analgesia or no tail pinch response if the animal shows some attention to pinch, bite, vocalize (make sounds). Unreactive animals (e.g., by vocalization or turning towards forceps) when pinched at the tip or base of the tail with forceps is noted as analgesic.
- Digital thermometer is used to measure rectal temperature by positioning 2 cm above the anal sphincter. The temperatures taken could be the same (euthermia), lower (hypothermia) or higher (hyperthermia) than the normal. Presence of hypothermia is recorded when baseline mean rectal temperatures are taken prior to the experiment for treated and the control groups and rats . Rats having mean temperatures decreased by 1 to 3 ^{o}C during the actual work compared to the baseline temperatures is deemed as hypothermia (Roux *et al*., 2004 and Redfern *et al*., 2019)
- Baseline mean rectal temperatures are taken prior to the experiment for treated and the control. Rat having mean temperatures increased by 1 to 3 ^{o} C during the actual work compared to the baseline temperatures is deemed as hyperthermia (Roux *et al*., 2004).

The above items in the grid are subjective to the experimenter depending on the type of research. Also, most endpoints are qualitative in nature, which requires training on the part of the experimenter to be able to distinguish 'normal' from 'abnormal' behaviors (Jackson *et al*., 2019). After period of administration and observation, the animals are sacrificed and their organs are harvested and examined for organ damages during toxicological studies (Ossato *et al*., 2016).

Compared to the automated system, the Irwin test is more labor intensive, performed in light phase and involves repetition of observations at short time intervals (Lynch III *et al*., 2011). Thus, experience observers or trained observers are needed in addition to the highly standardize procedure to reduce observational bias associated with qualitative studies and enhance it sensitivity and reproducibility (Castagne´ *et al*., 2014).

This Modified Irwin test was employed for safety screening of five herbal aphrodisiacs in community pharmacies within Greater Accra region. The profile for these five herbal aphrodisiacs sampled in pharmacies located in Greater Accra Metropolis were as follows:

1. ***Mr. Q***

Mr. Q is a Chinese herbal product indicated for enhancing the body's nature curing ability and promoting sexual coition.

Each 10 ml bottle of Mr. Q contains *Rehmanius* root, *Red ginseng*, *Achyrmthes* root, *Cnidium* fruit, herb of Korean *Epimedium*, *fruit* of *Puncturevine*, fruit of *Magnoliavine*, *Astragalus* root, *Rhodiola* root, *Songaria cynomorium* herb and *Aucklandia* root. *One bottle is taken orally each time before sex or two bottles (maximum for a day) each time before sexual intercourse.*

Figure 1. Image of Mr. Q herbal aphrodisiac

2. Ajanta's stamina capsule

Ajanta's stamina capsule is an herbal formulation that ensures relaxation, anti-stress and action thereby ensuring a person of healthy and happy sex life.

One capsule contains Silajeet, Bang bhasma, Ext. of Jaiphal (Mysritca fragrans), Ext. of Ashwagandha (Withania somnifera), and Ext. of Kavach (Macuna pruriens). Two capsules are recommended to be taken before sexual intercourse. It is produced by Ajanta Pharma Ltd.

Figure 2. Image of Ajanta's Stamina herbal aphrodisiac

3. Tinatett 230 Herbal Capsule

Tinatett 230 Herbal Capsule is indicated for enhancing energy and stamina for men.

One capsule of Tinatett 230 herbal capsule contains Roxb, *Ginkgo biloba*, *Tribulus terrestris*, *Cayenne panex* caltrop. Two capsules are taken orally 30 minutes before sexual intercourse. It is manufactured locally by Tinatett Herbal Manufacturing and Marketing Company Ltd.

Figure 3. Image of Tinatett 230 Herbal Capsule

4. Original Tiger Herbal

Original Tiger Herbal Formula distributed and marketed by Menzbek International Limited is indicated for premature ejaculation, sexual weakness, erectile dysfunction and improved sexual performance.

One capsule contains fruit of Chinese Wolfberry, Kudzu root, fruit of Psoralea, Wild Jujube, *Cinnamon lotus* seed, *Papaya*, leaf of *Epimedium*, Gorgon fruit, root of *Morinda officinalis*, Mulberry fruit, Chinese yam, *Fuling*, rhizome of *Polygonatum*, and Walnut kernel. One capsule is taken one hour before sex. It is manufactured by Luoyang Kanghua Biological Preparations Company ltd.

Figure 4. Image of Original Tiger Herbal Capsule

5. Odyssey herbal ***supplement***

Odyssey herbal supplement produced in Great Britain by alpha organics, is an herbal supplement indicated for male vitality.

One tablet contains, *Avena sativa, Gingko biloba, Tongkat ali, Chlorophylum, Borivilianum Withania somnifera, Capidium* and *Epimedium grandiflorum.* Two tablets are taken orally two hours before engaging in sexual intercourse. Best taken on an empty stomach and with warm water.

Figure 5. Image of Odyssey Herbal supplement.

Chapter 3

VALIDATION

INTRODUCTION

The Irwin test as a systematic, comprehensive, qualitative observational assessment tool was introduced to assess the neurobehavioral effects of drugs on mice (Irwin, 1968). The model has since been modified for use in rats and is now used in safety pharmacological studies recommended by the International Conference on Harmonization (ICH) S7A guidelines for evaluating the effects of new chemical entities, to help protect clinical trials participants and patients from potential adverse effects ("International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2000),", Redfern and Wakefield, 2006).

Validation of the model is very essential in determining the probable pharmacological effects of drugs in rats.

Once the model is validated, the certainty of any results obtained in the actual test will not be in doubt. Validation is mostly performed using two drugs that give opposing pharmacological effects which will aid in observing varying parameters. Mostly, a drug that gives sedating and calming effect such as Diazepam or Chlorpromazine and a drug that gives a central nervous system (CNS) stimulating effect such as amphetamines and caffeine (Redfern *et al*., 2019). In this validation, two established drugs, chlorpromazine a sedative and caffeine a stimulant will be used. The drugs will serve as positive controls on which effects of the test agents can be compared.

Chlorpromazine hydrochloride is a dimethylamine derivative of phenothiazine. It binds well with plasma proteins, with protein binding capacity of 90 – 99 %. Onset of action is 30 - 60 minutes and half-life is 30 hours and duration of action is 4 - 6 hours. It is mostly used as an antipsychotic drug and exerts its effect by antagonizing dopamine D2 receptors in the brain resulting in the inhibition of the release of hormones in the hypothalamic and hypophyseal. It possesses sedative, antiemetic, strong anti-adrenergic, weak peripheral anticholinergic, slight antihistaminic and anti-serotoninergic activity (Mokrushin, 2017).

Generally, caffeine is a CNS stimulant which improves wakefulness, reduce fatigues and span attention. Varying consumption of caffeine has different effects on the body. Temporal increase in heart rate and blood pressure are induced by moderate consumption whilst adverse effects such as severe hypertension, vomiting, nausea, tachycardia, convulsions and even death can be induced by excessive consumption of caffeine (Schepici *et al*., 2020).

Caffeine is absorbed orally in the gastrointestinal tract and metabolized by the liver cytochrome P450 enzymes into three metabolic products namely; theobromine, paraxanthine and theophylline. Caffeine and its metabolites cross all biological membranes and are found in all body fluids but do not accumulate in tissues or organs and is excreted through urination (Arnaud, 2011 and Che *et al*., 2012). Caffeine is lipid soluble thus crossing the blood-brain barrier (BBB) influencing the CNS and inducing respiratory and skeletal stimulation (Monteiro *et al*., 2016).

The mechanism of action of caffeine involves accumulation of intracellular cyclic adenosine monophosphate (cAMP) by the inhibition of phosphodiesterase (PDE) resulting in vasodilation, increasing nutrient and oxygen availability in the brain and muscle (Sansone *et al*., 2017).

In this experiment, caffeine and chlorpromazine were the drugs used to validate the model and served as the positive controls for all screening exercises with the herbal aphrodisiacs.

MATERIALS AND METHOD FOR THE VALIDATION

Acquisition and acclimatization of male adult Sprague Dawley rats

Male Sprague Dawley rats (n =25, 100g - 200 g;) were purchased and housed at Noguchi Memorial Institute for Medical Research, University of Ghana, Accra. The animals were housed in groups of 5 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with standard feed and tap water *ad libitum* except during early periods of the test. All animals used were experimentally naïve and were handled in accordance with the Guide for the Care and Use of Laboratory Animals (Nih *et al*., 2011).

Drugs

Chlorpromazine hydrochloride was purchased from Ernest Chemist Limited, Ghana, and anhydrous caffeine was purchased from G.R. Lane Health Products Limited (Gloucester, UK).

EXPERIMENT

The Irwin test was validated by randomly assigning the animals to five groups (n = 5) each. The sample size of n = 5 per treatment group was selected to have sufficient power to detect the effects reported on the modified Irwin test (Ewart *et al*., 2013).

Solutions of chlorpromazine and caffeine were prepared by dissolving appropriate quantities in normal saline. Rats in Group 1 were given saline 1 ml/Kg, p.o. as vehicle control, Groups 2 and 3 was given chlorpromazine, 30 and 60 mg/Kg, p.o. respectively; Group 4 and 5 received caffeine, 25 and 50 mg/Kg, p.o. respectively. Animals were monitored for effects of the drugs at the following times after drug administration: The first 0 -15 mins, then at 15 min, 30 min 1 h, 2 h, 4 h, 8 h, 24 h and 48 h. Specifically, animals were observed for changes in behaviours in three functional domains: motor/neuromuscular, autonomic and CNS activity (sedation, excitation, stereotypy) activities and death. Table 2.0 represent parameters added in the observations.

Table 2: Domains and associated parameters in which animals were assessed post drug administration.

	Domain	**Parameters**	
1	Autonomic	• Lacrimation • Salivation • Diarrhoea/defecation	• Urination • Piloerection • Pupil reflex
2.	Neuromuscular/motor	• Catalepsy • Akinesia • Abnormal gait	• Loss of traction • Loss of grasping • Loss of righting reflex

		• Loss of corneal reflex	
3.1	CNS: Sedation	• Decreased reactivity to touch • Decreased activity • Decreased fear/startle	
3.2	CNS: Excitation	• Convulsions • Tremors • Straub tail phenomenon. • Aggression	• Increased fear/startle • Jumping • Increased activity
3.3	CNS: Stereotypy	Fore-paw treading Head twitches Catalepsy sniffing	Head movements Chewing Scratching

Number of animals that exhibited a particular behavior was recorded and expressed as a percentage of total number of rats (n=5) in the cage.

At the end of the assay, animals were anesthetized with sodium pentobarbitone 100 mg/kg intraperitoneally, killed by cervical dislocation and incinerated.

RESULTS OBTAINED FROM VALIDATION

3.3.1. Animal behavior post chlorpromazine administration.

Figure 6 represents effects of chlorpromazine on functional domains in male Sprague-Dawley rats. The figure represents aggregates of behaviours observed over 48 hours at specified times. Appendix B contains the raw data collected at the following times t_0: 0-15 mins, t_1: 15 mins; t_3: 30mins; t_4: 1h; t_5: 2 h; t_6: 4 h; t_7: 8 h; t_8: 24 h; t_9:48 h.

Behavior traits markedly affected following chlorpromazine administration were indicative of sedation. Within 30 minutes' post drug administration, all rats treated with both 30 and 60 mg/kg, p.o. indicated marked decrease of fear, reactivity to touch as well as decreased activity/movement.

There was also piloerection, an indication of autonomic involvement, after chlorpromazine administration at both dose levels. No other behavioral parameter suggestive of autonomic involvement like lacrimation, defecation, salivation and ptosis was observed.

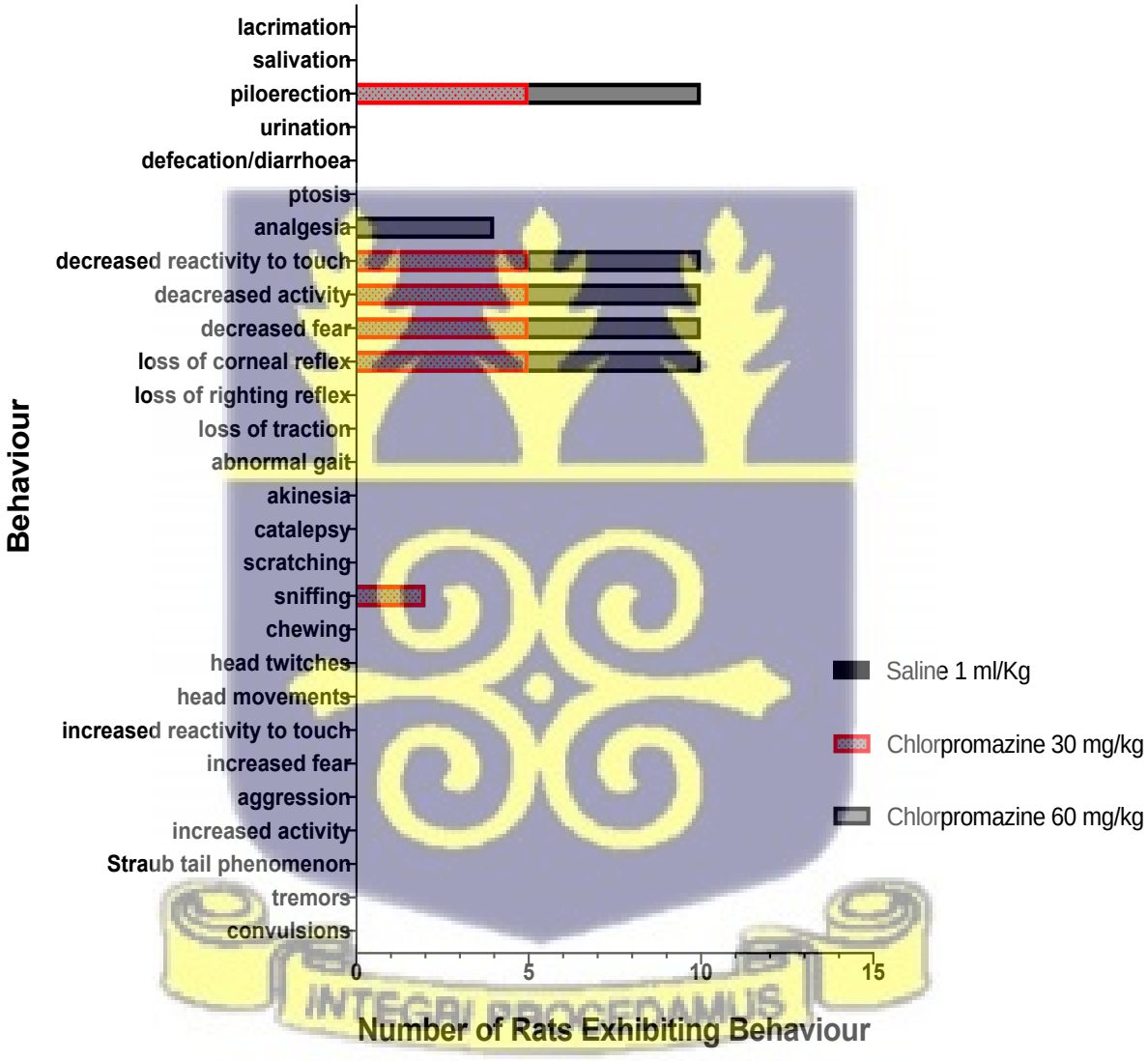

Figure 6. Effect of Chlorpromazine, administered p.o. at 30 and 60 mg/Kg body weight, on male Sprague-Dawley rats. Behavioral indices were determined according to the

modified Irwin test. The results are an aggregate of observations over 48 hrs. Raw data is presented in Appendix 2.

Animal behaviour post caffeine administration.

Figure 7 indicates the effects of caffeine administered at 25 and 50 mg/Kg, p.o. in male Sprague-Dawley rats.

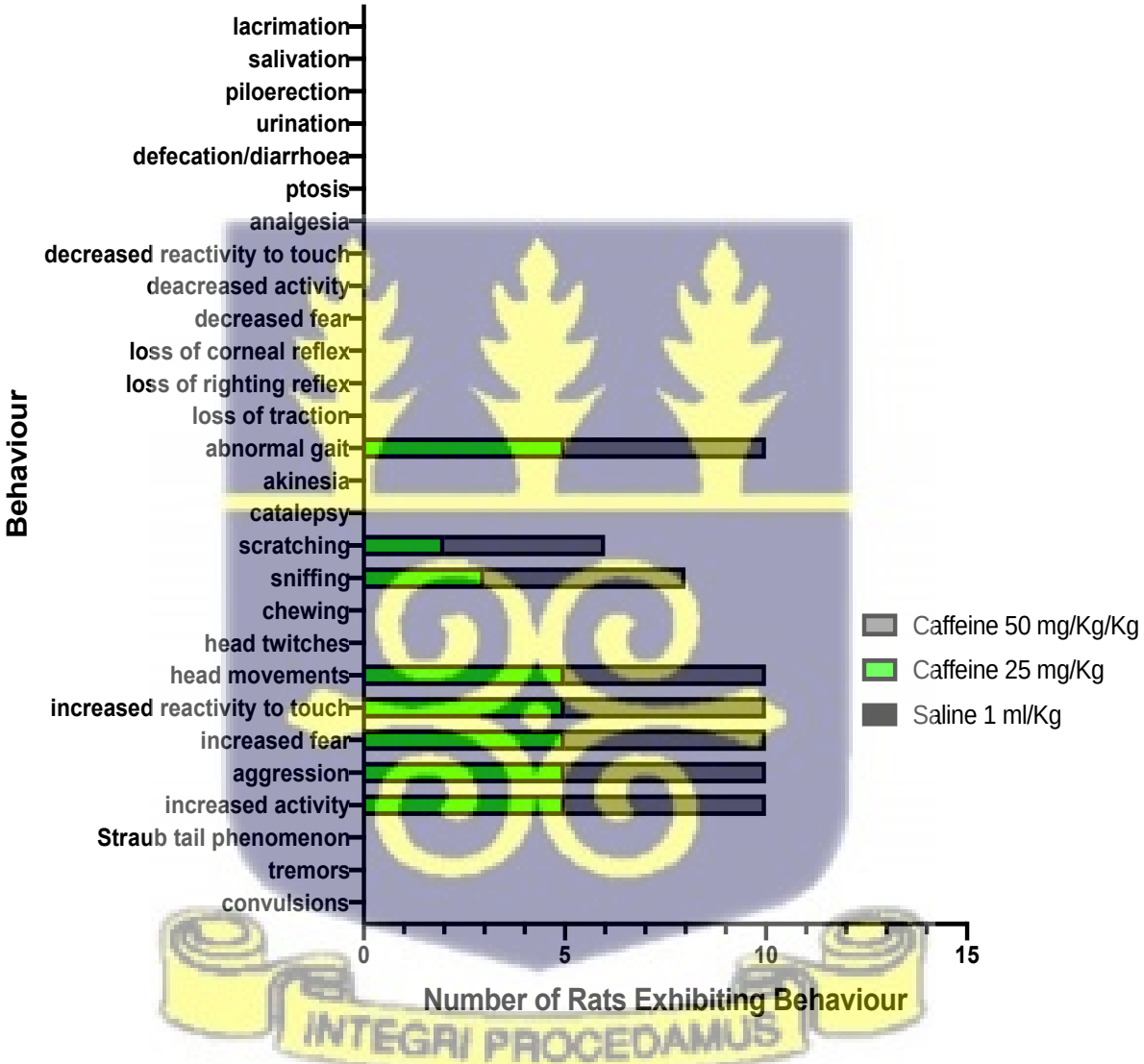

Figure 7. Effects of caffeine (administered at 25 and 50 mg kg^{-1}, p.o.) on male Sprague Dawley rats. These results are aggregates of observations over 48 h. The raw data indicating time-course effects is presented in Appendix 3.

There is concordance with effects of caffeine administered at 25 and 50 mg kg^{-1}, p.o. in the rats. Behaviours prominently indicating excitation were observed as: increased aggression, increased reactivity to touch, increased activity, increased fear/startle on snapping of observer's fingers. Though all animals exhibited these behaviours, rats treated with the higher dose exhibited the behaviours earlier.

CONCLUSION

In this validation experiment, dose- and time-related responses to chlorpromazine and caffeine were evaluated. The model has been shown to be suitable for further screening of test substances for peripheral and central nervous system effects.

The Irwin test is a validated approach for comprehensively observing and measuring the behavioral, neurological and autonomic nervous system responses of animals in pre-clinical pharmacological safety evaluation of test compounds. It is an established fact that single behavioral units of measure are not only costly but also time-consuming and offer little relevance for predicting estimates or making inferences regarding the relative safety of drugs administered, for extrapolation to safety evaluation in humans (Irwin, 1968a, 1968b).

Chapter 4

SCREENING OF PRODUCT

ETHICAL STATEMENT

The University of Ghana Institutional Animal Care and Use Committee (Protocol ID: UG-IACUC 009/18-19) approved the experimental protocol and surgical procedures. National Institute of Health Guidelines for the care and use of laboratory animal procedures and techniques were adhered to in this study. The certification is presented as APPENDIX A.

MATERIALS AND METHODS

HERBAL PRODUCTS SELECTION

Most herbal aphrodisiacs in pharmacies located in the Greater Accra metropolis are taken on daily basis as supplements for male vitality. Products selected for this study resulted from the following survey:

Criteria for the selection of herbal aphrodisiacs for acute toxicity screening

An online survey was made in December, 2020 for the selection of the five herbal aphrodisiacs in Community Pharmacies within Greater Accra Metropolis.

Research Question

Which herbal aphrodisiacs are of high demand in Community Pharmacies in the Greater Accra Metropolis?

Research Participants

Community Pharmacists in the Greater Accra Metropolis.

Place of study

Online Pharmacist's Platforms

Results

12 herbal aphrodisiacs were pooled from the platform.

Below is the table with the listed herbal aphrodisiacs and their respective constituents from online survey.

Table 3: list of herbal aphrodisiacs from survey

PRODUCT	INGREDIENTS
Mr. Q	*Rehmanius* root, *Achyrmthes* root, fruit of *Puncturevine, Cnidium* fruit, herb of Korean *Epimedium,* fruit of *Magnoliavine, Songaria cynomorium* herb, *Red ginseng, Astragalus* root, *Rhodiola* root, *Aucklandia* root.
New Kingdom Ginseng Power capsule	*Radix ginseng* 100%, *Ginko biloba, Daminana* ,
Original Tiger Herbal capsules	fruit of Chinese Wolfberry, Kudzu root, fruit of Psoralea, Wild Jujube, *Cinnamon lotus* seed, *Papaya*, leaf of *Epimedium*, Gorgon fruit, root of *Morinda officinalis,* Mulberry fruit, Chinese yam, *Fuling*, rhizome of *Polygonatum*, and Walnut kernel.
Givers P Capsules	*Penianthus zenkeri, Clausina anista*
Odyssey tablets	*Avena sativa, Gingko biloba, Tongkat ali, Chlorophylum, Borivilianum Withania somnifera, Capidium* and *Epimedium grandiflorum*
Ziipman Herbal Capsules	*Paulina pinnata*
Vigomax Forte	*Withania somnifera* 500mg, *Mucuna pruriens* 200mg, *Ricinus communis* 150mg, *Chlorophytum arundinaceum* 100mg
Pa-Kum Capsules	*Cissus popunea, Cyperus esculentus, Musa paradisiaca, Epimedium grandiflorum /Horney goat weed extract*
Today Man Capsule	*Penianthus zenkeri, Clausina anista*

Gidi Powa Herbal	*Capparis erythrocarpus, Khaya ivorensis, Clausina anista, Paulina pinnata*
Ajanta Stamina	Silajeet, Bang bhasma, Ext. of Jaiphal (Mysritca fragrans), Ext. of Ashwagandha (Withania somnifera), and Ext. of Kavach (Macuna pruriens)
Tinatett 230 Herbal capsule	Roxb, *Ginkgo biloba*, *Tribulus terrestris*, *Cayenne panex* caltrop

Selection of five herbal aphrodisiac products for screening was based on the following criteria

1. Is it a registered product with the Food and Drugs Authority?
2. Is it purely an herbal product or it contains other ingredients e.g., vitamins?
3. What is the Manufacture's dosage recommendation, thus is it a daily dose product or should be taken when needed?
4. Have there been any issues in time past with the product e.g., adulteration
5. Can the product be used as a one-time dose and desired effect obtained?

Based on the above criteria, these products were sampled out for screening.

Tinatett 230 herbal capsule

Ajanta Stamina

Odyssey tablets

Mr. Q

Original Tiger Herbal capsules

These products were randomly coded as Product 1, 2, 3, 4, and 5 for this project. The identity of each product is known to only the supervisor of this project.

ACQUISITION AND ACCLIMATIZATION OF EXPERIMENTAL ANIMALS

Male Sprague Dawley rats (n =100, 100g - 200 g;) were purchased and housed at Noguchi Memorial Institute for Medical Research, University of Ghana, Accra. The room temperature was 28^{o}C + 2, humidity 60%. Lighting was maintained at 12 h light, 12 h dark. The animals

were housed in groups of 5 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding. The animals allowed unrestricted access to standard feed and tap water except during early periods of the test. All animals used were experimentally naïve and were handled in accordance with the Guide for the Care and Use of Laboratory Animals (Nih *et al*., 2011).

SCREENING

The five herbal aphrodisiac products were screened using a combination of criteria under the modified Irwin test and the Organization for Economic Cooperation and Development (OECD) chemical tests guideline no. 420.

Each of the five products were screened as follows:

PRODUCT 1

The recommended dose of two capsules of product 1 was emptied, weighed and recorded as 0.81g. Based on 70 kg man, this weight gave a working standard dose of 12 mg/kg. The content was transferred into a mortar and with an aid of a pestle it was grounded into a smooth powder. A solution of 1.2 mg/ml was prepared with normal saline and administered as low dose to the first set of rats (n=5) in relation to their respective weights. The medium and high dosed sets of rats (n=5) received 12 mg/ml and 1200 mg/ml respectively. Test substance (herbal products) were administered orally in a single dose with a gavage. Rats in the saline control group were administered a single oral dose of normal saline (1ml/kg, p.o.)

PRODUCT 2

Two capsules of product 2 were emptied, weighed and recorded as 1.04g (equivalent to the manufactures' recommended daily dose for 70 kg man). This weight gave a working standard dose of 15 mg/kg. The content was transferred into a mortar and with an aid of a pestle it was grounded into a smooth powder. A solution of 1.5 mg/ml was prepared with normal saline and administered as low dose to the first set of rats (n=5) in relation to their respective weights. The

medium and high dosed sets of rats (n=5) received 15mg/ml and 1500mg/ml respectively. Test substance (herbal products) were administered orally in a single dose with a gavage. Rats in the saline control group were administered a single oral dose of normal saline (1ml/kg, p.o.)

PRODUCT 3

Two caplets of product 4 were emptied, weighed and recorded as 1.1g (equivalent to the manufactures' recommended daily dose of 70kg man). This weight gave a working standard dose of 16 mg/kg. The content was transferred into a mortar and with an aid of a pestle it was grounded into a smooth powder. A solution of 1.60mg/ml was prepared with normal saline and administered as low dose to the first set of rats (n=5) in relation to their respective weights. The medium and high dosed sets of rats (n=5) received 16mg/ml and 1600mg/ml respectively. Test substance (herbal products) were administered orally in a single dose with a gavage. Rats in the saline control group were administered a single oral dose of normal saline (1ml/kg, p.o.)

PRODUCT 4

Two capsules of product 4 were emptied, weighed and recorded as 0.28g (equivalent to the manufactures' recommended daily dose of 70kg man). This weight gave a working standard dose of 4.0mg/kg. The content was transferred into a mortar and with the aid of a pestle it was grounded into a smooth powder. A solution of 0.4 mg/ml was prepared with normal saline and administered as low dose to the first set of rats (n=5) in relation to their respective weights. The medium and high dosed sets of rats (n=5) received 4.0 mg/ml and 400mg/ml respectively. Test substance (herbal products) were administered orally in a single dose using a gavage. Rats in the saline control group were administered a single oral dose of normal saline (1ml/kg, p.o.)

PRODUCT 5

Two ampoules of product 5 was emptied into a measuring cylinder giving a volume of 20ml (recommended daily dose of 70kg man). This volume gave a working standard dose of 0.3ml/kg. 0.3ml/kg was administered as low dose to the first set of rats (n=5) in relation to their respective

weights. The medium and high dosed sets of rats (n=5) received 3ml/kg and 15ml/kg respectively. Test substance (herbal products) were administered orally in a single dose using a gavage. Rats in the saline control group were administered a single oral dose of normal saline (1ml/kg, p.o.)

Prior to dosing of any of the above herbal products, the animals were allowed free access to water but feed was withheld overnight. Following the fasting period, the animals were weighed and the test substances administered. After the test substances were administered, food was furtherly withheld for 2 hours.

Each Animal was observed for effects of the test substances at the following times after test substance administration: 0-15 mins, 15 min, 30 min 1 h, 2 h, 4 h, 8 h, 24h and daily for 14 days. Specifically, animals were observed for changes in behaviours at three functional domains: neuromuscular/motor; autonomic and CNS. Death in any group was also recorded. The body weights of the animals were taken on days 0 and 14.

The study was qualitative using behavioral changes as end-points. Number of animals exhibiting a particular behavior were recorded and expressed as a percentage of total number (5) in the cage in comparison to that of the control animals.

MEASUREMENT OF BODY WEIGHTS

The animals were weighed on the first day and the 14^{th} day of the experiment. Difference in body weights was determined and compared with the control using a two-tailed paired students t-test.

BLOOD ANALYSIS

On day 14, the animals were anesthetized with sodium pentobarbitone (100 mg/kg intraperitoneally). Blood (10 ml) was collected once through cardiac puncture and placed in bottles containing ethylene diamine tetra-acetic acid (EDTA), an anticoagulant and serum separating tubes for hematological and biochemical analysis respectively.

HEMATHOLOGICAL ANALYSIS

Automated hematology analyzer (Mytaic 22, Germany) was used in performing the hematological analysis. Analyzed parameters were erythrocyte (RBC), platelet count (PLT), total leukocyte (WBC), differential leukocyte, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean platelet volume (MPV), hemoglobin (Hgb), mean corpuscular volume (MCV), hematocrit (HCT), platelet distribution width (PDW) and red distribution width (RDW).

BIOCHEMICAL ANALYSIS

Serum separating tubes containing blood were centrifuged at 3000 rpm at 4 ◦C for 10 min to obtain the serum and transferred into Cryo valves. The serum was then stored at -20 ◦C for biochemical analysis. Analyzed parameters were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) indicative of liver function, creatinine (Cr) and Urea indicative of renal function and creatinine kinase (CK-MB) indicative of brain function using URIT 880 Semi-automatic Chemistry Analyzer.

MEASUREMENT OF RELATIVE ORGAN WEIGHTS

After euthanisation, the brains, livers, hearts, spleen and kidneys, were excised and quickly weighed and compared to the body weights to determine the Relative organ weight of each animal. This was mathematically expressed as follows: Relative organ weight (%) = (Organ weight / final body weight) x 100. All carcasses were disposed of by incineration.

TISSUE PROCESSING AND HISTOLOGY

These organs of interest for each animal were maintained in 10 % (v/v) neutral buffered formalin for a week. It was afterwards washed with water. They were trimmed into 3mm thick slices using a scalpel, the slices obtained from the organs under investigation were dehydrated using increasing concentrations of alcohol (70 %, 80 %, 90 % and 100 %). The tissues were cleared

with xylene to remove the alcohol to enable infiltration with paraffin wax. The tissues were embedded in molten paraffin wax, slice sections of 4 µm thickness were made from the paraffin embedded tissues using a rotary microtone and scooped with glass slides after being floated on water of 46 ^{o}C of temperature. The frosted sides of the tissue slides were labelled with codes and oven dried at a temperature between 40 ^{o} C - 50 ^{o} C for 3 hours followed by deparaffinization in xylene and subsequent hydration with descending series of alcohol (100 %, 95 % and 80 %). These tissues were thereafter stained with hematoxylin and eosin dyes, dried and mounted on a light microscope for histopathological examination. Scoring used were 0-none of the above changes, 1- equivocal (uncertain), 2-slight and 3-severe.

STATISTICS

The results were analyzed statistically using GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Data from the study was expressed as mean±SEM (n = 5). Comparison of test groups with that of the vehicle-control was made using a nonparametric paired sample t-test and a one-way ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

Chapter 5

RESULTS

Figure 8 indicates the effects of Product 1 administered at 12 mg/kg, p.o., 120 mg/kg p.o. and 1.2 g/kg, p.o. on behaviours under the modified Irwin's test to monitor for the involvement of autonomic and neuromuscular involvement. There was no indication of autonomic or neuromuscular activation by the Product at the dose levels administered even though two rats in the group treated with 1200 mg/kg had watery stools within 24 hours (Figure 8, Appendix 4).

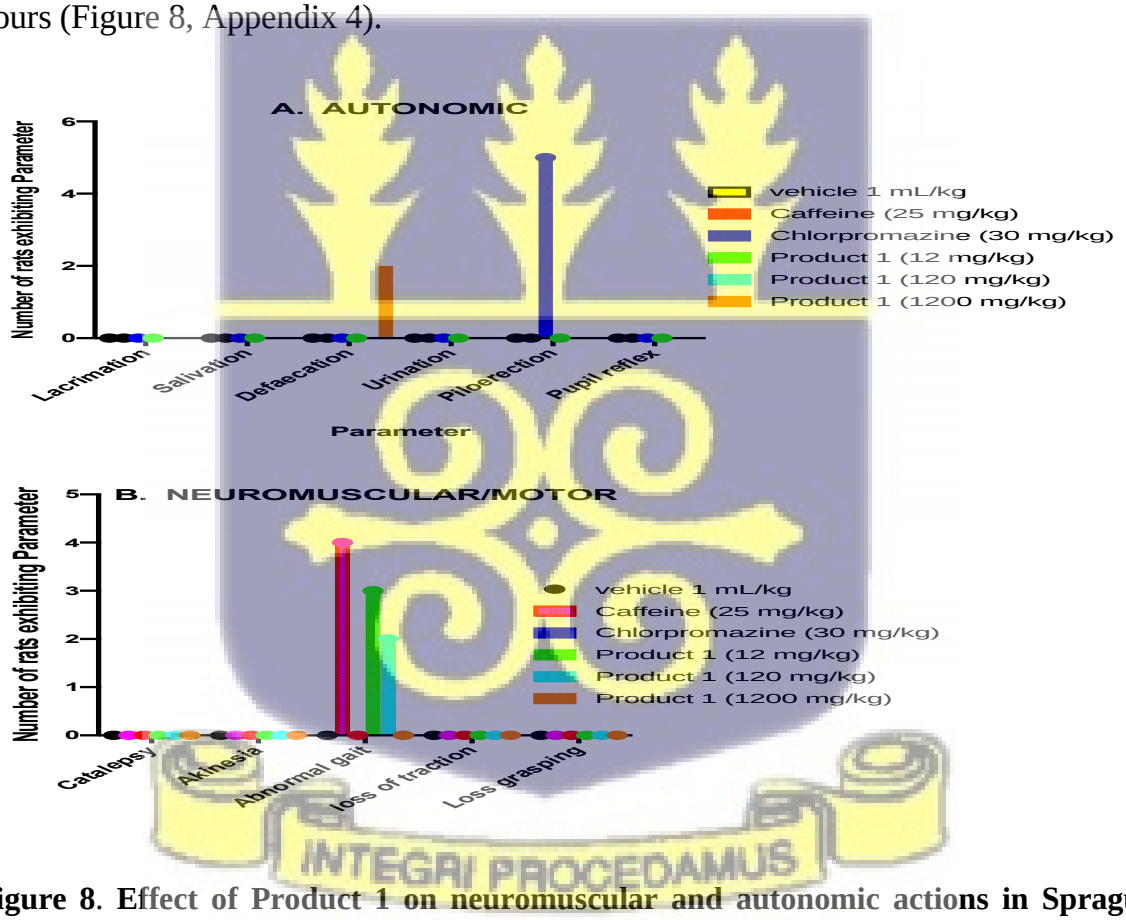

Figure 8. Effect of Product 1 on neuromuscular and autonomic actions in Sprague-Dawley rats. Rats were administered 12 mg/kg, p.o., 120 mg/kg p.o. and 1.2 g/kg, p.o. and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24 h and daily for 14 days. Results presented are number of rats exhibiting any parameter within the

first 24 h. The positive controls were Caffeine (red), Chlorpromazine (Blue) and vehicle (black) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

Figure 9 indicates effects of, Product 1 on the parameters indicating CNS activation as measured by effects on parameters indicating sedation, excitation and stereotypy in rats. The Product induced sedation at dose levels of 12 and 120 mg/kg, p.o. Out of the four parameters (decreased reactivity to touch, decreased fear, decreased activity and loss of righting reflex). Three out of 5 rats (60 %) in each test group did not react when touched (Fig 9, Appendix 4). All rats (5/5) given 12 mg/kg, p.o. of the Product showed decreased activity and fear when fingers were snapped. 80 % of rats given 120 mg/kg also showed reduced activity and fear. There was no loss of righting reflex in any group (Figure 9).

Though the Product at a dose level of 12 and 120 mg/kg sedated rats, Figure 9 show aggression, increased fear and activity and aggression when given the Product at 1200 mg/kg. Considering that the Product clearly induced sedation in rats, these paradoxical effects of aggression, increased fear and activity could not be attributed to CNS excitation as there was no indication of convulsions, tremors, straub tail phenomenon, aggression, and jumping all parameters for confirming CNS excitation.

Similarly, in Figure 9 (appendix 4) the Product increased sniffing over the dose range 12 mg/kg – 1.2 g/kg, p.o. Though this is one of the parameters among seven others (sniffing, fore-paw treading, catalepsy, head twitches, scratching, head movements and chewing) used to monitor for stereotypy, the evidence of ascribing stereotypy as one of the effects of the Product is weak.

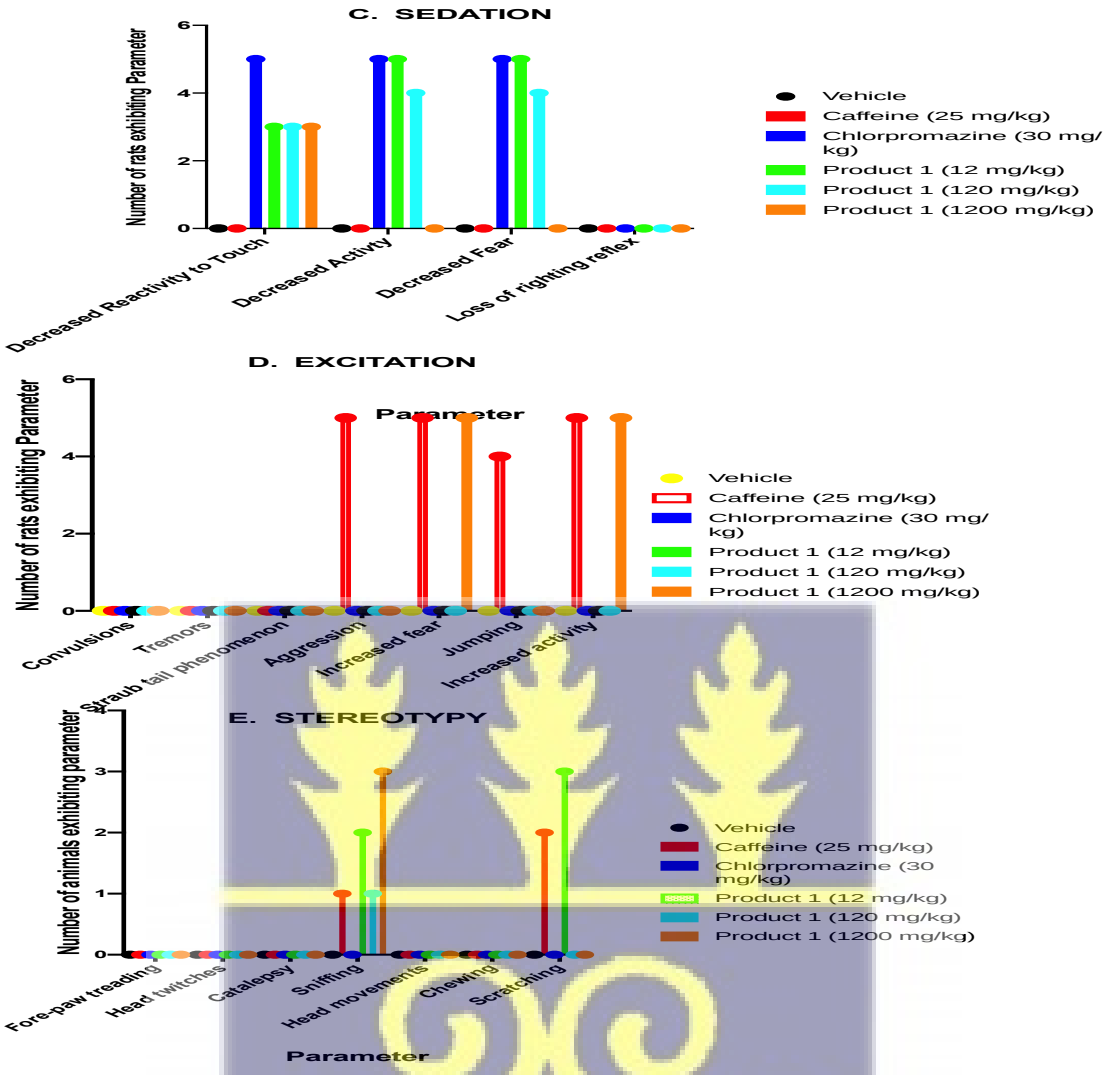

Figure 9. Effect of Product 1 on CNS activity in Sprague-Dawley rats. Rats were administered 12 mg/kg, p.o., 120 mg/kg p.o. and 1.2 g/kg, p.o. and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24 h and daily for 14 days. Results presented are number of rats exhibiting any parameter within the first 24 h. The positive controls were Caffeine (red), Chlorpromazine (Blue) and vehicle (black) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

All effects of Product 1 observed were of short duration, wearing off within 24 hrs. Details of the data are presented in Appendix 4.

EFFECT OF PRODUCT 2 ON SPRAGUE-DAWLEY RATS

Product 2 was administered, p.o., rats at 15, 150 and 1500 mg/kg (**Figure 10** and **Appendix 5**. For effects on CNS, rats were examined for evidence of excitation, sedation and stereotypy. Indicators for excitation were convulsions, tremors, Straub tail phenomenon, aggression, increased fear, jumping and increased activity. Caffeine, administered at 25 mg/kg, p.o., caused 4 rats in each case to convulse, tremor and exhibit Straub tail phenomenon. Four rats each in the groups treated with Product 2 at 150 and 1500 mg/kg increased activity/movements. There were no observable changes on the other parameters (convulsions, tremors, Straub tail phenomenon, aggression, increased fear, jumping) for excitement. Similarly, chlorpromazine had no observable effects on the indicators for excitation.

Indicators for sedation were decreased activity, decreased fear, decreased reactivity to touch and loss of righting reflex. As expected, chlorpromazine (30 mg/kg, p.o.) induced 4 rats, decreased reactions to touch, fear and activity. The extract, at all doses (15 mg/kg, 150 mg/kg and 1500 mg/kg) sedated the rats as evidenced by the number of animals showing decreased activity (15 mg/kg: 5 (100 %); 150 mg/kg: 5 (100 %) and 1500 mg/kg: 4 (80 %) and decreased fear (15 mg/kg: 5 (100 %); 150 mg/kg: 2 (20 %) and 1500 mg/kg: 2 (40 %). No rat on either the control drugs or herbal product lost the righting reflex over the dose ranges administered.

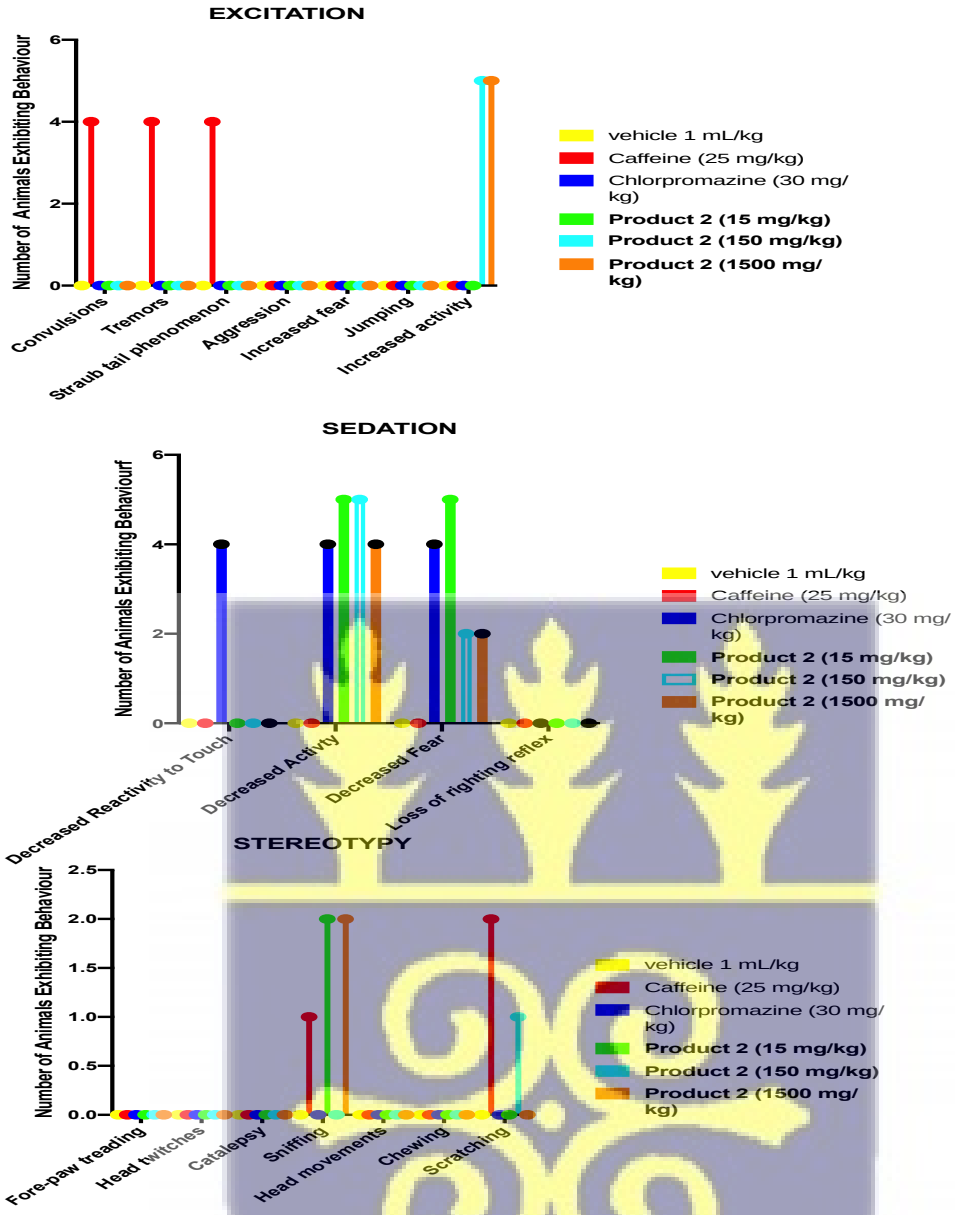

Figure 10: **Effect of Product 2 administered at 15, 150 and 1500 mg.kg, p.o., on the central nervous system of Sprague Dawley rats.**

Figure 11 indicates effects of Product 2 on manifestations of autonomic and motor activation. Two rats in the group treated with the high dose (1500 mg/kg) of the Product 2 had watery stools. There was otherwise no evidence of autonomic involvement with the effects of the product on the animals.

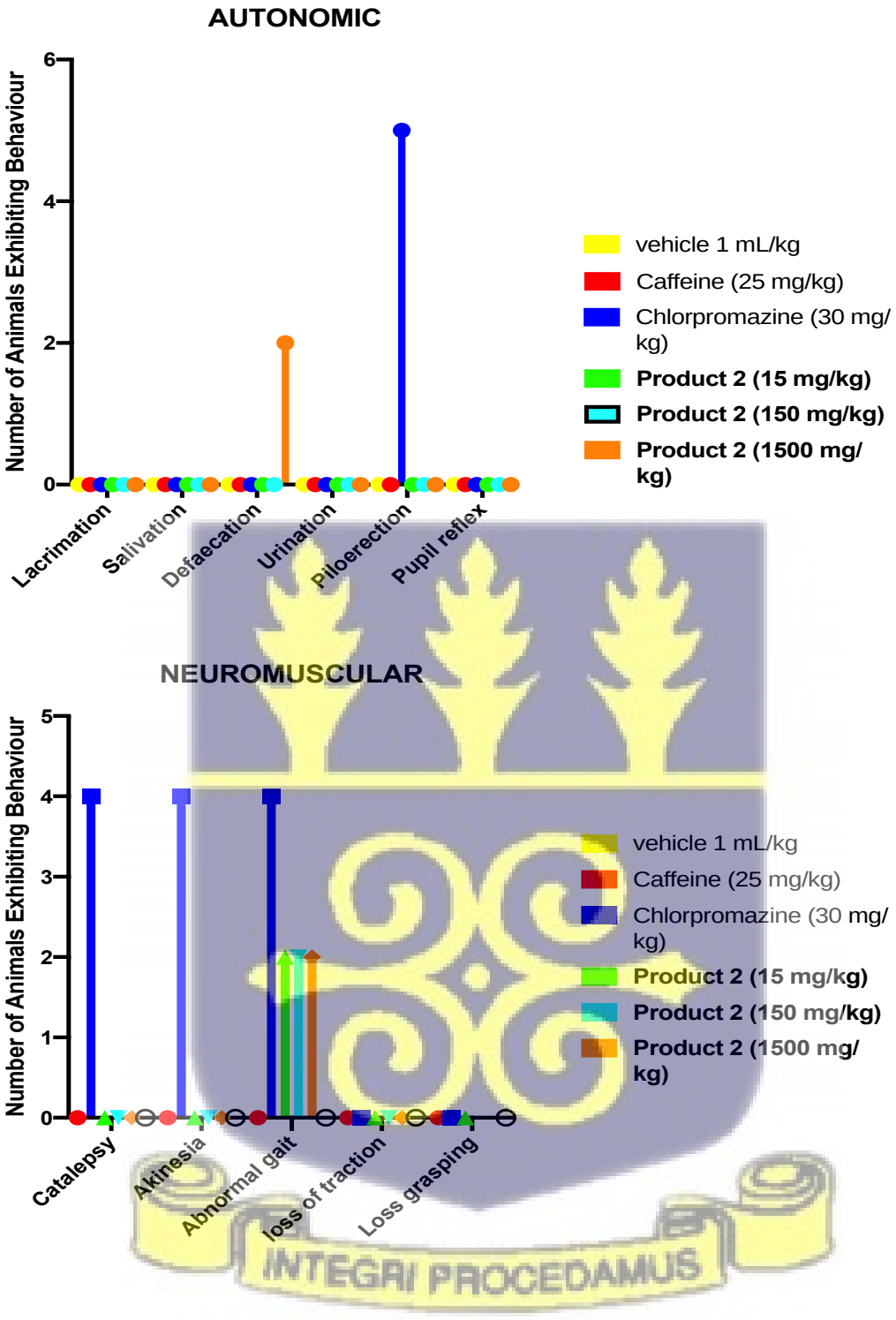

Figure 11. Effect of Product 2 on autonomic and motor systems. Rats (n = 5) were given p.o.; 15, 150 and 1500 mg/kg doses of Product 2.

EFFECT OF PRODUCT 3 ON SPRAGUE-DAWLEY RATS

Product 3 was administered, p.o., rats at, 16, 160 and 1600 mg/kg (**Figure 12** and **Appendix 6**). There were some evidences of neuromuscular and autonomic involvement with the effects of the product on the animals. The neuromuscular activity were abdominal gait and loss of grasping. Abdominal gait was observed to be increasing with increasing doses as 2, 3 and 5 rats in the groups treated with 16, 160 and 1600 mg/kg exhibited that respectively. Similarly, loss of grasping was not observed in the group treated with 16mg/kg of the product but rather in the groups treated with 160 mg/kg: 2 (40 %) and 1600 mg/kg: 3 (60 %) of the product.
The only autonomic behavior observed was defecation of which 1, 2, 1 rats out of 5 rats in the groups treated with 16, 160 and 1600 mg/kg respectively exhibited that.

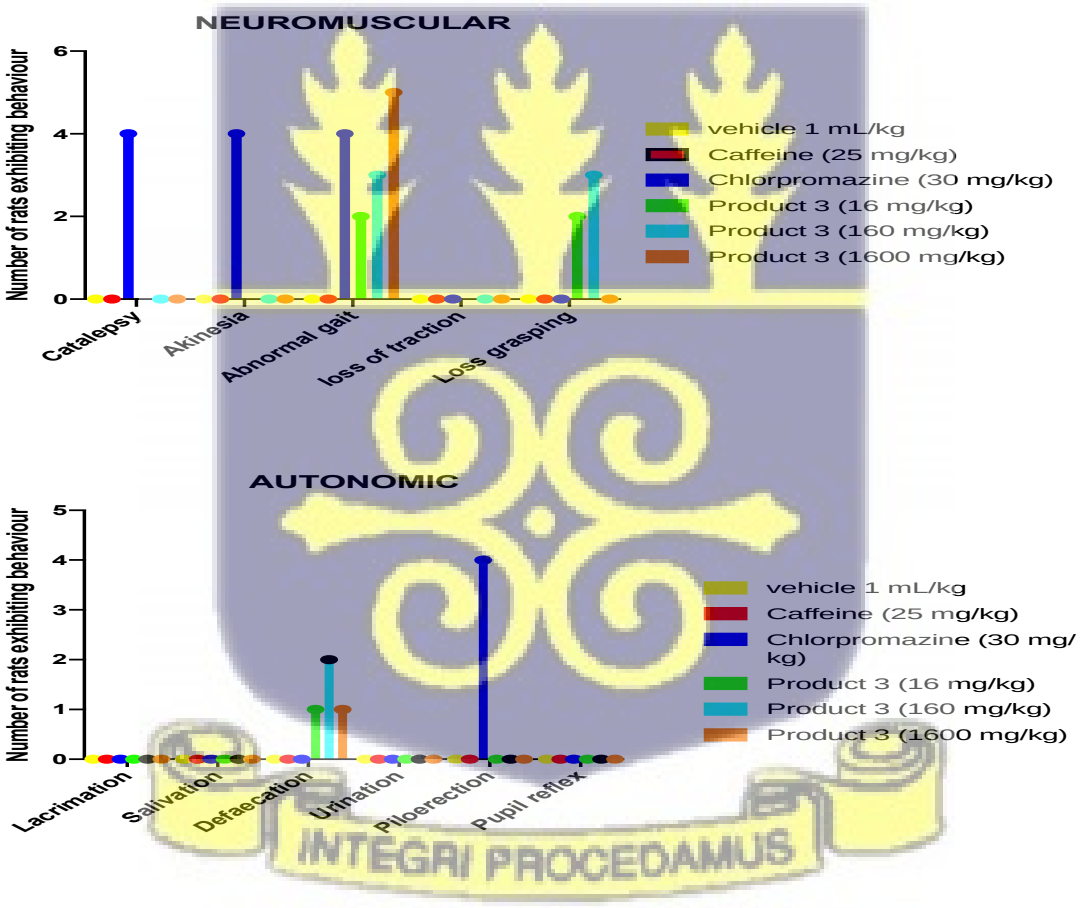

Figure 12. Influence of Product 3 on neuromuscular and autonomic actions in Sprague-Dawley rats. Rats were administered 16 mg/kg, p.o., 160 mg/kg p.o. and 1600 mg/kg, p.o. and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24 h and

daily for 14 days. Results presented are number of rats exhibiting any parameter within the first 24 h. The positive controls were Caffeine (red), Chlorpromazine (Black) and vehicle (white) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

Also as shown in Figure 13, rats were examined for evidence of excitation, sedation and stereotypy. Indicators for excitation were convulsions, tremors, Straub tail phenomenon, aggression, increased fear, jumping and increased activity. The only excitatory parameter observed out of the above was straub tail phenomenon which is unique for drugs that has opioid receptor activation. Nevertheless, this excitatory parameter was observed reducing with increased dosed of the product. Evidentially, 3 and 1 rats from the group treated with 16 and 160mg/kg respectively and none for the group treated with 1600mg/kg. Caffeine, administered at 25 mg/kg, p.o., caused 4 rats in each case to convulse, tremor and exhibit Straub tail phenomenon. There were no observable changes on the other parameters (convulsions, tremors, aggression, increased fear, jumping) for excitement.

Indicators for sedation were decreased activity, decreased fear, decreased reactivity to touch and loss of righting reflex. As expected, chlorpromazine (30 mg/kg, p.o.) induced 4 rats, decreased reactions to touch, fear and activity. The extract, at all doses (16 mg/kg, 160 mg/kg and 1600 mg/kg) sedated the rats as evidenced by the number of animals showing decreased reactivity to touch (16 mg/kg: 5 (100 %); 160 mg/kg: 5 (100 %) and 1600 mg/kg: 5 (100 %) decreased activity (16 mg/kg: 4 (80 %); 160 mg/kg: 5 (100 %) and 1600 mg/kg: 5 (100 %) and decreased fear (160 mg/kg: 5 (100 %) and 1600 mg/kg: 5 (100 %). No rat on either the control drugs or herbal product lost the righting reflex over the dose ranges administered. These sedation parameters were similar to that observed in rats treated with chlorpromazine 25mg/kg p.o.

In addition, the Product increased sniffing over the dose range 16 mg/kg – 1.6 g/kg, p.o. and chewing over a dose range of 160mg/kg – 1.6g/kg. Though this is two of the parameters among seven others (fore-paw treading, head twitches, catalepsy, sniffing, head movements, chewing and scratching) used to monitor for stereotypy, the evidence of ascribing stereotypy as one of the effects of the Product is weak.

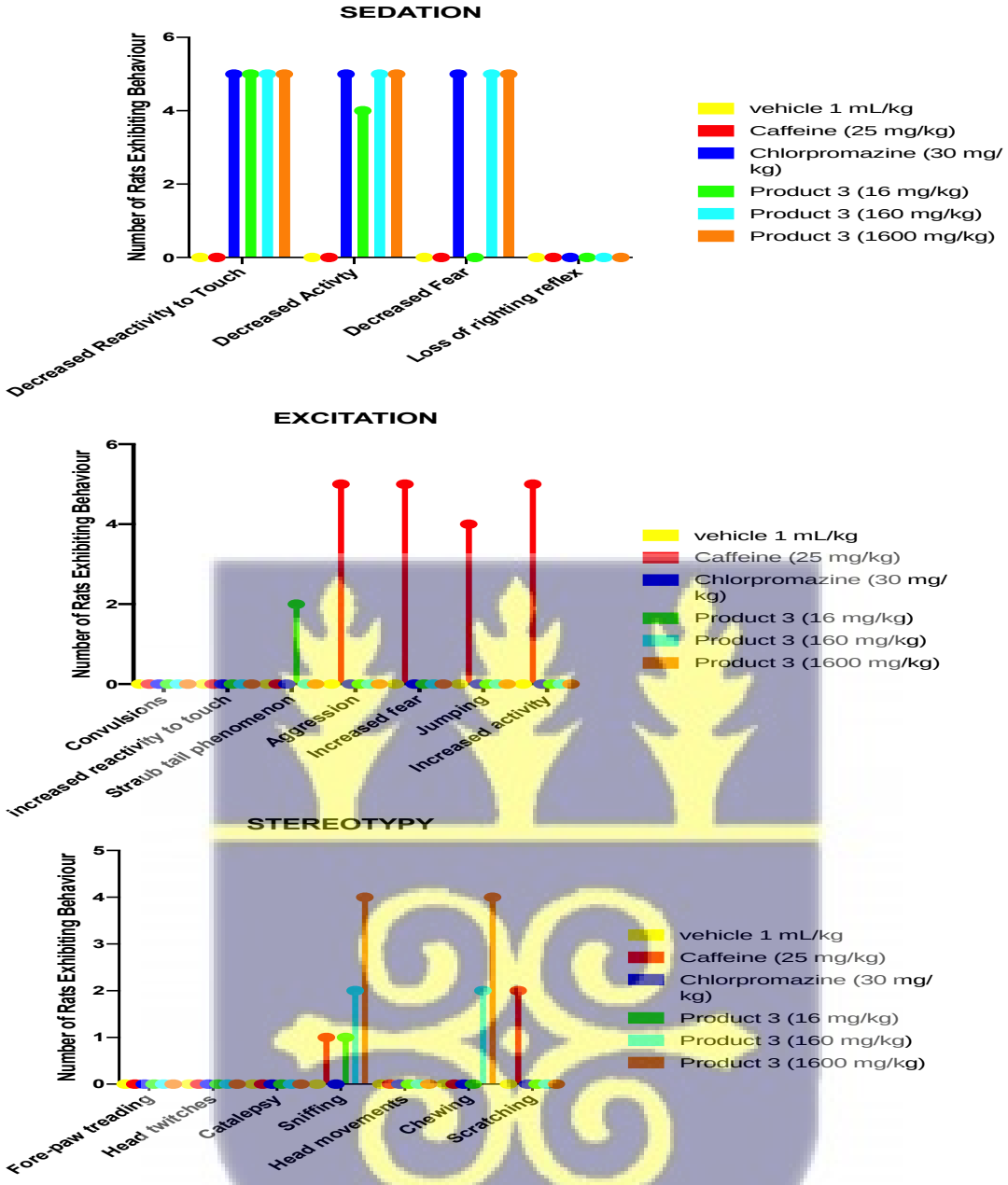

Figure 13. Influence of Product 3 on CNS activity in Sprague-Dawley rats. Rats were administered 16 mg/kg, p.o., 160 mg/kg p.o. and 1.6 g/kg, p.o. and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24h and daily for 14 days. Results presented are number of rats exhibiting any parameter within the first 24 h. The positive controls were Caffeine (red), Chlorpromazine (Black) and vehicle (white) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

EFFECT OF PRODUCT 4 ON SPRAGUE-DAWLEY RATS

Product 4 was administered, p.o., rats at 4.4, 44 and 440 mg/kg (**Figure 14** and **Appendix 7**). For effects on CNS, rats were examined for evidence of excitation, sedation, autonomy and sedation. Indicators for sedation were decreased activity, decreased fear, decreased reactivity to touch and loss of righting reflex. As expected, chlorpromazine (30 mg/kg, p.o.) induced 4 rats, decreased reactions to touch, fear and activity. The extract, at all doses (4.0 mg/kg, 40 mg/kg and 400 mg/kg) sedated the rats as evidenced by the number of animals showing decreased activity (4.0 mg/kg: 5 (100 %); 40 mg/kg: 4 (80 %) and 400 mg/kg: 5 (100 %), decreased fear (4.0 mg/kg: 5 (100 %); 40 mg/kg: 5 (100 %) and 400 mg/kg: 4 (80 %) decreased reactivity to touch :4.0 mg/kg is 5 (100 %). No rat on either the control drugs or herbal product lost the righting reflex over the dose ranges administered. These are similar to observable effect with chlorpromazine (30mg/kg p.o.) induced animal effects on the indicators for sedation.

Indicators for excitation were convulsions, tremors, Straub tail phenomenon, aggression, increased fear, jumping, increased activity and increased reactivity to touch. All rats in the group treated with Product 4 at 400mg/kg increased fear whilst 2 rats treated with 40mg/kg increased fear. 4 and 3 rats treated with 4 and 400 mg/kg of the product increased activity/movements. Despite the increase in fear and increase in activity observed by animals induced with the product, there was no indication of convulsions, tremors, straub tail phenomenon, aggression, and jumping to typically confirm CNS excitation as in the case of caffeine administered at 25mg/kg, p.o.

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The Product increased head twitches, sniffing and scratching over the dose range 4.0 mg/kg – 400mg/kg, p.o. Though this is two of the parameters among seven others (fore-paw treading, head twitches, catalepsy, sniffing, head movements, chewing and scratching) used to monitor for stereotypy, the evidence of ascribing stereotypy as one of the effects of the Product is also weak for this product.

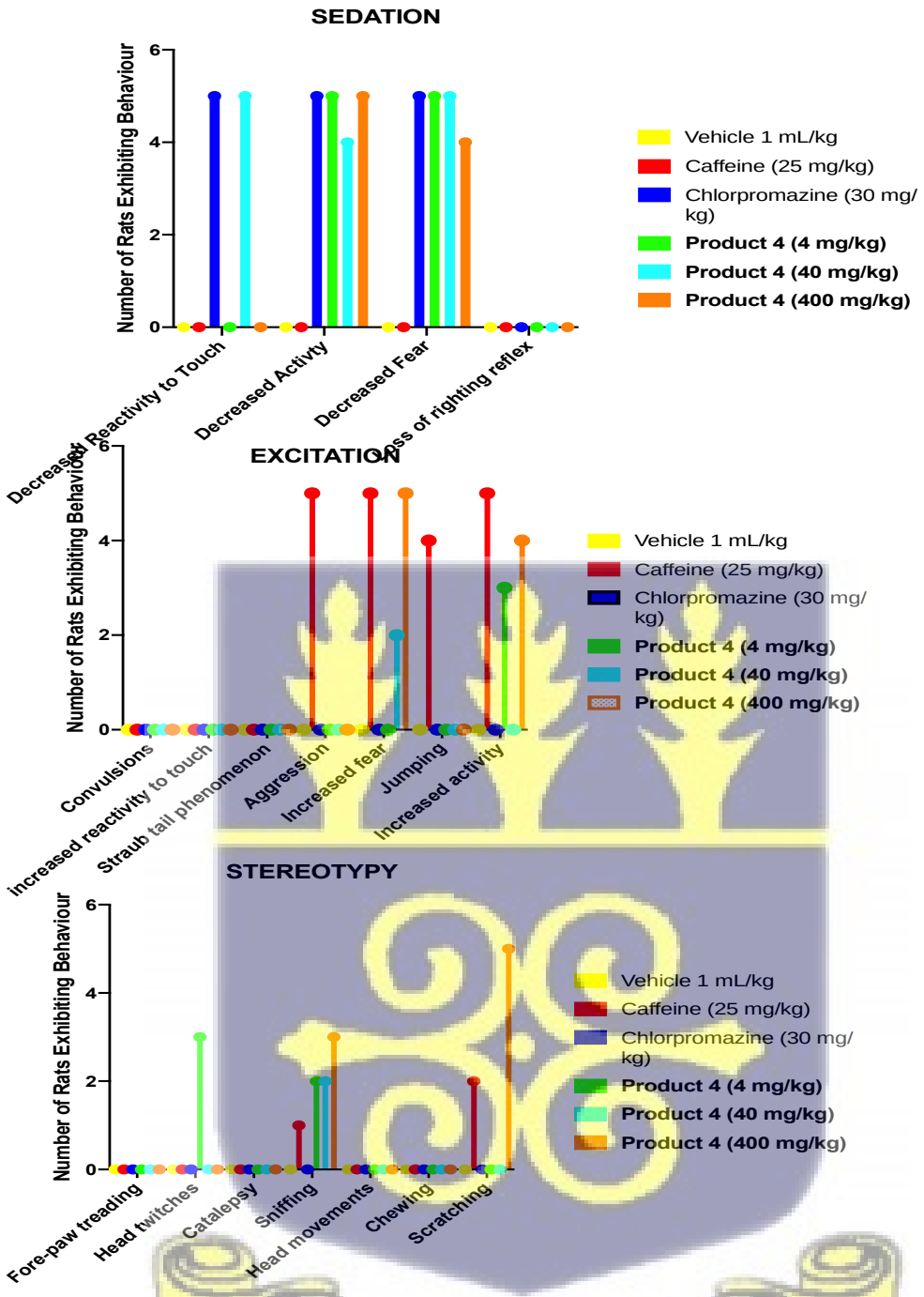


Figure 14. Effect of Product 4 on sedation, excitation, stereotypy and motor systems. Rats (n = 5) were given p.o.; 4, 40 and 400 mg/kg doses of Product 4. The positive controls

were Caffeine (red), Chlorpromazine (Black) and vehicle (white) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

There was some indication of neuromuscular and autonomic activation by the Product at the dose levels administered. Two rats in the group treated with 4.0 mg/kg exhibited abdominal gait (tip-toe). 4 rats in the group treated with 40mg/kg of the product had lacrimal secretion within 24 hours post administration (**Figure 15** and **Appendix 7**).

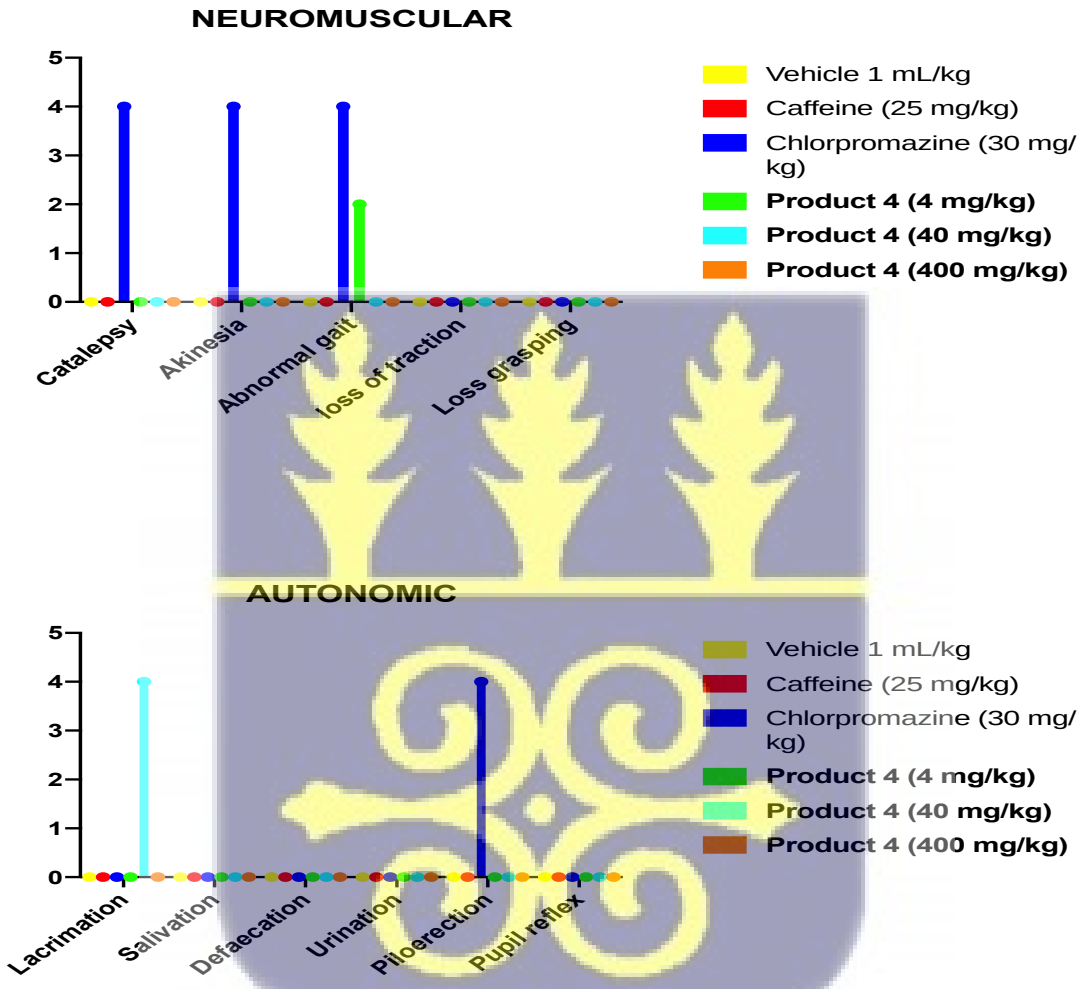


Figure 15. Influence of Product 4 on neuromuscular and autonomic actions in Sprague-Dawley rats. Rats were administered 4 mg/kg, p.o., 40 mg/kg p.o. and 400 mg/kg, p.o., and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24h and daily for 14 days. Results presented are number of rats exhibiting any parameter within the first 24 h. The positive controls were Caffeine (red), Chlorpromazine (Black) and vehicle (white) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively

EFFECT OF PRODUCT 5 ON SPRAGUE-DAWLEY RATS

Product 5 was administered, p.o., rats at 0.3, 3 and 30ml/kg (**Figure 16** and **Appendix 8**. For effects on CNS, rats were examined for evidence of sedation, excitation and stereotypy.

Indicators for sedation were decreased activity, decreased fear, decreased reactivity to touch and loss of righting reflex. As expected, chlorpromazine (30 mg/kg, p.o.) induced 4 rats, decreased reactions to touch, fear and activity. Only the group treated with 0.3ml/kg of the product sedated the rats as evidenced by the number of animals showing decreased activity 4 (80 %), decreased fear 4 (80 %) and decrease reactivity to touch 3 (60 %). No rat on either the control drugs or herbal product lost the righting reflex over the dose ranges administered.

Indicators for excitation were convulsions, tremors, Straub tail phenomenon, aggression, increased fear, jumping and increased activity. Caffeine, administered at 25 mg/kg, p.o., caused 4 rats in each case to convulse, tremor and exhibit Straub tail phenomenon. The only excitatory parameter observed with the product was increased activity over a dose range 0.3 ml/kg – 3ml/kg, p.o. evidenced with 2 and 1 rat exhibiting that respectively. There were no observable changes on the other parameters (convulsions, tremors, Straub tail phenomenon, aggression, jumping) for excitement. Similarly, chlorpromazine had no observable effects on the indicators for excitation.

The Product increased sniffing and scratching at all doses (0.3 ml/kg, 3ml/kg and 15ml/kg). The number of animals showing sniffing (0.3ml/kg: 5 (100 %); 3 ml/kg: 3 (60 %) and 15ml/kg: 5 (60 %) and scratching (0.3ml/kg: 2 (40 %); 3ml/kg: 1 (20 %); 15ml/kg: 3 (60 %). Thus, two of the parameters among seven others (fore-paw treading, head twitches, catalepsy, sniffing, head movements, chewing and scratching) used to monitor for stereotypy were observed. Therefore, ascribing stereotypy as one of the effects of the Product is weak.

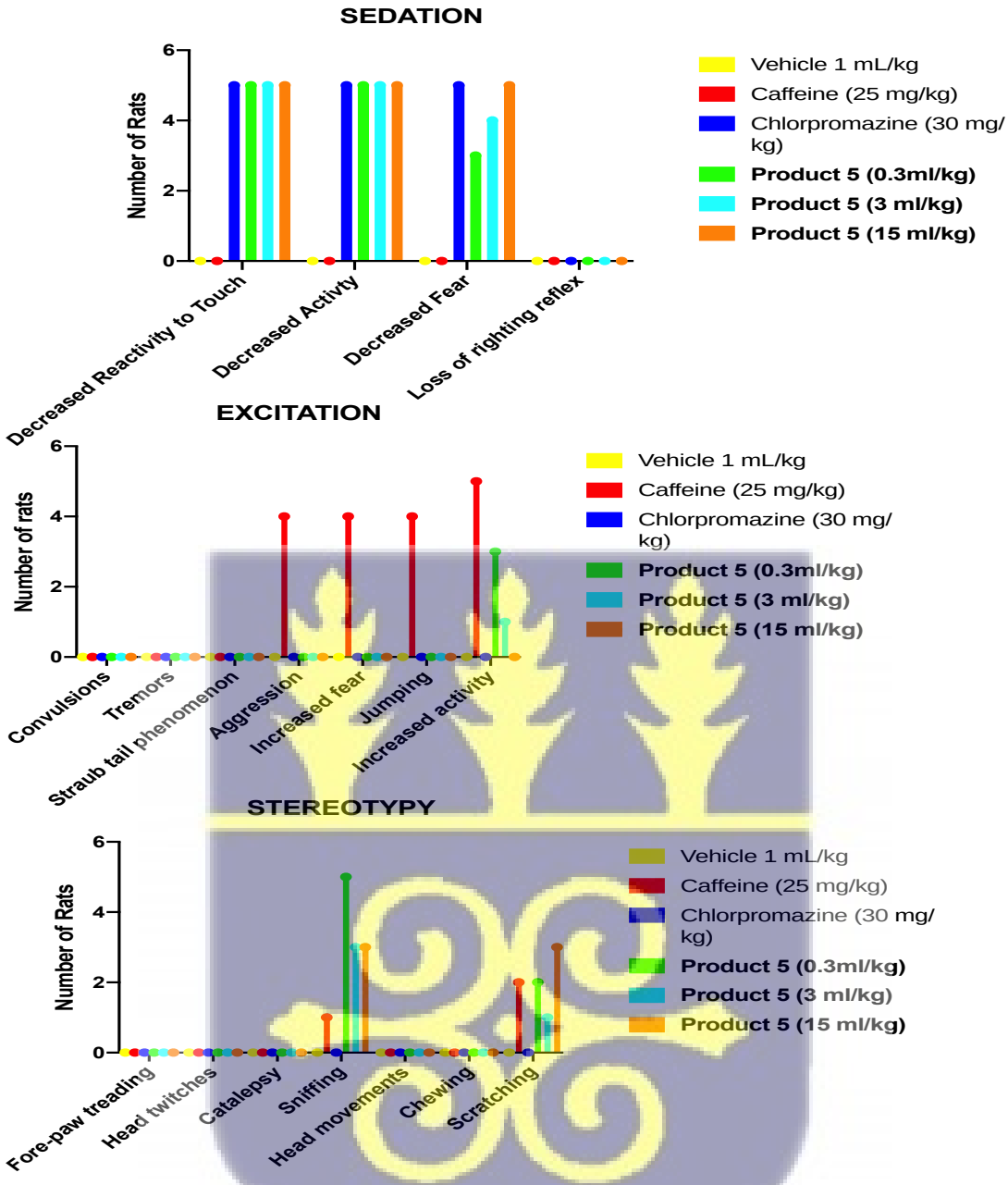


Figure 16. Influence of Product 5 on CNS activity in Sprague-Dawley rats. Rats were administered 0.3ml/kg, p.o., 3ml/kg p.o. and 15ml/kg, p.o. and observed for the first 15 minutes then at 30 mins, 1 h, 2 h, 4 h, 8 h, 24h and daily for 14 days. Results presented are number of rats exhibiting any parameter within the first 24 h. The positive controls

were Caffeine (red), Chlorpromazine (Blue) and vehicle (yellow) administered at 25 mg/kg, 30 mg/kg and 1 ml/kg; p.o. respectively.

Similarly, **Figure 17** showed a very weak neuromuscular and autonomic effect of the product compared to chlorpromazine 30 mg/kg p.o. treated rats. There was no neuromuscular activity observed in rats treated with this product unlike chlorpromazine 30mg/kg treated rats that showed loss of attraction of 80 %. The only autonomic activity amongst the rats treated with this product was defecation and it occurred with rats (20 %) administered with 0.3ml of the product. Caffeine 25mg/kg p.o. did not exhibit autonomic activity.

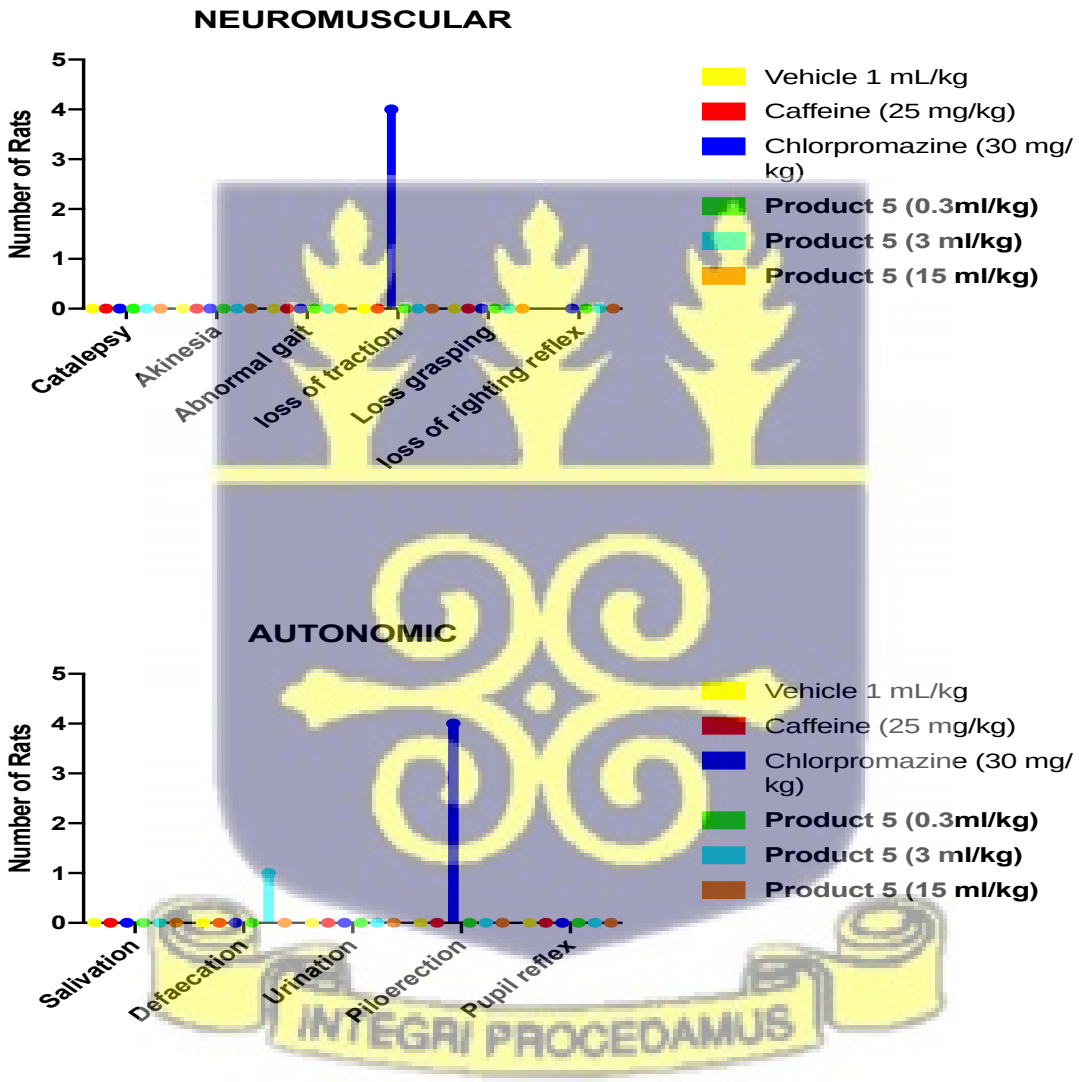


Figure 17 Effect of Product 5 on neuromuscular and autonomic systems. Rats (n = 5) were given p.o.; 0.3, 3 and 15 ml/kg doses of Product 5.

EFFECT OF PRODUCTS ON WEIGHT

Weights of rats were recorded before treatment with the test substances as described under methods. Two weeks following a single dose administration of the test substances, the weight of each rat was again recorded. The weights (before and after treatment) were then compared using a nonparametric paired sample t-test. The test results are presented in table 4:

Table 4: Comparison of weights of rats before and two weeks after treatment with test substances. At the end of week 2, weights of rats treated with Products 4 was not significantly different from their original weights.

Treatment	P-value	T value	df	Comment
Vehicle treated				
Product 1 (12 mg/kg)	0.035*	3.148	4	Significant
Product 1 (120 mg/kg)	0.114	2.018	4	Not Significant
Product 1 (1200 mg/kg).	0.005**	5.76	4	Highly Significant
Product 2 (15 mg/kg)	0.026*	3.462	4	Significant
Product 2 (150 mg/kg)	0.021*	3.692	4	Significant
Product 2 (1500 mg/kg)	0.005**	10.52	4	Highly Significant
Product 3 (16 mg/kg)	0.027*	3.394	4	Significant
Product 3 (160 mg/kg)	0.002**	7.347	4	Highly Significant
Product 3 (1600 mg/kg)	0.044*	2.910	4	Significant
Product 4 (4 mg/kg)	0.061	2.588	4	Not Significant
Product 4 (40 mg/kg)	0.167	1.684	4	Not Significant
Product 4 (400 mg/kg)	0.799	0.273	4	Not Significant
Product 5 (0.3ml/kg)	0.117	1.994	4	Not Significant
Product 5 (3ml/kg)	0.002**	13.270	4	Highly Significant
Product 5 (300 ml/kg)	0.197	3.823	4	Not Significant

RELATIVE ORGAN WEIGHT

Table 5: Relative weights of vital organs of Sprague Dawley male rats after 14 days of observation of Product 1 post administration.

Organ	Dose of Administered Product 1/Relative organ weight (%)				
	Vehicle	**(12 mg/kg)**	**(120 mg/kg)**	**(1200 mg/kg)**	**P-VALUE**
Brain	0.88±0.01	0.78±0.05	0.89 ±0.04	0.86 ±0.05	0.1480
Heart	0.43±0.02	0.37±0.01	0.39 ±0.02	0.39±0.01	0.0061
Liver	3.44±0.16	2.86±0.57	2.53±0.51	2.11±0.62**	0.1468
Kidney 1	0.34±0.01	0.32 ±0.01	0.32±0.02	0.33±0.02	0.1638
Kidney 2	0.34±0.01	0.32±0.01	0.32±0.02	0.33 ±0.02	0.1014
Spleen	0.34±0.00	0.32 ±0.00	0.33 ±0.01	0.33 ±0.01	< 0.001

Values are presented as Mean ± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test

There was a significant reduction (p< 0.01) in the relative weight of the liver at high dose of the extract when compared to the control as shown in table below. There was no significant increase or reduction in weights of the other vital organs irrespective of the varying administrative doses of product 1.

Table 6 indicates the effects of Product 2 on organ weights of rats two weeks after receiving a single dose of Product 2.

Table 6: **Relative weights of organs from rats 14 days after treatment with single dose of Product 2.**

ORGAN	Dose of Administered Product 2/Relative organ weight (%)				
	Vehicle	**15 mg/kg**	**150 mg/kg**	**1500 mg/kg**	**P-VALUE**
BRAIN	0.88±0.01	0.75±0.0.04	0.83±0.06	0.80±0.01	0.1480
HEART	0.43±0.02	0.31±0.08	0.61±0.05*	0.48±0.04	0.0061
LIVER	3.44±0.16	3.03±0.14	3.06±0.16	3.16±0.07	0.1468
KIDNEY 1	0.34±0.01	0.32±0.02	0.38±0.03	0.37±0.02	0.1638
KIDNEY 2	0.34±0.01	0.32±0.02	0.38±0.03	0.37±0.02	0.1014
SPLEEN	0.34±0.00	0.31±0.01	0.39±0.03	0.35±0.02	< 0.0001

Values are presented as Mean ± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test

There was a significant increase (p< 0.05) in the relative weight of the heart at medium dose of Product 2 when compared to the control as shown in table below. However, this increase was not dose dependent. There was no significant (P < 0.05) increase or reduction in weights of the other vital organs irrespective of the varying administrative doses

Table 7 indicates **r**elative weights of vital organs of Sprague Dawley rats after 14 days of one-time treated with Product 3.

Table 7: **Relative weights of organs from rats 14 days' post treatment with a single dose of Product 3.**

Organ	**Dose of Administered Product 3/Relative organ weight (%)**				
	Vehicle	**16 mg/kg**	**160 mg/kg**	**1600 mg/kg**	**P-VALUE**
BRAIN	0.88±0.01	0.85±0.04	0.83±0.04	0.92±0.05	0.8421
HEART	0.43±0.02	0.52±0.09	0.47±0.04	0.55±0.05	0.0061
LIVER	3.44±0.16	3.16±0.07	3.19±0.0.05	3.31±0.14	0.1468
KIDNEY 1	0.34±0.01	0.35±0.03	0.34±0.01	0.38±0.02	0.1638
KIDNEY 2	0.34±0.01	0.35±0.03	0.34±0.01	0.38±0.02	0.1014
SPLEEN	0.34±0.00	0.36±0.03	0.36±0.01	0.39±0.02	< 0.0001

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

There were no significant changes in weights of vital organs following administrative of Product 3 over the dose range 16 – 1600 mg/kg, p.o.

Table 8 indicates relative weights of vital organs of Sprague Dawley rats 14 days after administering a single dose of Product 4.

Table 8: Relative weights of organs from rats 14 days after treatment with different doses of Product 4 administered

Organ	Dose of Administered Product 4/Relative organ weight (%)				
	VEHICLE	4 mg/kg	40 mg/kg	400 mg/kg	P-VALUE
BRAIN	0.88±0.01	0.87±0.07	0.88±0.07	0.95±0.10	0.8421
HEART	0.43±0.02	0.48±0.05	0.48±0.06	0.50±0.04	0.0061
LIVER	3.44±0.16	3.10±0.20	3.24±0.21	2.96±0.18	0.1468
KIDNEY 1	0.34±0.01	0.38±0.03	0.35±0.02	0.36±0.03	0.1638
KIDNEY 2	0.34±0.01	0.38±0.03	0.35±0.02	0.36±0.03	0.1014
SPLEEN	0.34±0.00	0.36±0.03	0.00±0.00***	0.38±0.03	< 0.0001

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

The was a very significant increase (p< 0.001) in the relative weight of the spleen at medium dose of Product 4 when compared to the control as shown in the table below. However, this increase was not dose dependent. There was no significant increase or reduction in weights of the other vital organs irrespective of the varying administrative doses as shown below.

Table 9 shows the relative weights of vital organs of Sprague Dawley rats 14 days' post administration of 3 different doses of Product 5. There were no significant increase or reduction in weights of vital organs irrespective of the doses of Product 5 as indicated below

Table 9: Relative weights of vital organs of Sprague Dawley rats 14 days' post administration of 3 different doses of Product 5

ORGAN	**Dose of Administered Product 4/Relative organ weight (%)**				
	Vehicle	**0.3 ml/kg**	**3 ml/kg**	**15 ml/kg**	**P-VALUE**
BRAIN	0.88±0.01	0.89±0.06	0.94±0.02	0.82±0.02	0.8421
HEART	0.43±0.02	0.54±0.05	0.53±0.07	0.51±0.07	0.0061
LIVER	3.44±0.16	3.35±0.15	3.23±0.14	2.93±0.07	0.1468
KIDNEY 1	0.34±0.01	0.36±0.03	0.36±0.03	0.38±0.02	0.1638
KIDNEY 2	0.34±0.01	0.38±0.02	0.36±0.03	0.38±0.02	0.1014
SPLEEN	0.34±0.00	0.39±0.02	0.38±0.03	0.37±0.01	< 0.0001

Values are presented as Mean±S.E.M, n = 5; Significant in relation to control at *p < 0.05, One-way ANOVA followed by Dunnett's post hoc test.

BIOCHEMISTRY

PRODUCT 1

Serum biochemical parameters revealed below were grouped as those indicating liver function, renal function and cardiac function. There was a very significant increase ($p< 0.001$) with regards to ALP between the treated group and the vehicle for the low dose of product 1 (Table 10). Effect on the kidney is inconsistent; whereas creatinine levels were low, urea was significantly (0.02) elevated.

Table 10 Table **: Influence of Product 1 on liver, kidney and cardiac enzymes of SD rats**

Parameters	**Vehicle**	**Product 1 (12mg/kg)**	**Product 1 (120mg/kg)**	**Product 1 (1200mg/kg)**
Liver functioning test				
AST(IUL^{-1})	174.30±7.98	138.60±33.15*	195.40±19.01	108.80±16.14*
ALT (UL^{-1})	119.00±6.60	148.90±50.75	138.00±14.71	104.00±17.80
ALP (UL^{-1})	232.10±24.38	582.40±102.50***	301.60±54.5*0	410.90±79.12**
Renal functioning test				
CR	62.40±5.37	36.04±6.98*	41.64±6.85*	42.54±7.03*
UREA	12.65±0.42	12.26±1.99	12.90±1.74	15.15±1.10*
Cardiac functioning test				
CK-MB	122.40±12.41	186.50±26.64	136.10±17.65	115.30±17.65

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p< 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test

PRODUCT 2

Table 11 indicates serum biochemical parameters of rats treated with Product 2. ALP was significantly ($p < 0.0001$) increased by the lower dose (15 mg/kg) but AST levels were significantly ($p < 0.0.017$) reduced.

Table 11: Influence of Product 2 on liver, kidney and cardiac enzymes of SD rats

Parameter	Vehicle	Product 2 (15 mg/kg)	Product 2 (150 mg/kg)	Product 2 (1500 mg/kg)
Liver functioning test				
AST (IUL^{-1})	174.30±7.98	140.00±16.14	124.80±21.37	126.40±22.30
ALT (UL^{-1})	119.00±6.60	78.00±8.08	130.20±19.68	88.80±14.52
ALP (UL^{-1})	232.10±24.38	373.80±98.59*	240.80±13.97	221.60±18.35
Renal functioning test				
CR	62.40±5.37	41.20±9.05*	33.62±4.87*	93.40±51.40
UREA	12.65±0.42	12.16±1.36	11.59±0.80	13.93±1.63
Cardiac functioning test				
CK-MB	122.40±12.41	222.10±51.46	163.80±75.96	174.90±67.09

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

PRODUCT 3

Table 12 indicates treatment with Product 3 produced no significant changes in the markers for liver, kidney and cardiac functions of rats. Except for a very significant increase in ALP levels, and a significant ($p < 0.017$) lowering of AST, there was no remarkable change in the other enzymes.

Table 12: Influence of Product 3 on liver, kidney and cardiac enzymes of SD rats

Parameter	Vehicle	Product 3 (16 mg/kg)	Product 3 (160 mg/kg)	Product 3 (1600 mg/kg)
Liver functioning test				
AST (IUL^{-1})	174.30±7.98	139.40±24.19*	135.40±15.78*	153.00±25.70
ALT (UL^{-1})	119.00±6.60	74.60±14.86	61.00±16.46	83.80±14.76
ALP (UL^{-1})	232.10±24.38	340.60±82.00*	347.50±87.27*	241.60±17.21
Renal functioning test				

CR	62.40±5.37	36.02±8.41*	60.72±11.25	45.46±2.626
UREA	12.65±0.42	15.20±0.66	16.59±2.46	16.68±1.05
Cardiac functioning test				
CK-MB	122.40±12.41	124.70±55.24	136.60±37.35	48.78±8.72

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test

PRODUCT 4

There was no indication of damage to the heart as measured by changes in cardiac enzymes. From the liver, AST were significantly elevated. There was a significant decrease in creatinine.

Table 13: **Influence of Product 4 on liver, kidney and cardiac enzymes of SD rats.**

Parameter	**Vehicle**	**Product 4 (4 mg/kg)**	**Product 4 (40 mg/kg)**	**Product 4 (400 mg/kg)**
Liver functioning test				
AST (IUL^{-1})	174.30±7.98	97.00±17.82**	157.20±25.14	138.00±19.36
ALT (UL^{-1})	119.00±6.60	93.60±28.00	125.20±20.54	124.40±26.82
ALP (UL^{-1})	232.10±24.38	246.80±31.40	163.50±26.01	227.20±19.02
Renal functioning test				
CR	62.40±5.37	24.04±6.52**	53.44±16.95	33.72±8.57*
UREA	12.65±0.42	11.80±.59	12.01±0.96	11.14±0.75
Cardiac functioning test				
CK-MB	122.40±12.41	92.90±22.61	148.10±82.77	77.00±12.17

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test

PRODUCT 5

AST levels in rats treated with 3 ml/kg of Product 5 were significantly ($p < 0.01$) depressed. All other enzyme levels from kidney and cardiac tissue not significantly ($p < 0.05$) affected.

Table 14: Effect of Product 5 on organs from rats treated with the Product

Parameter	Vehicle	Product 5 (0.3 ml/kg)	Product 5 (3 ml/kg)	Product 5 (15 ml/kg)
Liver functioning test				
AST (IUL^{-1})	174.30±7.98	147.80±24.49	94.00±9.96**	132.80±20.37
ALT (UL^{-1})	119.00±6.60	113.60±13.77	81.40±9.14	116.60±18.61
ALP (UL^{-1})	232.10±24.38	205.40±19.72	223.70±46.12	231.60±39.09
Renal functioning test				
CR	62.40±5.37	15.92±6.96*	3.90±5.71	29.10±3.827
UREA	12.65±0.42	13.93±0.91	13.54±2.05	14.83±1.15
Cardiac functioning test				
CK-MB	122.40±12.41	88.62±11.54	119.60±12.79	71.70±8.22

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

HEMATOLOGY

Table 15 indicates effects of a single oral administration of Product 1 at dose levels of 12, 120 and 1200 mg/kg, on hematological parameters of SD rats after a 14.

The data revealed that, compared with the vehicle group, the low dose of Product 1 caused significant ($p < 0.01$) increase in lymphocytes, RBC ($10^{6}\mu L^{-1}$) and PDW (fl) but a significant decrease ($p < 0.01$) in RDW_SD (fl).

With the exception of medium dose showing significant increase ($p < 0.05$) in mean corpuscular volume (MCV), all other hematological parameters pertaining to this dose were not significantly affected with respect to the vehicle group.

Table 15: **Hematological parameters of rats treated with Product 1 at 12, 120 and 1200 mg/kg, p.o.**

Parameters	**Vehicle**	Product 1 **(12 mg/kg)**	Product 1 **(120 mg/kg)**	Product 1 **(1200 mg/kg)**
WBC ($10^3\mu L^{-1}$)	9.24±0.41	11.34±3.41	8.38±1.58	7.27±0.95
LYM# ($10^3\mu L^{-1}$)	3.73±0.32	6.96±1.30*	4.32±1.25	2.84±0.32
MON# ($10^3\mu L^{-1}$)	0.70±0.07	1.04±0.60	0.92±0.43	0.66±0.16
NEU# ($10^3\mu L^{-1}$)	3.90±0.14	3.17±1.87	2.79±1.36	5.46±1.81
EOS# ($10^3\mu L^{-1}$)	0.19±0.70	0.07±0.05	0.54±0.50	0.23±0.04
BASO# ($10^3\mu L^{-1}$)	0.02±0.00	0.10±0.05	0.30±0.26	0.02±0.00
LYM%	44.23±1.83	68.45±10.71**	55.37±8.54	42.12±1.88
MON%	12.04±0.39	7.38±2.37	15.89±7.10	9.42±1.39
NEU%	39.99±1.37	22.47±8.19	32.54±10.04	44.70±2.07
EOS%	2.55±0.66	0.94±0.66	5.16±4.20	3.55±0.64
BASO%	0.22±0.04	0.76±0.16	0.61±0.23	0.21±0.06
RBC ($10^6\mu L^{-1}$)	5.92±0.21	7.51±0.21**	6.63±0.47	6.73±0.28
HGB (g dL^{-1})	14.89±0.13	14.16±0.52	14.86±0.44	14.84±0.14
HCT (%)	40.38±0.23	39.40±0.67	41.00±1.85	42.16±1.11
MCV (fl)	56.20±0.80	53.94±1.44	65.04±8.38*	59.10±3.31
MCH (pg)	22.11±0.26	18.92±0.86	24.10±3.89	22.78±1.76
MCHC (g dL^{-1})	35.48±0.24	34.92±0.60	36.04±0.39	36.24±1.36
RDW_CV (%)	17.01±2.18	15.56±1.25	14.40±1.08	12.48±0.39
RDW_SD (fl)	44.63±0.33	37.34±2.84**	44.78±4.00	44.40±0.74
PLT ($10^3\mu L^{-1}$)	726.8±90.50	659.80±193.80	849.60±78.90	859.60±94.70

MPV (fl)	8.24±0.27	7.30±0.24	7.96±0.89	7.86±0.29
PDW (fl)	12.08±0.21	14.52±0.68**	12.40±1.26	11.96±0.85
PCT	0.81±0.04	0.63±0.09	0.62±0.06	0.65±0.11
P_LCR (%)	21.42±1.56	11.50±1.50	16.79±2.18	28.41±5.01

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01; One-way ANOVA followed by Dunnett's post hoc test.

The effects of a single administration of Product 2 at 15, 150 1500 mg/kg on haematological parameters are shown in table 16.

There was a dose-dependent increase (in RBCs in comparison with the vehicle. Thus, a significant increase (p<0.05), (p<0.01) and (p<0.01) in RBC for low, medium and high dosed SD rats respectively compared to the vehicle dosed rats. In addition, the low dose of Product 2 caused significant increase ($p < 0.01$) in number of Eosinophils (EOS#) with all other hematological parameters pertaining to this dose revealing non-significant differences compared with the vehicle group.

Rats treated with high dose of Product 2 also revealed significant increase ($p < 0.05$) in MCHC compared with the vehicle group. All other hematological parameters in relation to this dose revealed non- significant differences.

Table 16: Hematological parameters of rats treated with Product 2 at 15, 150 and 1500 mg/kg, p.o.

Parameter	**Vehicle**	**Product 2 (15 mg/kg)**	**Product 2 (150 mg/kg)**	**Product 2 (1500 mg/kg)**
Parameter	Vehicle	Low dose	Medium dose	High dose
WBC ($10^3\mu L^{-1}$)	9.24±0.41	9.75±1.92	9.79±1.46	6.32±1.05
LYM# ($10^3\mu L^{-1}$)	3.73±0.32	3.11±0.54	6.02±1.58	3.08±0.93
MON# ($10^3\mu L^{-1}$)	0.70±0.07	1.01±0.20	0.89±0.28	0.60±0.16
NEU# ($10^3\mu L^{-1}$)	3.90±0.14	5.46±1.81	2.65±0.49	2.43±0.54
EOS# ($10^3\mu L^{-1}$)	0.19±0.70	1.66±1.50**	0.10±0.04	0.20±0.04

BASO# ($10^3\mu L^{-1}$)	0.02±0.00	0.01±0.00	0.14±0.10	0.01±0.00
LYM%	44.23±1.83	35.91±6.135	58.79±7.24	45.10±8.35
MON%	12.04±0.39	8.84±2.09	9.71±2.99	10.39±3.85
NEU%	39.99±1.37	52.01±6.93	29.31±7.24	41.00±10.64
EOS%	2.55±0.66	1.55±1.17	1.11±0.47	3.37±0.69
BASO%	0.22±0.04	0.14±0.04	1.08±0.61	0.20±0.06
RBC ($10^6\mu L^{-1}$)	5.92±0.21	7.15±0.12*	7.39±0.19**	7.54±0.55**
HGB (g dL^{-1})	14.89±0.13	14.70±0.58	15.02±0.30	15.52±0.29
HCT (%)	40.38±0.23	41.00±1.85	42.06±1.11	40.54±0.80
MCV (fl)	56.20±0.80	57.44±2.51	56.40±1.84	55.00±0.89
MCH (pg)	22.11±0.26	20.52±0.77	20.06±0.65	20.70±0.42
MCHC (g dL^{-1})	35.48±0.24	36.00±0.63	35.66±0.96	37.80±0.37*
RDW_CV (%)	17.01±2.18	13.26±0.67	14.16±1.16	12.08±0.30
RDW_SD (fl)	44.63±0.33	46.30±1.11	41.90±2.20	43.98±0.85
PLT ($10^3\mu L^{-1}$)	726.8±90.50	959.80±39.94	850.20±80.00	820.40±38.28
MPV (fl)	8.24±0.27	7.66±0.24	7.78±0.21	7.64±0.62
PDW (fl)	12.08±0.21	11.92±0.47	13.18±0.79	11.68±0.39
PCT	0.81±0.04	0.73±0.02	0.67±0.05	0.63±0.03
P_LCR (%)	21.42±1.56	22.84±4.07	18.43±5.42	24.14±2.55

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01; One-way ANOVA followed by Dunnett's post hoc test.

Table 17 indicates the influence of a single oral administration of Product 3 in increasing dose levels (low dose, medium dose and high dose) on hematological parameters of SD rats 14 days after product administration.

The data below revealed that, compared with the vehicle group, there was a very significant increase ($p < 0.001$) in RBC for low and high dose of Product 3. The medium dose revealed significant increase ($p < 0.05$) in RBC compared with the vehicle group. Aside the high dose revealing significant increase ($p < 0.01$), ($p < 0.01$), ($p < 0.05$) in MON%, HGB and HCT

respectively, all other hematological parameters revealed non-significant differences compared with the vehicle group.

Table 17: **Hematological parameters of rats treated with Product 3 at 16, 160 and 1600 mg/kg, p.o.**

Parameter	Vehicle	Product 3 (16 mg/kg)	Product 3 (160 mg/kg)	Product 3 (1600 mg/kg)
WBC ($10^3\mu L^{-1}$)	9.24±0.41	7.02±0.47	7.77±0.96	6.22±1.48
LYM# ($10^3\mu L^{-1}$)	3.73±0.32	3.91±0.53	2.10±0.53	1.97±0.51
MON# ($10^3\mu L^{-1}$)	0.70±0.07	0.51±0.13	0.78±0.17	1.08±0.14
NEU# ($10^3\mu L^{-1}$)	3.90±0.14	2.02±0.48	4.41±1.11	2.69±1.05
EOS# ($10^3\mu L^{-1}$)	0.19±0.70	0.33±0.23	0.41±0.23	0.25±0.23
BASO# ($10^3\mu L^{-1}$)	0.02±0.00	0.06±0.05	0.02±0.00	0.29±0.25
LYM%	44.23±1.83	58.29±7.51	31.80±10.21	31.97±5.45
MON%	12.04±0.39	7.23±1.78	10.06±2.00	23.93±7.34**
NEU%	39.99±1.37	28.98±5.28	54.37±9.22	36.69±5.68
EOS%	2.55±0.66	4.84±3.32	4.73±2.49	4.02±2.25
BASO%	0.22±0.04	0.81±0.57	0.25±0.07	0.50±0.12
RBC ($10^6\mu L^{-1}$)	5.92±0.21	7.54±0.24***	7.12±0.24*	7.88±0.37***
HGB ($g\ dL^{-1}$)	14.89±0.13	15.92±0.53	14.94±0.40	16.40±0.51**
HCT (%)	40.38±0.23	43.36±2.12	41.12±1.75	44.20±2.47*
MCV (fl)	56.20±0.80	55.96±1.07	57.80±1.37	56.10±0.95
MCH (pg)	22.11±0.26	20.14±0.69	19.92±0.32	20.84±0.34
MCHC ($g\ dL^{-1}$)	35.48±0.24	36.40±1.06	35.04±0.43	37.08±0.88
RDW_CV (%)	17.01±2.18	13.76±0.74	13.76±1.00	12.38±0.45
RDW_SD (fl)	44.63±0.33	44.16±2.90	46.70±1.62	44.76±1.10
PLT ($10^3\mu L^{-1}$)	726.8±90.50±	986.40±60.98	922.20±56.69	603.60±137.00

MPV (fl)	8.24±0.27	7.64±0.62	7.32±0.43	7.14±0.23
PDW (fl)	12.08±0.21	13.34±0.94	11.00±0.52	10.94±0.76
PCT	0.81±0.04	0.74±0.05	0.66±0.05	0.43±0.09
P_LCR (%)	21.42±1.56	24.28±8.55	21.68±3.34	25.49±4.81

Values are presented as Mean±S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

Table 17 is the effect of a single administration of Product 4 at dose levels 0f 4, 40 and 400 mg/kg, p.o. on haematological parameters 14 days after product administration.

As the doses of product 4 increases, the LYM% and HCT also significantly increased compared to the vehicle. There was a very significant difference ($p<0.001$) in BASO%, RBCs and RDW-SDs for all doses and a general increase in HGB for all doses but only the high dosed revealed a very significant increase ($p<0.001$) compared with the vehicle. Contrarily, there was a slight significant reduction ($p<0.05$) in NEU% for medium and high dosed SD rats compared with the vehicle. The low dosed group also showed a reduction in NEUT% but was not significant. Similarly, there was a slight significant reduction in MCH in low and medium dose respectively compared to the vehicle. A very significant increase ($p<0.01$) in MPV for low dose whilst the medium and high dose revealed non-significant reduction compared to vehicle. A non-significant reduction in the MON% for low and medium dosed rats but the high dosed rats revealed significant reduction ($p<0.05$) compared with the vehicle.

All other hematological parameters pertaining to this dose revealed non- significant difference with respect to the vehicle group.

Table 18: Hematological parameters of rats treated with Product 4 at 4, 140 and 400 mg/kg, p.o.

Parameter	**Vehicle**	**Product 4 (4 mg/kg)**	**Product 4 (40 mg/kg)**	**Product 4 (400 mg/kg)**
WBC ($10^3\mu L^{-1}$)	9.24±0.41	9.89±2.65	6.52±0.96	7.73±0.93
LYM# ($10^3\mu L^{-1}$)	3.73±0.32	6.09±0.81	4.84±0.72	5.87±0.80
MON# ($10^3\mu L^{-1}$)	0.70±0.07	0.84±0.79	0.26±0.7	0.15±0.05

NEU# ($10^3\mu L^{-1}$)	3.90±0.14	2.76±1.48	1.24±0.33	1.58±0.22
EOS# ($10^3\mu L^{-1}$)	0.19±0.70	0.00±0.00	0.01±0.00	0.00±0.00
BASO# ($10^3\mu L^{-1}$)	0.02±0.00	0.19±0.07	0.17±0.04	0.14±0.03
LYM%	44.23±1.83	71.12±9.46**	74.26±3.47***	75.06±2.58***
MON%	12.04±0.39	4.60±3.93	3.94±0.83	2.00±0.70*
NEU%	39.99±1.37	22.34±5.68	19.08±3.40*	21.18±2.91*
EOS%	2.55±0.66	0.06±0.04	0.16±0.04	0.08±0.04
BASO%	1.88±0.54	2.56±0.55***	1.68±0.34***	1.00±0.25***
RBC ($10\ ^6\mu L^{-1}$)	5.92±0.21	8.73±0.17***	8.96±0.24***	9.34±0.48***
HGB ($g\ dL^{-1}$)	14.89±0.13	15.74±0.32	16.08±0.42	17.48±0.82***
HCT (%)	40.38±0.23	45.66±0.59**	46.44±0.57***	49.24±1.84***
MCV (fl)	56.20±0.80	52.40±0.56	51.90±1.05	52.92±0.95
MCH (pg)	22.11±0.26	18.8±0.39*	17.96±0.33**	18.78±0.39
MCHC ($g\ dL^{-1}$)	35.48±0.24	34.44±0.43	34.60±0.56	35.48±0.45
RDW_CV (%)	17.01±2.18	16.66±0.85	17.08±1.22	15.06±0.25
RDW_SD (fl)	44.63±0.33	35.34±1.52***	35.86±2.68***	32.32±0.44***
PLT ($10^3\mu L^{-1}$)	726.8±90.50	1010.00±68.96	922.4±32.97	659.40±149.50
MPV (fl)	8.24±0.27	145.2±138.9**	6.38±0.18	6.56±0.26
PDW (fl)	12.08±0.21	15.24±0.07***	15.18±0.08***	15.38±0.16***
PCT	0.81±0.04	0.65±0.05	0.59±0.03	0.42±0.09
P_LCR (%)	21.42±1.56	6.96±0.99 *	6.64±0.74 *	18.02±8.94

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

The effects of a single administration of three doses of Product 5 (3ml, 30 ml, 15 ml/kg, p.o.) on the haematopoietic system of SD rats is shown on Table 19.

Compared with the vehicle-treated group, Product 5 caused a very significant increase (p < 0.001) in RBC, HCT, PDW and LYM% and a very significant reduction (p<0.001) in

RDW_SD. There was a significant increase ($p<0.05$) in HGB for low and medium dosed SD rats compared with the vehicle. On the contrary, a significant reduction, in MON% ($p < 0.01$) and NEU% ($p<0.05$) for low and high dose was recorded and a significant reduction ($p<0.01$) in BASO% for high dosed SD rats was also recorded compared with the vehicle. Also, a significant reduction ($p<0.05$) in MCH and P_LCR was revealed for all dose levels compared with the vehicle group. All other doses revealed a non-significant difference compared with the control.

Table 19: Hematological parameters of rats treated with Product 5 at 0.3, 3 and 15 ml/kg, p.o.

Parameter	Vehicle	Product 5 (0.3 ml/kg)	Product 5 (3 ml/kg)	Product 5 (15 ml/kg)
WBC ($10^3\mu L^{-1}$)	9.24±0.41	11.34±7.38	8.83±1.32	5.35±1.47
LYM# ($10^3\mu L^{-1}$)	3.73±0.32	4.42±1.18	6.27±0.68	4.30±1.60
MON# ($10^3\mu L^{-1}$)	0.70±0.07	0.03±0.01	0.32±0.20	0.08±0.04
NEU# ($10^3\mu L^{-1}$)	3.90±0.14	0.83±0.25*	2.16±0.68	0.90±0.20*
EOS# ($10^3\mu L^{-1}$)	0.19±0.70	0.00±0.00	0.00±0.00	0.00±0.00
BASO# ($10^3\mu L^{-1}$)	0.02±0.00	2.52±2.45***	0.09±0.02	0.06±0.02
LYM%	44.23±1.83	82.78±2.10***	73.34±5.90***	77.72±2.62***
MON%	12.04±0.39	0.50±0.11**	3.30±2.12	1.50±0.45*
NEU%	39.99±1.37	15.66±1.86**	22.32±4.14	19.34±2.91*
EOS%	2.55±0.66	0.06±0.02	0.06±0.02	0.08±0.08
BASO%	0.22±0.04	1.00±0.25	0.98±0.15	1.36±0.39**
RBC ($10^6\mu L^{-1}$)	5.92±0.21	8.80±0.15***	8.87±0.14***	8.70±1.17***
HGB ($g\ dL^{-1}$)	14.89±0.13	16.28±0.37*	16.22±0.34*	16.12±0.37
HCT (%)	40.38±0.23	46.90±0.84***	47.20±0.48***	46.08±0.94***
MCV (fl)	56.20±0.80	53.32±0.55	53.28±0.91	52.94±0.65

MCH (pg)	22.11±0.26±	18.50±0.20*	18.32±0.50*	18.52±0.19*
MCHC (g dL^{-1})	35.48±0.24	34.70±0.19	34.40±0.40	34.96±0.24
RDW_CV (%)	17.01±2.18	15.36±0.25	16.90±1.09	16.16±0.32
RDW_SD (fl)	44.63±0.33	33.36±0.87***	36.24±1.70***	34.52±0.88***
PLT ($10^3 \mu L^{-1}$)	726.8±90.50	916.00±43.79	1007.00±82.66	844.8±50.37
MPV (fl)	8.24±0.27	6.28±0.10	18.10±11.73	6.70±0.11
PDW (fl)	12.08±0.21	15.12±0.10***	15.18±0.06***	15.28±0.06***
PCT	0.81±0.04	0.46±0.10	0.64±0.05	0.57±0.04
P_LCR (%)	21.42±1.56	6.12±0.43 *	6.36±0.38 *	6.36±0.38 *

Values are presented as Mean± S.E.M, n = 5; Significant in relation to control at *p < 0.05, **p < 0.01, ***P<0.001; One-way ANOVA followed by Dunnett's post hoc test.

HISTOPATHOLOGY

Histological examinations of liver, kidney and heart from representative male SD rats treated with vehicle (control) or herbal aphrodisiac product generally did not show any prominent abnormalities with the exception of some observed morphological changes on SD rats administered with Product 1 and Product 2. These morphological changes were as follows;

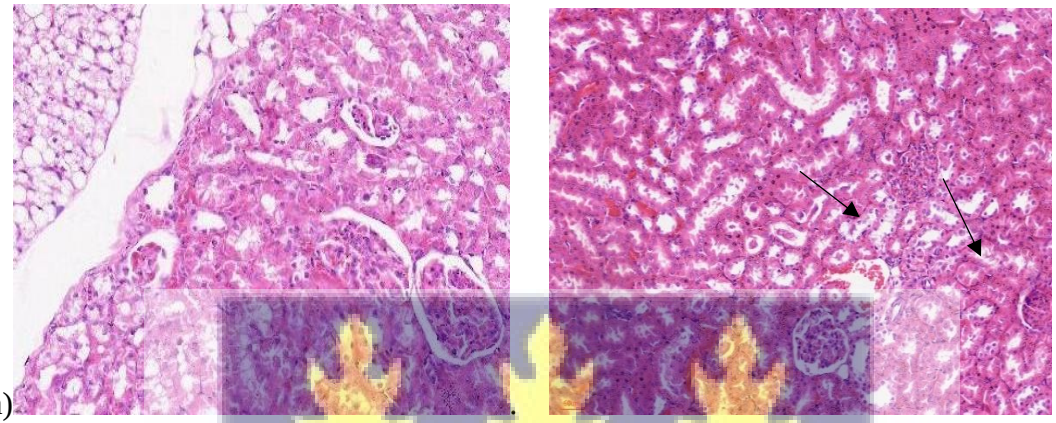
Low dosed of Product 1 administered SD rats presented with equivocal cytoplasmic accumulation of the liver and tubular deposit (hyaline cast) of the kidneys as showed in Fig. 19 and Fig.18 respectively.

Similarly, the medium dosed administered Product 1 rats showed equivocal tubular deposit (hyaline cast) in the kidneys, cytoplasmic accumulation, equivocal inflammation and slight steatosis of the liver tissues as represented in Fig. 18 and Fig. 19 respectively.

Also, Fig. 18 and Fig. 19 showed both low and medium dosed of Product 2 administered SD rats with mild equivocal tubular deposits (hyaline cast) in the kidneys and equivocal cytoplasmic accumulation of lymphocytes (cytoplasmic clumping) in the liver. There was also

equivocal focal acute inflammation of the heart compared with the control as shown in Fig. 20 for the medium dosed SD rats of Product 2.

In addition, high dosed Product 2 revealed equivocal hyaline cast in the tubules of the kidneys and equivocal focal acute inflammation of the heart compared with the control as shown in Fig. 1 and Fig.20 respectively.



(a) (b)

Figure 18. Photomicrograph of sections of SD male rats in 14 days' acute toxicity study. (a) healthy kidney (x10), (b) kidney presented with equivocal hyaline cast of the tubules (x10) evidential in low and medium dose of Product 1 administered SD rats and also in the medium dose of product 2 administered SD rats.

(c) (d)

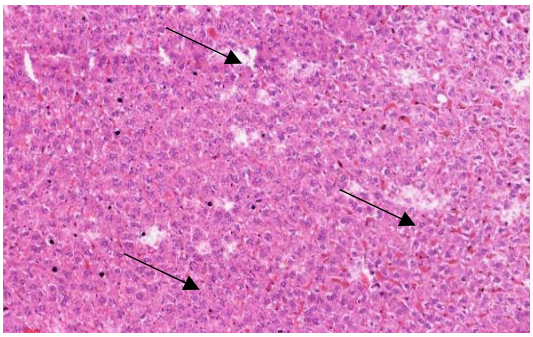
(e) 

Figure 19. Photomicrograph of sections of SD male rats in 14 days' acute toxicity study. (c) healthy liver (x20), (d) liver presented with mild focal steatosis (x20) observed in medium doses of Product 1 and Product 2 administered SD rats and (e) liver presented with cytoplasmic clamping (x20) as observed in low and medium doses of product 1 administered SD rats.

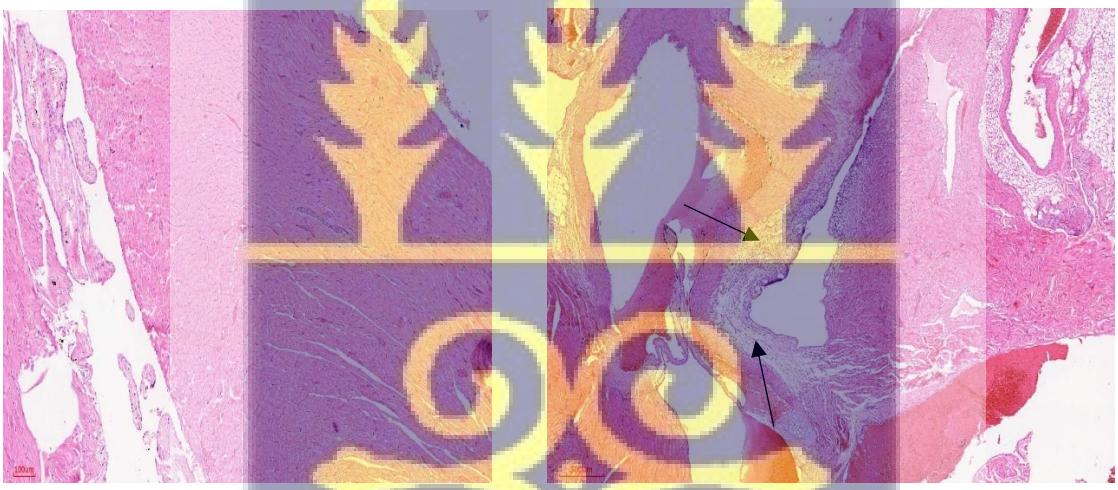


Figure 20. Photomicrograph of sections of SD male rats in 14 days' acute toxicity study. (f) healthy heart (x10) and (g) heart presented with equivocal focal acute inflammation of the heart (x4) as observed in both medium and high doses of Product 2 administered SD rats.

Chapter 6

DISCUSSION

Sexual dysfunction is very common, affecting about 31 % of men and 43 % of women (Rosen, 2000). Underactive sexual desire disorder has been reported in about 15 % of men and 30 % of women in population-based studies (Rosen, 2000). The quality of life and interpersonal relationships of individuals with sexual dysfunction are significantly adversely affected (Idung *et al*., 2012). Unfortunately, sudden cardiac arrest associated with sildenafil administration, for erectile dysfunction, has been reported in many cases, especially in patients with co-morbidities (Huber *et al*, 2011, Cohen, 2001). Thus, in the search for safer and effective treatment, herbal products have been frequently patronized. In this project, five products of herbal origin, marketed for treatment of erectile dysfunction, were screened for potential toxicity in male Sprague-Dawley rats.

A global neurobehavioral assessment, on male Sprague-Dawley rats, in the form of the functional observational battery (FOB) or modified Irwin Test was employed. This test is frequently used in safety pharmacology for detection of drug effects on the nervous system (including the central nervous system; CNS). It relies on a systematic, multi-parameter observational assessment, involving a battery of tests and observations conducted on the same animals, covering a broad range of neurological and behavioral functions. The modified Irwin tests is used in safety pharmacology studies recommended by the International Conference on Harmonization (ICH) S7A guideline for assessing the effects of new chemical entities, to help protect participants in clinical trials and patients from potential undesired effects ("International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2000); Redfern & Wakefield, 2006).

Effects of the products on biochemical and haematological parameters of SD rats and histopathological evaluation on brain, livers, hearts, spleen and kidneys of the experimental animals were also conducted.

There was no mortality recorded throughout the study period.

NEUROBEHAVIOURAL EVALUATION OF PRODUCTS

After successfully validating the model using chlorpromazine and caffeine, the test substances were then subjected to the evaluation. The Irwin parameters for assessing each product included incidence of rats exhibiting specific behaviours. Influence of the products on CNS was assessed by incidence of rats exhibiting parameters indicative of Excitation, Sedation and Stereotypy. In comparison with caffeine 25 mg/kg which caused excitement in all rats (n = 5) measured as increased head movements, fear, aggression, and increased reactivity to touch, only rats treated with the high dose of Product 1 (1200 mg/kg) increased reactivity to touch and fear. Product 4 also exhibited a potential for causing excitement by increasing fear and reactivity to touch in treated rats.

Penile erection comes about through relaxation of smooth muscle in the penis controlled by a spinal reflex. It involves processing and integration of olfactory, tactile, mental stimuli and auditory by the central nervous system. The reflex involves both autonomic and somatic afferents and modulated by supraspinal influences peripherally (Kota *et al.*, 2013).
Product 5 at all dose levels showed sedative effects comparable with chlorpromazine with all rats showing decreased reactivity to touch, decreased activity and fear. Effects of Product 1, 2, 3 and 4 similarly sedated the animals but no animal lost its righting reflex. The failure to induce loss of righting reflex is suggestive of anxiolytic rather than hypnotic actions in the products. The observation may be beneficial for the products as aphrodisiac actions of depression and anxiety are often associated with sexual dysfunction (Laurent and Simons, 2009).

BIOCHEMISTRY

Biochemical analyses were employed to establish whether these five herbal aphrodisiacs could have an alteration in renal, hepatic and cardiac function (indices of drug toxicity) in Sprague Dawley rats. Regarding the biochemical parameters as measured to reflect the liver function namely (ALT, AST and ALP), the renal function namely, (creatinine and urea) and cardiac function namely, (CK-MB) of the SD rats, there was a very significant elevation ($p < 0.001$)

with regards to ALP between the treated group and the vehicle for the low dose of product 1. However, this significant increase was not dose dependent as subsequent higher dose did not show any significant differences. Though ALP is produced in the liver, they are also found in other organs such as bone marrow, kidneys and guts. Therefore, such significant elevation may be attributed to bone disease (most common cause) even in the presence of a healthy liver.

There was a significant decrease ($p<0.05$ and $p< 0.01$) with regards to AST between the treated group and the vehicle for the low dose of product 4 and medium dose of product 5 respectively. However, this significant decrease was not dose dependent. Though AST is found also in the heart and muscles aside the liver, a reduction in serum AST as in this case may indicates hepatocellular enzyme production inhibition or reduction in enzyme activity (Zainal *et al*., 2020).

Clearance of creatinine is an indicator of glomerular filtration rate and is used for assessing functionality of the kidney (Ugwah-Oguejiofor *et al*., 2019). It is also produced constantly as a byproduct of muscle's creatinine phosphate and excreted solely by the kidneys. Low creatine levels show high blood cleansing function of the kidneys (Seriana *et al*., 2021) as in the case of the significant decrease ($p< 0.05$) observed between the treated group and the vehicle for the low administered dose of product 5.

HEMATOLOGY

Highly sensitive system to toxicants is the hematopoietic system. Studies pertaining to this system are important indices of the pathophysiological status of humans and animals (Zainal *et al*., 2020).

The low dosed treated group of Product 1 revealed significant increase ($p < 0.01$) in LYM% and PDW compared with the vehicle but a significant decrease ($p < 0.01$) in RDW_SD. There was a significant increase ($p<0.05$) of LYM# for low dose compared with the vehicle. LYM# reduced slightly with increasing dose in relation to the low dose but not significant ($p< 0.05$) in relation to vehicle. Lymphocytes are very dynamic cells and regulate immune response to evading substances (Pearce *et al*., 2013). In addition, they are also responsible of producing antibodies for the destruction of cancer cells and intracellular microbes and cancer cells

(Ugwah-Oguejiofor *et al.*, 2019). Therefore, the increase in lymphocytes is suggestive of possible immune stimulatory effect of the product at low dose.

With the exception of medium dose showing significant increase ($p < 0.05$) in MCV, all other hematological parameters pertaining to this dose revealed non- significant difference with respect to the vehicle group. The size of the red blood cells which is MCV determines the category of anemia. MCVs below normal value indicates red blood cells smaller than normal and is termed as microcytic anemia whilst MCVs elevated than normal indicates RBC larger than normal and is described as macrocytic but normal size RBCs are termed normocytic (Patrick-Iwuanyanwu and Nkpaa, 2015). With the medium dose revealing a significant increase of MCV is indicative of macrocytic anemia indicating a lack of vitamin B12 or folic acid deficiency tendencies in relation to the drug.

There was significant increase ($p<0.01$) in RBCs for the low dosed rats compared to the vehicle. The medium and high dose revealed slight increase in RBCs but not significant. The increase in RBC is an indicative of the positive product effect in hemopoiesis which may reduce with increasing dose levels. However, the rats treated with high dose did reveal a slight reduction of all hematological parameters but it was non- significant compared with the vehicle.

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Regarding Product 2, the significant increase ($p<0.05$), ($p<0.01$) and ($p<0.01$) in RBC for low, medium and high dosed SD rats respectively compared to the vehicle dosed rats also shows that the product has effect in hemopoiesis. The low dosed rats significant increase ($p < 0.01$) in number of Eosinophils (EOS#) indicates that there may be some slight hypersensitivity reaction to some constituent of the product. A significant increase ($p < 0.05$) in MCHC for high dose compared with the vehicle group indicates the depletion of hemoglobin concentration as the dose increases.

Regarding product 3, a very significant increase ($p < 0.001$) in RBCs and a significant increase ($p < 0.05$) in RBC compared with the vehicle group is also indicative that this product is involve in hemopoiesis. Monocytes which is a type of WBC increase in numbers in response to infections. (Amoateng *et al.*, 2016). The high dosed SD rats revealing significant increase ($p < 0.01$) in MON% indicative of possible response to infection or inflammation. The high dosed

SD rats revealed significant increment ($p < 0.01$), ($p < 0.05$) in HGB and HCT respectively. Hematocrit (Hct) also known as Packed Cell Volume (PCV) is the red blood cells percentage (%) in blood and involved in the oxygen transport and nutrients absorption. Therefore, raised HCT results in an increased primary and secondary polycythemia because it indicates a better transportation (Etim *et al.*,2014). There was a general increase in raised HGB is termed as polycythemia and could be as a result of reduce plasma volume or increase red blood cells as in this case.

Regarding product 4, there was a very significant increase ($p<0.01$), ($p<0.001$) and ($p<0.001$) in LYM% and HCT for low, medium and high dosed SD rats respectively compared to the vehicle dosed rats. Thus, a very significant increase ($p<0.01$), ($p<0.001$) and ($p<0.001$) in LYM% for low, medium and high dosed SD rats respectively compared to the vehicle dosed rats suggests the possible immune stimulatory effect of the product (Ugwah-Oguejiofor *et al.*, 2019).

The very significant difference ($p<0.001$) in BASO% especially with the significant reduction for the medium and high dose compared with the control is indicative of allergic reaction, chronic myelogenous leukemia, and polycythemia vera. Contrarily, there was a slight significant reduction ($p<0.05$) in NEU% for medium and high dosed SD rats compared with the vehicle. The low dosed also gave a reduction in NEUT% just that the reduction was not significant. Neutrophils defend the body against infections and the reduction in their percentage is indicative of neutropenia induced by the product which increases with increasing doses. A non-significant reduction in the MON% for low and medium dosed rats but significant reduction ($p<0.05$) with the high dosed rats compared with the vehicle is termed monocytopenia. It could be due to suppression of WBCs production in general by the product. There was a very significant increase ($p<0.01$), ($p<0.001$) and ($p<0.001$) in HCT for low, medium and high dosed SD rats respectively compared to the vehicle dosed rats. Haematocrit (Hct) also known as Packed Cell Volume (PCV) is the red blood cells percentage (%) in blood and involved in the oxygen transport and nutrients absorption. Therefore, raised HCT results in an increased primary and secondary polycythemia because it indicates a better transportation (Etim *et al.*, 2014). There general increase in HGB for all doses but only the high dosed revealed a very significant increase ($p<0.001$) compared with the vehicle this is termed as

polycythemia and could be as a result of reduce plasma volume or increase red blood cells as in this case. There was a very significant increase ($p<0.001$) in RBCs for all doses which affirms the raised HGB. The increase in RBCs is suggestive of the product inducing hemopoiesis even at the lower dose.

Also, a significant reduction ($p < 0.5$), ($p<0.01$) in MCH in low and medium dose respectively compared to the vehicle is indicative of possible iron deficiency anemia. Suggestive of possible inhibition of iron absorption needed for hemoglobin generation by the product. A very significant decrease ($p<0.001$) in RDW-SDs for all doses compared to the vehicle indicating variations in size of the RBCs caused by product in all doses.

Also, a slight significant reduction ($p<0.5$) in P_LCR with low and medium dose but non-significant reduction in high dose. A significant increase ($p<0.01$) in MPV for low dose whilst the medium and high dose revealed non-significant reduction compared to vehicle was revealed. Blood platelets place a role in blood clotting. Prolong clotting formation is associated with low platelet concentration hence excess loss of blood through bleeding in the case of injury and vice versa. The revealed significant reduction in P_LCR indicates drug induce thrombocytopenia which may lead to prolong bleeding in the case of an injury (Etim *et al.*, 2014).

Regarding Product 5, a very significant increase ($p < 0.001$) in PDW and significant reduction ($p<0.05$) in P_LCR revealed in all doses compared with the vehicle. The significant increase in this platelet indices shows that the product shortens clotting formation and reduces bleeding. A significant reduction ($p < 0.01$), ($p<0.05$) in MON% and NEU% for low and high dose indicates suppression of the immune system by the product. A significant increase ($p<0.001$) in BASO# for low dose and a significant reduction ($p<0.01$) in BASO% in high dosed SD rats compared with the vehicle revealed possible induction of allergic response at low dose and a suppression of hypersensitivity reaction at high dose by the same product. On the contrarily, a very significant increase ($p<0.001$) of LYM% of all doses indicates a possible immune stimulatory effect to infection specifically viral infection by the product.

HGB revealed a significant increase ($p<0.05$) in low and medium dosed SD rats compared with the vehicle this could be as a result of facilitation of production of red blood cells by the product

which gave a very significant increase ($p < 0.001$) of RBC and HCT. Nevertheless, the significant reduction ($p<0.05$) of MCH for all doses despite increase in HGB shows that the average weight of the hemoglobin was less. MCH values increase and decrease with MCV values respectively. The results obtain reveal a reduction in MCV from the vehicle but was non-significant for all doses. Therefore, the significant reduction of MCH in relation to MCV can be termed as microcytic anemia induced by the product. All other doses revealed a non-significant difference compared with the control. group a very significant reduction ($p<0.001$) in RDW_SD for all doses indicating that the red blood cells size varies little and it could be as a result of microcytic or macrocytic anemia in relation to the product intake.

WEIGHT DIFFERENCE AND RELATIVE ORGAN WEIGHT

Generally, all the five herbal aphrodisiac products altered the weight of the animals after the 14-day post-administration observation except for product 4. This indicates that Product 1, 2, 3 and 5 may possess some ability to cause carbohydrate or fat metabolisms abnormalities (Amoateng *et al.*, 2016) which could lead to body fat accumulation or it could be a possible aphrodisiac effect on muscular development during the treatment (Sundaram et al., 2021). Product 4 did not reveal any significant difference ($p < 0.05$) between the weights of the animals treated with the product and that treated with the vehicle at all dose levels.

Another sensitive indicator of toxicity is changes in organ weights. The kidneys, spleen, liver, lungs and heart are the first vital organs to exhibit metabolic responses to poisonous chemicals. Therefore, determining the relative organ weights help diagnose possible harmful changes to organs exposed to poisonous chemical (Ugwah-Oguejiofor *et al.*, 2019). The principle behind relative organ weight is that in most cases, aside the brain, the weight change of the organ is proportional to the whole-body weight. If the size of the organ changes in relation to the total body weight of the animal, it indicates adverse chemical effects. It is in the light of this, that a study to investigate the effects of these herbal aphrodisiacs on the relative body weight as well as on histopathological parameters of the kidneys, liver, brain, heart and spleen was carried

out. Changes in organ weight depicts physiological disturbances, effect on enzymes and target organ injury. A decrease in organ weight suggests target organ necrosis and occurrence of hypertrophy is vice versa (Zainal *et al.*, 2020 and Nigatu *et al*., 2017).

The significant decrease ($p<0.01$) in weight of liver compared with the vehicle observed in the high dosed SD rats orally administered with Product 1 could be an indication of toxicity. Specifically target organ necrosis. There was some reduction of liver weights observed with increasing doses as in the case of low and medium dose of the same product but this reduction was not significant compared with the vehicle. This suggests that Product 1 may be slightly harmful to the liver with increasing doses.

Again, the weight of the heart was significantly increased ($p<0.05$) with the medium dose of Product 2 when compared to the vehicle. However, this increase was not dose dependent. This may suggest a hypertrophy of the heart. There was no significant increase or reduction in weights of the other vital organs irrespective of the varying administrative doses.

Finally, there was a very significant reduction ($p<0.001$) of the weight of the spleen of the animals administered with medium dose of product 4 compared to that of the vehicle. This reduction is suggestive of increase necrosis of the spleen. However, the reduction was not dose dependent as the low and medium dosed animals did not exhibit any significant reduction in comparison to the vehicle.

All other organs for the rest of the products did not exhibit any significant alterations compared to the vehicle irrespective of the varying dose levels.

HISTOPATHOLOGY

Histological screening identifies changes in the structure of the cell of internal organs irrespective of the changes detected by biochemical and hematological investigations (Zainal *et al*., 2020)

Histopathological analysis performed on the liver concentrated on major changes of cellular cytoplasmic changes like bile and fat accumulation or clumping, cell death or damages. Changes observed for the kidneys were inflammation, cytoplasmic accumulation, cell death including tubular necrosis and tubular deposit or casts and glomerular changes. That of the

heart were inflammation, cytoplasmic diseases including contraction bands and cell death. Changes observed for the brain were inflammation, cytoplasmic changes including accumulations, cell death and extracellular deposits and lastly, that for the spleen were inflammation, microphage/cell accumulation and cell death.

Histopathological examinations of the kidneys, livers, hearts, spleens and brains of the test animals presented no major significant pathological changes with respect to the control. The control SD rats presented with normal liver, renal, spleen, cardiac and brain tissues but there were some minor pathological variations (equivocal and slight) presented by the test SD rats subjected to acute toxicity.

Low and medium dosed rats of Product 1 presented with equivocal cytoplasmic accumulation of the liver and tubular deposit (hyaline cast) of the kidney. Indicative of likely renal and hepatotoxicity effect of the product. ALP is produced in the liver, they are also found in other organs such as bone marrow, kidneys and guts therefore, its elevation in all doses of product 1 with significant elevation ($p< 0.001$) for the low dose compared to the control during the biochemical analysis could confirm a slight renal or hepatic toxicity.

There was equivocal inflammation and mild focal steatosis of the liver for rats administered with medium dose of product 1. This could indicate mild liver toxicity induce by the drug at such dose.

Product 2 low and medium dosed SD rats presented with mild equivocal kidney tubular deposits (hyaline cast) indicates that such doses could affect the renal function. Also, equivocal cytoplasmic accumulation of lymphocytes termed as cytoplasmic clumping in the liver presented by the medium dose of Product 2 indicates that it is likely this dose can induce hepatotoxicity.

There was also equivocal focal acute inflammation of the heart of medium dosed SD rat compared with the control for Product 2. The equivocal focal acute inflammation of the heart reflects the weight of the heart that was significantly increased ($p<0.05$) with the medium dose of Product 2 when compared to the vehicle, indicating likely cardiac toxicity by the product or it could be from the evasive cardiac puncture employed in the collection of blood.

Product 3,4,5 histopathological tissues were not different from that of the vehicle and therefore could be said that such doses of the product given could not alter the morphology or cause lesions or pathological changes of the vital organs and could be safe in this aspect.

Chapter 7

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

CONCLUSION

In conclusion, the present study showed that all the five herbal aphrodisiacs did not cause any mortality in the rats. However, there were clinical signs of toxicity such as autonomic, stereotypic, excitatory and motor activity for all the five herbal aphrodisiac products screened using the modified Irwin test. These signs of toxicity were dose dependent for most of the products. The biochemical, hematological, body and organ weights indicated some evidence of toxicity at different dose levels of the products administered. Histopathological examinations did not reveal significant changes in the isolated organs of all the five herbal aphrodisiac products in comparison to the control even with a high dose (x 100 of the low doses). Nevertheless, there were minor pathological variations amongst product 1 and 2 in relation to the liver, kidney and heart when compared to the control which had some correlation with hematological and biochemical changes.

LIMITATONS

- This study depicts instant excessive high intake of herbal aphrodisiac therefore, data gathered cannot be all that linked to a daily constant abuse of the recommended doses of these products.
- Observation made during modified Irwin test is subjective to the researcher and therefore could have slight variation in interpretation or analysis by another researcher.
- Conventional manual observations used in this experiment is disadvantaged to the automated systems due to susceptibility to observer bias, shorter duration and performed only in light phase. However, automated system with single housing rats affects their behavior.

RECOMMENDATION

- Further sub-chronic and chronic toxicity study needs to be performed to ensure that the minor variations in the liver, kidney and heart in addition to the hematological and biochemical indices are affected by product 1 and product 2.
- Ministry of Health and Food and Drug Authority must intensify education on sexuality and health implications from herbal aphrodisiac abuse.
- Media advertisements of herbal aphrodisiacs must be seriously scrutinized and random screening of herbal aphrodisiacs in pharmacies must be intensified by FDA.

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APPENDIX

Appendix 1: ETHICAL CLEARANCE CERTIFICATE

UNIVERSITY OF GHANA

University of Ghana Institutional Animal Care and Use Committee (UG-IACUC)

Phone:
Email: UG-IACUC@ug.edu.gh

P.O. Box LG 581
Legon, Accra
Ghana

Office Location: Department of Animal Experimentation Building, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana

13th April, 2021

ETHICAL CLEARANCE
(UG-IACUC 009/18-19)

Your protocol for an amendment has been reviewed by the University of Ghana Institutional Animal Care and Use Committee and has been **approved** as follows:

TITLE OF PROTOCOL: ACUTE TOXICITY SCREENING OF SOME HERBAL APHRODISIACS IN PHARMACIES WITHIN GREATER ACCRA USING THE MODIFIED IRWIN TEST

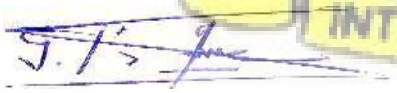
PRINCIPAL INVESTIGATOR: MR DENNIS AMANKWAH,

Please note that the final review report must be submitted to the Committee at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to UG-IACUC for review and approval prior to implementation.

Please report all serious adverse events related to this study to UG-IACUC within seven days verbally and in writing within fourteen days.

This certificate is valid till 31st December, 2020. You are to submit annual reports for continuing review.



Signature of Chairperson
Prof. Major (Rtd.) George A. Asare

Appendix 2: EFFECT OF CHLORPROMAZINE

t_1			Saline 1ml/kg p.o. Number of rats responding								Chlorpromazine (30 mg/kg p.o.). Number of rats responding									Chlorpromazine 60mg/kg p.o. Number of rats responding								
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	Excitation	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Increased reactivity to touch		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Aggression		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Fore-paw treading	Stereotypy	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Head movements		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	**	-	-	-	-	-	-	-	-	-	-
Chewing		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sniffing		-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	**	-	-	-	*	-	-	-	-	-
Scratching		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Catalepsy	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-

Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	*	-	-	**	**	******	-	-	-	-	-	-	******	*****	******	-	-
Decreased fear/startle	Sedation	-	-	-	-	-	-	-	-	-	-	-	*****	*****	******	******	******	-	-	-	-	*****	*****	******	*****	******	-	-
Decreased activity		-	-	-	-	-	-	-	-	-	-	******	******	******	******	******	******	-	-	-	*****	*****	******	******	*****	******	-	-
Decreased reactivity to touch		-	-	-	-	-	-	-	-	-	***	******	******	******	******	******	******	-	-	**	*****	*****	******	******	*****	******	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	*****	-	-
Ptosis	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection		-	-	-	-	-	-	-	-	-	-	******	******	******	******	******	******	-	-	-	*****	*****	*****	*****	*****	*****	*****	-
Salivation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Other	Blackish red patches were observed on the eyelids of three rats at 24 hours post administration for 30mg/kg dose of chlorpromazine																										
		Blackish red patches disappeared on the eyelids of the three rats at 24 hours post administration for 30mg/kg dose of chlorpromazine																										

Appendix 3: EFFECT OF CAFFEINE

			Saline 1ml/kg p.o. Number of rats responding								Caffeine 25mg/kg p.o.). Number of rats responding								Caffeine 50mg/kg p.o. Number of rats responding							
Observation time		t 0	t_1	t_2	t 3	t 4	t 5	t 6	t 7	t 8	t_0	t 1	t 2	t 3	t 4	t 5	t 6	t 7	t 8	t 0	t 1	t 2	t 3	t 4	t 5	t 6	t 7	t 8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	-	-	-	-	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	* * * * *	* * * * *	* * *	-	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * *	-	-
Increased reactivity to touch		-	-	-	-	-	-	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	-	-	-
Aggression	Excitation	-	-	-	-	-	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	-	-	-	-	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	-	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	* * * * * *	* * * * * *	* * * * * *	* * * * * *	-	-	-	-	-	* * * * * *	* * * * * *	* * * * * *	* * * * * *	* * * * * *	-	-	-
chewing		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
sniffing		-	-	-	-	-	-	-	-	-	-		* * * *		* * * *	-	-	-	-	-	* * * * *	* * * * *	-	-	-	-	-	-
Scratching	Stereotypy	-	-	-	-	-	-	-	-	-	-				* *	-	-	-	-	-	* * * *	* * * *	-	-	-	-	-	-
Catalepsy		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-

Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)		-	-	-	-	-	-	-	-	-	***	****		**	******	******	-	***	-	***	*****	**	*******	******	******	***	***	-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle	Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Decreased activity	Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Decreased reactivity to touch	Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Urination	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Salivation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
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Appendix 4: EFFECT OF PRODUCT 1 (12MG/KG,120MG/KG AND 1200MG/KG)

			Product 1 (12mg/kg p.o.) Number of rats responding									Product 1 (120mg/kg p.o.). Number of rats responding									Product 1 (1200 mg/kg p.o.). Number of rats responding								
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*****	*****	*****	*****	*****	*	-	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***	*****	*****	*****	-	-
Increased reactivity to touch		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	***	***	*		-	-
Aggression	Excitation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	******	*****	*****	*****	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chewing		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sniffing		***	**	*	***	-	-	-	-	-	-	*	*	-	-	-	-	-	-	*	***	***	***	***	***	***	-	-
Scratching	Stereotypy	-	-	-	***	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalepsy		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abnormal gait (tip-toe)		-	**	***	****	***	****	**	-	-	-	*	*	*	**	*	**	-	-	-	-	-	-	-	-	-		-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle	Sedation	-	-	-	-	*****	*****	*****	-	-	-	*****	*****	-	-	-	*****	-	-	-	-	-	-	-	-	-	-	-
Decreased activity	Sedation	-	-	-	*****	*****	*****	*****	-	-	-	-	*****	*****	*****	*****	-	-	-	-	-	-	-	-	-	-	-	-
Decreased reactivity to touch	Sedation	-	-	-	-	*****	*****	-	-	-	-	-	-	-	*****	*****	-	-	-	-	-	-	***	-	-	-	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	**	-	-	-	-	-
Urination	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 5: EFFECT OF PRODUCT 2 (15MG/KG,150MG/KG AND 1500MG/KG)

			Product 2 (15mg/kg p.o.) Number of rats responding									Product 2 (150mg/kg p.o.). Number of rats responding									Product 2 (1500 mg/kg p.o.). Number of rats responding								
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	****	****	****	-	-	-	-	-	-	****	****	****
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased reactivity to touch	Excitation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aggression		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chewing		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sniffing	Stereotypy	**	**	*	**	-	*	-	-	-	-	-	-	-	-	-	-			-	*	***	****	***	***	*	-	-
Scratching		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-
Catalepsy		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)		**	**	*	*	-	*	-	-	-	-	*	**	**	-	-	-			-	**	**	-	-	-	-	-	-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of righting reflex	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle	Sedation	-	-	****	******	******	-	-	-	-	-	-	**	**	-	-	-	-	-	-	**	**	-	-	-	-	-
Decreased activity			***	*****	******	******	******	-	-	-	-	-	******	******	******	-	-	-	-	-	*****	****	*****	-	-	-	-	-
Decreased reactivity to touch		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 6: EFFECT OF PRODUCT 3 (16MG/KG,160MG/KG AND 1600MG/KG)

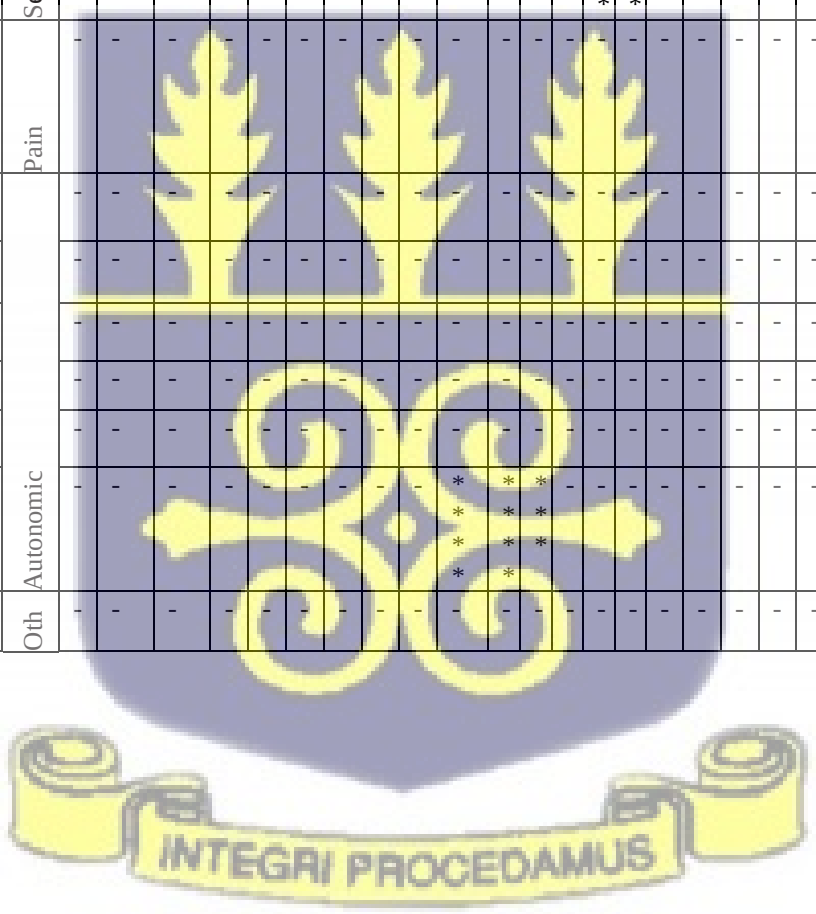
			Product 3 (16mg/kg p.o.) Number of rats responding									Product 3 (160mg/kg p.o.). Number of rats responding									Product 3 (1600 mg/kg p.o.). Number of rats responding								
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		**	**		*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased reactivity to touch	Excitation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aggression		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chewing		-	-	-	-	-	-	-	-	-			* *		-	-	-	-	-	-	-	* * * * *	-	-	-	-	-	-
sniffing		*	*	*		-	-	-	-	-	*	* *	* *	*	-	-	-			* * *	* * * * *	* * * * *	* *	-	-	-	-	-
Scratching	Stereotypy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalepsy		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)		* *	**	*	*	-	-	-	-	-	-	-	* * *	*	-	-	-	-	-	-	* * * * *	* * * *	* *	-	-	-		-
Loss of balance	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping		-	-	-	**	*	*	-	-	-	-	-	-	***	-	-	-	-	-	-	-	-	-	-	-	-
Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle		-	-	-	-		-	-	-	-	*****	*****	*****	**	-	-	*	-	-	-	*****	*****	-	-	-	-	-	-
Decreased activity		-	-	**	****	*****		-	-	-	*****	*****	*****	*	*	-	-	-	-	*****	*****	*****	*****	**	**	-	-	-
Decreased reactivity to touch	Sedation	-	-	*****	*****	*****	-	-	-	-	*****	*****	*****	**	*	-	-	-	-	*****	*****	*****	**	**	-	-	-	-
Writing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea		-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	**	-	-	-	-	*	-	-	-	-	-
Urination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 7: EFFECT OF PRODUCT 4 (4.0MG/KG,40MG/KG AND 400MG/KG)

			Product 4 (4.0mg/kg p.o.) Number of rats responding								Product 4 (40mg/kg p.o.). Number of rats responding								Product 4 (400 mg/kg p.o.). Number of rats responding							
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	**	***	*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	****	***	***	**	-	-	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						-	-	-	-
Increased fear/startle		-	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	*****	***	-	-	-	-	-
Increased reactivity to touch	Excitation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
Aggression		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	***	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chewing		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sniffing	Stereotypy	-	*	-	-	**	-	-	-	-	*	*	-	-	-	-	-			***	****	***	***	***	**	-	-	-
Scratching		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***	*	*	*	*	-	-	-
Catalepsy		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)			*	-	-	**	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-		-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle	Sedation	-	-	-	****		***	******	-	-	***	****	-	-	******	****	-	-	-	-	-	-	-	****	****	-	-	-
Decreased activity		******	***	**	******	****	****	******	-	-	******	*******	*******	*******	*******	*******	-	-	-	-	-	-	-	***	*******	*******	-	-
Decreased reactivity to touch		-	-	**	-	-	-	-	-	-	-	-	-	-	******	*******	-	-	-	-	-	-	-	-	-	-	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defecation/diarrhea		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	Autonomic	-	-	-	-	-	-	-	-	-	*****	***	***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Appendix 8: EFFECT OF PRODUCT 5 (0.3ml/kg, 3ml/kg and 15ml/kg)

			Product 5 (0.3ml/kg p.o.). Number of rats responding									Product 5 (3ml/kg p.o.). Number of rats responding									Product 5 (15ml/kg p.o.). Number of rats responding								
Observation time		t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
PARAMETERS	Death	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
convulsions		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tremor		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased activity		-	***	***	****	***	*	-	-	-	-	*	*	*	*		-	-	-	-	*	-	-	-	-	-	-	-
Jumping		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased fear/startle		-	-	**	-	-	*	-	-	-	-	-	-	-	**	*	-	-	-	-	-	-	-	-	-	-	-	-
Increased reactivity to touch	Excitation	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aggression		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fore-paw treading		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
head movements		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chewing		-	-	-	-	-	-	-	-	-	-			*		-	-	-	-	-	-	-	-	-	-	-	-	-
sniffing	Stereotypy	*****	**	** *	* *	* *	*	-	-	-	-	*	* *	*	* * *		-	-	-	* * *	* * *	* *	-	-	-	-	-	-
Scratching		-	-	** *	-	-	*	-	-	-	*	*	*	*	-	-	-	-	-	* * *	* * *	*	-	-	-	-	-	-
Catalepsy		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (tip-toe)		****	*	-	*	*	-	-	-	-	*	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-		-
Loss of balance		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of grasping	Motor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Loss of righting reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreased fear/startle	Sedation	-	-	-	-	-	***	-	-	-	*	*	****	-	-	****	*****	-	-	-	***	*****	*****	*****	*****	*****	-	-
Decreased activity		-	-	-	-	-	*****	*****	-	-	-	***	*****	*****	*****	*****	*****	-	-	*****	*****	*****	*****	*****	*****	*****	-	-
Decreased reactivity to touch		-	-	-	****		*****		-	-	-	-	*****	*****	*****	*****	*****	-	-	-	*****	*****	*****	*****	*****	*****	-	-
Writhing Analgesia	Pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	Autonomic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
Defecation/diarrhea		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-		-	-
Urination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-