Sudan University of Science and Technology
College of Graduate Studies

Some Aspects of Pharmacotoxicity of Goro *(Cola nitida)* In Rats

بعض الجوانب الاقربانية والسمية لنبات القورو وأثرها في الفئران

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Dedication

This Thesis is dedicated to my late father, my mother, wife and children with love .

Abdel Bagy Mustafa Ahmed

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I wish to give my family the biggest acknowledgement. My wife, Tahani, always supported me. I had to leave her alone with kids. Thanks to her and to my daughters Salma, Sulafa and Sons Awab & Osama for their love and support they gave me.

ABSTRACT

The Pharmacotoxicty effects of oral administration of 100, 200 and 400 mg/kg body weight for 28 days to rats were investigated. The result showed that goro significantly decreased water and food intake and body weight of rats. Significant increase in locomotor activity such as line crossing, rearing, grooming and light/dark transition was shown at the dose of 100mg/kg, but activity was decreased at 200 and 400 mg/kg. Goro at the dose of 200 and 400 mg/kg decreased platelet count, increased serum urea, creatinine and alanine and aspartate aminotransferase suggesting liver and renal injury in treated rats. Goro, further, at a dose of 200mg/kg improved sexual desire and performance, but decreased sperm count, morphology, motility and functional testicular marker enzymes. In the case of female rats, goro significantly blocked ovulation, altered estrous cycle and produced some teratogenic effects in foetal rats. Goro at a dose of 100mg/kg caused induction of hepatic microsomal mixed function oxidase in rats, but at the dose of 200 and 400 mg/kg inhibited the drug metabolizing enzymes. It is likely that goro may produce pharmacological effects at the dose of 100mg/kg and toxicology effects at higher doses.

الملخص بالعربى

لقد تم دراسة التأثيرات الدوائية والسمية لنبات القورو عند إعطائه بواسطة الفم بجرعات مقدارها 100 و 200 و 400 ملجرام للكيلوجرام وزن حي ولمدة 28 يوماً للفئران لقد أوضحت النتائج أن القورو قد أدى وبدلاله إحصائية إلى نقص في أوزان الفئران ونقص في استهلاك الماء والغذاء . وزاد نشاط الفئران العضوي عند جرعة 100 ملجرام للكيلوجرام ونقص نشاطها عند جرعات 200 و 400 ملجرام للكيلوجرام . ولقد أدى إعطاء القورو بجرعات 200 و 400 ملجرام للكيلو جرام إلى نقص في عدد الصفائح الدموية وزيادة اليوريا والكريتين وإنزيم الالنين- والاسبارتيت امينوتر انسفيريز مما يدل على تأثيرات سمية للقورو على الكبد والكلى . ولقد أدى إعطاء القورو في جرعة 200 ملجرام للكيلوجرام للفئران إلى زيادة النشاط الجنسي- لكن في نفس الوقت أدى إلى نقصان الحيوانات المنوية والصفات التشريحية والحركة وإنزيمات الخصية أما بالنسبة لإناث الفئران فقد أدت الجرعة عند 200 ملجرام للكيلوجرام إلى نقص في عملية التبويض وتغيرات في دورة الشبق وإحداث تشوهات خلقية في الجنين- . لقد أدى اعطاء القورو بجرعة 100 ملجرام للكيلوجرام إلى زيادة في نشاط إنزيمات ايض الأدوية وأدت الجرعات عند 200 و 400 ملجرام للكيلوجرام إلى تثبيط إنزيمات الأدوية . يتضح من هذه التجارب إلى ان تأثيرات القورو تعتمد على مقدار جرعته فهى دوائية عند 100 ملجرام للكيلوجرام وذات مفعول سمي عند 200 و 400 ملجرام للكيلوجرام .

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INTRODUCTION

Toxins occur in virtually all grasses, herbs shrubs, and trees throughout the world (Cheeke 1998).

There are many of classes of toxins-alkaloids, terpenoids, tannins,cyanogenic glycosides to name a few (D'NeLLo 1997).

The use of plants for producing central nervous system stimulation is well known and is attributed to the presence of active constituents, such as caffeine, theobromine and theophylline in cola nitida and C acuminata (Sterculiaceae), Camphor in Ocimum Canum (Labiatae), and the alkaloids dilhydroiscorine in Dioscorea dumetorum (Dioscoreaceae) and ellipticne and strychnine in the loganiaceous strychnos spinosa (Bakhiet and Adam 1995).

Goro, Cola nut (Cola nitida), a central nervous system stimulant has been shown to mediate some physiological effects that are similar to the action of caffeine (Carrillo and Bennitez, 2000). Cola nuts have been used in folk medicine as an aphrodisiac and an appetite suppressant, enabling African soldiers who chew them to travel long distances without food. (Trindall, 1997). Other uses include increasing the capacity for physical exerition and for enduring fatigue without food, stimulating a weak heart, despondency, brooding, anxiety and sea sickness (The Psychoactive Encyclopedia. (T.P.E) 2008).

Goro is found everywhere in Sudan. Specially in western Sudan. An increasing number of people are now consuming the plant for the variety of reasons. However no detailed behavioral , toxicology or metabolic studies have been carried out.

Objectives:

The objectives of this study were to investigate some aspects of pharmacotoxicty of goro in rats as follows:

1. Effect of goro on water and food intake, haemtological and biochemical variables
2. Effect of goro on locomotor behaviour
3. Effects of goro on male sexual behaviour and reproduction
4. Effects of goro on female reproduction
5. Effects of goro on the activity of drug metabolizing enzymes in liver of rats

Chapter One

LITREATURE REVIEW

1. LITERATURE REVIEW

1.l Plants and Central Nervous System:-

Brain function and nervous system are the most important aspects of physiology that define the difference between humans and other species. Disorder of brain function and nervous system (Central and Peripheral) due to improper balance in neurotransmitter levels, whether primary or secondary, to malfunction of other system, are a major concern of human society and a field in which pharmacological intervention plays a key role (Jinka et al, 2011).

Although, a variety of agents for different central nervous system (CNS) activities, are available and symptomatic, but neither are effective, prophylactic nor possess proper cure compliance with medication is major problem because of the need for long term therapy together with the unwanted effects of many drugs. Hence, a lot of effort is being focused to investigate a new drug which overcome the limitations of currently used CNS active drugs.

The use of plants for producing central nervous system stimulation is well known and is attributed to the presence of active constituents, such as caffeine, theobromine, and theophylline in cola nitida and C acuminate (Sterculiaceae), camphor in ocimum canum (Labiatae), and the alkaloids dihydroioscorine in Dioscorea dumetorum (Dioscoreaceae) and ellipticine and strychnine in the loganiaceous strychnos spinosa (Bakhiet and Adam 1995).

1.2 Goro (*Cola nitida*)

Goro (*Cola nitida*) has been used in folk medicine as an aphrodisiac, an appetite suppressant, to treat morning sickness, migraine headache, and indigestion (Esimone *etal* 2007). It has also been applied directly to the skin to treat wounds and inflammations (Newall *etal* 1996). The tree's bitter twig has been used as well, as clean the teeth and gums (Esimone *etal* 2007). In Africa, duodenal and peptic ulcer is common among southern part of Africa, Burundi, Rwanda, and eastern Zaire, high land of Ethiopia, central Sudan and east Africa especially around Kilimanjaro Mountain. However, in Nigeria there is no record on the incidence of peptic ulcer, but sero-prevalence of *Helicobacter pylori* in patients with gastric and peptic ulcer

was carried out in the western part of Nigeria. Of the 92% patients screened 41% represented with peptic ulcer disease. The cola nut tree is native to West Africa. Cola nuts are obtained from cola trees. Cola nitida belongs to the genus cola and family Sterculiaceae. They are commonly used to counteract hunger and thirst, in some cases it is used to control vomiting in pregnant women also it is used as an principal stimulant to keep awake and withstand fatigue by students, drivers and other minerals (Haustein 1971; Chukwu et al., 2006). Cola nitida is not advised for individuals with stomach ulcers due both to its caffeine and its tannin content (Ibu *etal* 1986, Newall *etal* 1996). The colacuminata is more popular in the Igbo and Igedde tribes of eastern and middle regions of Nigeria, while the cola nitida is more common in the northern part of the country among the Hausa Fulani (Ibu *etal* 1986). Cola trees are best known for their seeds or nuts which are rich in caffeine. Thymolepte, antidepressant and antidiarrheal effects have been observed with its uses.

1.2.1 Botany:-

Goro (Cola nitida), also known as kola tree, cola nut, cola seed, and bissy nut is an ever green plant about 15m tall. The flowers have diameter of 2.5cm and fruit grow up to 20cm long and 5cm wide. The seeds are usually redish or red, occasionally white (Amori and Ashri 1994).

1.2.2 Habitat:-

The plant is indigenous to Togo, Sierra Leone, and Angola. It is found today in all tropical regions and is cultivated widely, including western Sudan (Amori and Ashri 1994).

Production:-The ripe fruit is harvested and the seeds are removed and dried. Cola nut is the endosperm freed from the testa of various cola species,

1.2.3 Constitutions of Cola:-

Cola contains the following compounds (Rainyake *etal* 2009);

a. Purine alkaloids: main alkaloids caffeine (0.6-3.7%), additionally theobromine, and theophylline.

b. (+)- catechin, (-)- epicatechin

c. Catechin tannis

d. Oligomeric proanthocyanidins

e. Starch.

1.3 Mechanism of action:-

Caffeine, 1,3,7-trimethyLxanthine, is the most widely consumed behaviorally active substance in the world. Both acute and especially chronic caffeine intake appear to have only minor negative consequence on health (Fredholm etal., 1999). It is a potent adenosine antagonist specially in low-doses and is a CNS stimulant that easily crosses the blood-brain-barrier due to its lipophilic properties (Davis *etal* 2003). Four distinct adenosine receptors, $A_{1,}A_{2A}$,A_{2B} and A_3 are likely to be the major targets for caffeine. The A_1 and A_{2A} adenosine receptors are the subtypes primarily involved in the caffeine effect, while A_{2B} and A_3 receptors play only a minor role (Fredholm *etal* 1994). It has been shown to counteract most of the inhibitory effects of adenosine on neuro-excitability, neurotransmitter release, arousal and spontaneous activity. Furthermore, caffeine can improve CNS function by inhibiting phosphodiesterase activity, blocking GABA receptors and mobilizing intracellular calcium (Garrett and Griffiths, 1997). However, higher doses can suppress behavioral activity and even performances associated with learning and memory (Howell *etal*., 1997).

Caffeine also acts to increases alertness, anxiety and hallucination due to it its blocking of adenosine receptors which normally inhibits glutamate release (Ekam, 2001; Obochi, 2006). Glutamate supplies the amino group for the biosynthesis of amino acids, and is a substance for glutamine and glutathione synthesis (Danbolt, 2001). Inhibition of glutamate release result in low protein synthesis and efficiency (Bertolini et al, 1980; Bathnager and Misra 1988). Adenosine receptors are linked with an interplay of release, reuptake, metabolism and excretion of neurotransmitters (Obochi, 2006). Thus, blockage of adenosine receptors by caffeine results in alternation in behavioral pattern by delaying neuronal in behavioural pattern by delaying neuronal tube closure (Eteng *etal* 1991, Obochi, 2006). Caffeine also activates phosphorylase and lipase, thus, enhancing glucogenolysis and lipolysis, resulting in loss of weight. Caffeine also inhibits androgen binding protein, resulting in decreased cauda epididymis sperm reserve, seminiferous tubular fluid volume, resulting in low sperm production and infertility (Eteng *etal* 1997; Obochi 2006).

Mechanism of action of caffeine involve interaction with hormones, peptides and receptors on the surface of the plasma membrane to generate signals since these molecules cannot cross the plasma membrane (blood-

brain-barrier). The amplification and subsequent transmission of such signals to the call interior require the participation of second messenger, usually a cyclic nucleotide, cAMP, while the hormones, peptides or receptors serve as the first messenger (Eteng et al 1997; Ekam 2001; Obochi, 2006).

1.4 Effects:-

Cola purine (Caffeine) content makes cola a strong CNS stimulant in humans. It acts as a respiratory analeptic, lipolytic, mildly positively chronotropic, and mild diuretic. In addition, it stimulates gastric acid and increases motility of the gastrointestinal tract. In animals tests, cola is also analeptic, lipolytic, stimulates production of gastric acid, and increases gastric motility. (Arnaud 1987).

1.4.1 Central nervous system

Although all levels of the CNS may be affected, regular doses of caffeine (100-150 mg) will stimulate the cortex and produce increased alertness but decreased motor reaction time to both visual and auditory events (Nelig et al 1992). Drowsiness and fatigue generally disappear. Larger doses may affect the medullar, vegus, vasomotor and respiratory centers, resulting in slowing of the heart rate, vasoconstriction and increased respiratory rate (Astorino and Roberson 2010).

Caffeine is thus useful for counteracting fatigue in shift worker and students and as cognitive enhancer. The mechanism is thought to be via antagonism of adenosine receptors and consequent enhancement of dopamine activity (Astorino and Roberson 2010). Caffeine also lifts the mood and may have antidepressant effects; it has been shown to reduce the risk of suicide, caffeine withdrawal leads to headaches; it has therefore been used clinically to relieve postoperative withdrawal symptoms and for post-dural puncture headache (Bishop 2010).

1.4.2 Respiratory effect

Although the mechanism of action is not clearly defined. Caffeine appears to stimulate the medullary respiratory centre and normalize autonomic function (Peters 1967). Thus it may be useful for treating apnoea in preterm infants and Cheyne-stokes respiration in adults, as an adjunct to non-drug measures and as an alternative to theophylline. The methylxanthines are an important

group of bronchodilator agents; in particular, aminophylline, a derivative of theophylline, is used in childhood asthma (Hackett 2010).

1.4.3 Cardiovascular system

In low doses caffeine is thought to enhance vagal stimulation and thus slow the heart. In higher doses, caffeine stimulates the myocardium, increasing both heart rate and cardiac output. Overstimulation may cause tachycardia and cardiac irregularities (Zhang *etal* 2011).

Depending on the dose, caffeine may cause vasodilatation, or a reflax increase in systemic vascular resistance, which can cause an increase in blood pressure. This latter effect may be secondary to stimulation of the sympathetic nervous system and blockade of adenosine-induced vasodilatation (Fredholm et al 1999).

1.4.4 Analgesic Adjunct and vascular effects

Overall, Caffeine has a weak vasodilator action, with little effect on blood pressure (Funk 2009). Caffeine is used in analgesic products and in combination with ergotamine for treating migraine and other headaches, to enhance pain relief. The enhanced effect of ergotamine may be a result of better absorption of the ergotamine in the presence of caffeine (Maughan and Griffin 2003) caffeine itself may also have some direct antimigraine action. Caffeine and theophylline cause potent cerebral vasoconsrtiction; the latter has been trialled in ischaemic stroke, on the rationale that decrease in blood flow in prefused areas in the brain may enhance development of collateral vessels in ischaemic areas after stroke. Clinical evidence for benefit is not yet convincing (Maughan and Griffin 2003).

1.4.5 Musculoskeletal system

Caffeine affects voluntary skeletal muscles to increase the force of contraction and decrease muscle fatigue. These effect are via activation of the ‘ryanodine receptor’ family, activation of which open calcium channels in the sarcoplasmic reticulum of skeletal muscels cells, causing calcium release and contraction of the muscle. Caffeine also has a general theromgenic action, increasing heat production, possibly via the hypothalamus or by enhacing catecholamine effects.

Recently: interesting effects of methylxantines have been demonstrated in bone. In animals some abnormalities of foetal bone and joint development have been shown. In humans there is some evidence that high caffeine intake

may increase urinary excretion of calcium, decreasing bone mineral density. This could have important implications for the development of oesteoporosis, especially in postmenopausal women. (Armstrong *etal* 2007).

1.5 Pharmacokinetics:

Caffeine is rapidly and totally absorbed after oral administration. Its is only 35-40% protein bound and is distributed to all body compartments. It crosses the blood-brain barrier and enter the CNS, and passes readily through the placenta. The peak plasma level is achieved within 50-75minutes, with therapeutic plasma levels at 5-25 mg/mL (Arnaud 2011, Verbeek 2008).

Caffeine is metabolized in the liver. In adults it is metabolised to theophylline and the theobromine whereas in the neonate only a small portion is metabolised to theophylline. Caffeine's half-life is 3-10hours (average 4hours) in adults and 65-130hours in neonates. In adults, caffeine is excreted by the kidneys, with only 1-2% excreted unchanged; in neonates it is excreted by the kidneys, with about 85% excreted unchanged (Newton *etal* 1981, Verbeek 2008).

1.6 Toxicity:

Caffeine, is consumed through processed cocoa, coffee, tea or cola nut based food and beverages, stimulants, drugs and cosmetic (Eteng *etal* 1997; Obochi, 2006). The metabolism and toxicity of caffeine are viewed to be dose dependent, resulting in non-linear accumulation of the methylxanthines, hence greater risk of cardiovascular diseases such as heart failure, high blood pressure and neuronal disorder such as schizophrenia (Cucinnel *etal* 1965; Shaffiner and Popper, 1996; Leon and Hedge, 1970). Metabolic derangement and subsequent toxicity of caffeine leads to weight loss, poor growth, low protein efficiency ratio and poor nitrogen retention, leading to death (Leon and Hedge 1970; Eteng *etal* 1997, Ekam 2001). Thus, The toxicity of caffeine is viewed as a disposition demonstrating that caffeine is rapidly absorbed but slowly excreted (Leon and Hedge, 1970; Eteng etal, 1997; Ekam 2001; obochi, 2006).

Common adverse reaction include increased nervousness or anxiety and irritation of the gastrointestinal tract, resulting in dyspepsia and nausea. More frequent adverse reactions in neonates include abdominal swelling or distension, vomiting, body tremors, tachycardia or nervousness(Rainyake et al 1995). Signs of overdose include increased temperature, headache, confusion, increased irritability and sensitivity to pain or touch, tinnitus, insomnia, palpitations, fine tremor, increased urination, dehydration, nausea and vomiting, abdominal pain and convulsions (Neligh *etal* 1992) A withdrawal syndrome of increased irritability,

headache and increased weakness has been reported when users of more than 600mg/day (about six cups of coffee) decreases or eliminate this intake. Caffeine has also been implicated in may adverse health effects, such as cancer, fibrocystic breast disease and birth defects (Gawin and Ellinwood 1988).

CHATPER TWO

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 Animals and Housing

2.1.1 Rats :

Male and female adult Wistar rats obtained from faculty of Pharmacy, University of Khartoum breeding unit and weighing between 190-200g were used in this study. The rats were kept in clean cages at room temperature (23-25ºC) and relative humidity was 40-70% with 12hrs light/dark cycle. Tap water and feed standard granules for mouse and rats, (ARASCO, Saudi Arabia) were available *ad libitum.*

2.2 Collection of Blood Samples

Blood samples were collected in heparniazed syringes for hematology or plain tubes for serum preparation. Blood samples were obtained by cardiac puncture in rats. Serum was stored at -30ºC until analysis.

2.3 Chemical and Drugs

All chemicals and drugs used were BDH Analar grade unless otherwise specified.

2.4 Preparation of the goro seed extract

The seeds were procured from the local market and botanically identified (by Prof.Bushra Al-Nour, Department of Botany, University of Khartoum). Seeds (photo 1)were washed thoroughly, weighed then macerated into a fine flour-like paste using a mortar and pestle. This paste was mixed with physiological saline solution and stirred thoroughly using electromagnetic stirrer. The mixture was left undisturbed for overnight at a room temperature. The clear supernatant thus obtained is aspirated and used in such a manner that constant volume (0.1 ml) used to produce doses of 100, 200 or 400-mg goro extract/ body weight which were given orally.

Goro *(Cola nitida)*

2.5 Hematological Methods

Packed cell volume (PVC) was determined by the microhaematocrit method. Hemoglobin was measured as cyanomethemoglobin erythrocyte (RBC)

and white blood cell (WBC) count, and mean corpuscular volume (MCV) mean corpuscular hemoglobin concentration (MCHC) were determined with a Coulter Model ZBI Counter (Coulter Electronics, Hialeah, FL). The Differential leukocyte counts were carried out using blood smears stained with Giemsa and May-Grunwald solution. At least 200 cells were counted.

2.6 Serobiochemical Methods

Serum protein concentration was determined by a refractometer (Bellman and Standly Ltd, Uk). Serum chemistry analysis was conducted on each sample using 32uL of serum per assay and examined using a clinical chemistry analyzer (Gilford Imp 400E, Ciba Corning Diagnostics, Oberlin, OH) according to the manufacturer's recommended procedure. Kits used for the assays were obtained from Worrthington Biochemicals Corporations (Freehold, NJ, USA). These included glutamine oxaloacetate transminase (GOT), alkaline phosphatase (ALP) and glutamate pyruvate aminotransferase (GPT), urea and creatinine.

2.7 Effects of goro on water and food intake, haemtological and biochemical variables:-

Animals were randomly divided into 4 groups of 10 animals per group. Group 1 animals were fed rat diet and kept as controls. Group 2, 3 and 4 animals were given in addition goro at doses of 100, 200 and 400 mg/kg body weight for 4 weeks. Blood samples and tissues were obtained and stored at -30ºC until analysis.

2.8 Effects of goro on locomotor behaviour:-

Thirty (30) adult wistar rats weighing between 150-160g were used. The animals were randomly assigned into four (4) groups of ten (10) animals per group. Each rat in a study group was individually housed in a plastic cage with iron guaze bottom grid and a wire screen top.

2.8.1 *Treatment Regimen*

The animals were either given saline (group 3 controls) or goro at a dose of 100, (group 2), 200 (group 3) and 400 (group 4) mg/kg body weight, orally for a period of 4 weeks.

2.8.2 Experimental protocol using the open field-maze:-

The open field maze test was used to provide measures of locomotion, exploration and anxiety (Walsh and Cummins, 1976). The experiment was performed in an enclosed laboratory to screen the animals from noise and provide dim light to avoid distraction of the animals. The animals were placed in the centre of the maze and allowed to explore the open field for 5minutes. Before introducing each animal, the floor of the maze was cleaned using 70% ethyl alcohol in order to eliminate olfactory influence. The following behaviours were scored during the 5minutes to assess locomotor and exploratory behviours, line crossing, rearing and grooming.

2.8.3 Experimental protocol using the light/dark (LD) transition box:

The light and dark transition box is a test of locomotion and exploratory behaviour. Each rat picked up using a plastic bucket and placed in the centre division of the large compartment facing the floor. The rat was allowed to explore the transition box for 5minutes. Entering into a chamber is defined as the placement of all four paws in the chamber. During the period of 5minutes, behaviour scored using a stopwatch was frequency of line crossing.

2.9 Effects of goro on male sexual behaviour and reproduction:-

2.9.1 Experimental model:

Adult male rats weighing about 200g were used in this study. Rats were allowed for three pre-experimental mating tests with sexually receptive females and those who achieved ejaculations in the three times within a period of <30min were chosen for this study. The chosen rats were divided randomly into two groups, control versus experimental and caged separately. Each group contained ten male rats. The animals were kept under controlled temperature of 21±1ºC and 12h light. 12 h darkness schedule (lights on 06.00 AM-18.00 PM). Food and water were available ad libitum. The rats were allowed for two weeks acclimatization period before starting the treatment.

2.9.2 Treatment:

Goro was given at a dose of 200mg/kg orally.

2.9.3 Sexual behaviour test:

The sexual behavior of male rats was monitored by two trained observes unaware of the experimental design in a sound-attenuated room according to the

standard procedure (Agmo, 1997). The test was performed 24 h after the last administration. Single male rat was placed in a rectangular Plexiglass observation chamber (45x40x30 cm height) and allowed to acclimate for 5min. Then, a sexually receptive female rat was introduced in the chamber. The following parameters of sexual behavior were measured as described by Agmo (1997).

2.9.4 Mount Latency (ML):-Time from the introduction of the female until the first mount.

2.9.5 Mount Latency (ML):- Time from introduction of the female until the first intromission (Vaginal penetration).

2.9.6 Ejaculation Latency (EL):- Time from the first intromission until ejaculation.

2.9.7 Postejaculatory Interval (PEI):- Time from ejaculation until the next intromission.

2.9.8 Mount Frequency (MP):- Number of mounts preceding ejaculation.

2.9.9 Intromission Frequency (IF):-Number of intromissions preceding ejaculation. Also, the following parameters were calculated on the basis of the above data.

2.9.10 Inter-Intromission Interval (III):- Average interval between successive intromission (calculated as ejaculation latency divided by intromission frequency).

2.9.11 Copulatory Efficacy (CE):- A measure of intromissive success (Calculated as intromission frequency divided by mount frequency + intromission frequency). Tests were normally ended immediately after the first post-ejaculatory intromission.

2.9.12 Testosterone assay:- Four rats from each group, which were not submitted to mating tests, were used to analyze the serum testosterone level. Animals were euthanized by ethyl ether 24h after the last dose.

Trunk blood was collected into centrifuge tubes and the serum was prepared by centrifugation (3000 rpm. For 30min) and stored frozen (-20ºC) until testosterone assay. The testosterone concentration determined in triplicate using the testosterone enzyme immunoassay test kit (BioCheck Inc., Foster city, California, USA) according to the manufacturer's instructions. The minimum detectable concentration of this assay was estimated to be 0.05 ng/m and cross reactivity with other corticosteroids was minimal (<0.05%).

2.9.13 Sperm motility assay

Animals were killed and the epididymis was minced in prewarmed normal saline (37ºC) . One drop of sperm suspension was placed on a slide glass (Morrisey *etal* 1988). The motility of epidiymal sperm was evaluated microscopically within 2-4min of their isolation from the epididymis and data were expressed as percentages.

2.9.14 Sperm count

Epididymal sperm was obtained by mincing the epididymis in normal saline and filtering through a nylon mesh. The sperm were counted using a hamocytometer following the methodology of Morrisey *etal* (1988).

2.9.15 Morphological abnormalities

A portion of the sperm suspension placed on a slide glass was smeared out with another slide, fixed in 95% ethanol and stained with eosin. A total of 200 sperm from each rat were examined for abnormalities in different regions of spermatozoa, according to the method described by Morrisey *etal* (1988).

2.9.16 Assay of enzymes

A portion of testis was homogenized (1:9) in 0.2 M TRIS.HCl buffer (pH 7.0, containing 0.1% eetyltrimethyl ammonium bromide) with potter Elvejham Homogenizer for the estimation of sorbitol dehydrogenase (SDH) and lactic (LDH) dehydrogenase (Gerlach, 1983). The second portion was homogenized in ice-cold water for the assay of acid phosphatase and B-glucuronidase (Walter and Schutt, 1974). Another portion of testis was homogenized (1:9) in 0.05mM TRIS.HCI buffer (pH7.4) for the assay of gamma-glutamyl transpeptidase (r-GT) (Roomi and Goldberg, 1981). Protein was also estimated in the homogenates (Lowry et al. 1951). Enzymes and protein were assayed using specific kits.

2.10 Effects of goro on female reproduction

50 adult female rats were collected from the animal house. They were acclimatized for 2 weeks in the rat control room under standard conditions of temperature and illumination (12hours dark: 12hours light) cycle. They Underwent 2 successive 4 or 5-day cycles. They were divided into subgroups of five each. Goro was give orally at a dose of 200mg/kg body weight.

The animals were divided into three experimental groups each with a control group. In the ovulation experiment, group I (n=20), animals were replicated into

control and three experimental subgroups a, b, c; of 5rats each which received the goro , 200mg/kg body weight at 10am, 2pm and 6pm respectively. The control group received distilled water. Vaginal smears were obtained daily by vaginal lavage to monitor ovulation and oestrous cycle. At the end of group 1 experiment, the animals were sacrified using either anesthesia and the fimbriated part f the oviduct was dissected out from the rats, suspended in normal saline and placed on a microscopic slide with a cover slip to count the number of ova in the oviduct.

In the oestrous cycle experiments, group II (n=6) the animals received 200mg/kg body weight of goro extract once daily for six weeks. The pattern of oestrus cycle before and after administration of extract was studied and the animals served as their control. In the teratogenic experiment, group III (n=24) the animals were mated during the proestrous to oestrus night and the presence of spermatozoa was determined by microscopic examination of the vaginal smear the next morning. The presence of spermatozoa indicated conception and represented day 1 of pregnancy (Oderinde et al., 2002). These pregnant rats were subdivided into groups a, b and c which received 200mg/kg body weight of extract on days 1 to 5 of gestation (Implantation studies) for group IIIa, 7-9 days of gestation for IIIb (Beginning of organogenesis), and 14^{th} and 15^{th} day of gestation for group IIIc. The control group 'd' received distilled water. Body weight food consumption, gross appearance and behaviour were monitored daily. On day 21 of gestation, fetuses were removed from pregnant rats by ventral laparatomy and examined. The number of total implants, resorption, live and dead fetuses recorded. Live fetuses were removed from the uterus and weighed, and examined for gross malformations. Foetal parameters such as foetal number, weight, crown-rump-length, and length of umbilical cord, and placental weight were measured.

2.11 Effects of goro on the activity of drug metabolizing enzymes in liver of rats

Samples of liver of rats from experiment 2.7 were used to study the effect of goro on the activity of drug metabolizing enzymes.

At the end of the experiment rats were killed and liver were immediately removed, weighed and homogenized in ice-cold isotonic KCI. The crude homogenates were then centrifuged at 10,000g for 15min. A microsomal and cytosolic fractions were prepared as described by Mazel (1976). Protein concentrations in these fractions were determined by the method of Lowry et al (1951). The activities of aminopyrine-N-demethylase and aniline-4-hydroxylase were determined using the method of Mazel (1976) by estimating the concentrations of formaldehyde and p-aminophenol, respectively. The method of

Dutton and Storey (1962) was used to determine UDP-glucuronyltransferase activity by estimating o-aminophenyl-glucuronide concentration using o-aminophenol as a substrate. The activity of glutathione-S-tranferase was determined in the cytosotic fraction by estimation of 2, 4-dinitrophenylglutathione concentration according to the method described by Habig et al. (1974). The concentration of cytochrome p-450 was determined in the microsomal fraction according to the method of Omura and Sato (1964). The enzyme activities were linear with time, protein and substrate concentration (EL-Sheikh et al 1991, 1992).

2.12 Statistical Analysis:-

Result are expressed as mean ±SD and presence of significant differences among means of groups was determined using one way analysis of variance (ANOVA) with turkey-kramer post-test for significance. Values were considered significant when $P < 0.05$.

Chapter Three

RESULTS

3.RESULTS

3.1 Water intake

The daily water intake for goro-fed rats as compared to control groups is given in Fig 1. The goro-fed groups of rats had significantly ($P<0.05$) less means water intake when compared to the controls.

3.2 Food intake and body weight changes

The food intake (Fig 2) of goro-fed rats was significantly ($P<0.001$) lower when compared to control rats. There was a marked ($P<0.001$) decrease in body weight (Fig 3) of rats fed goro when compared to controls.

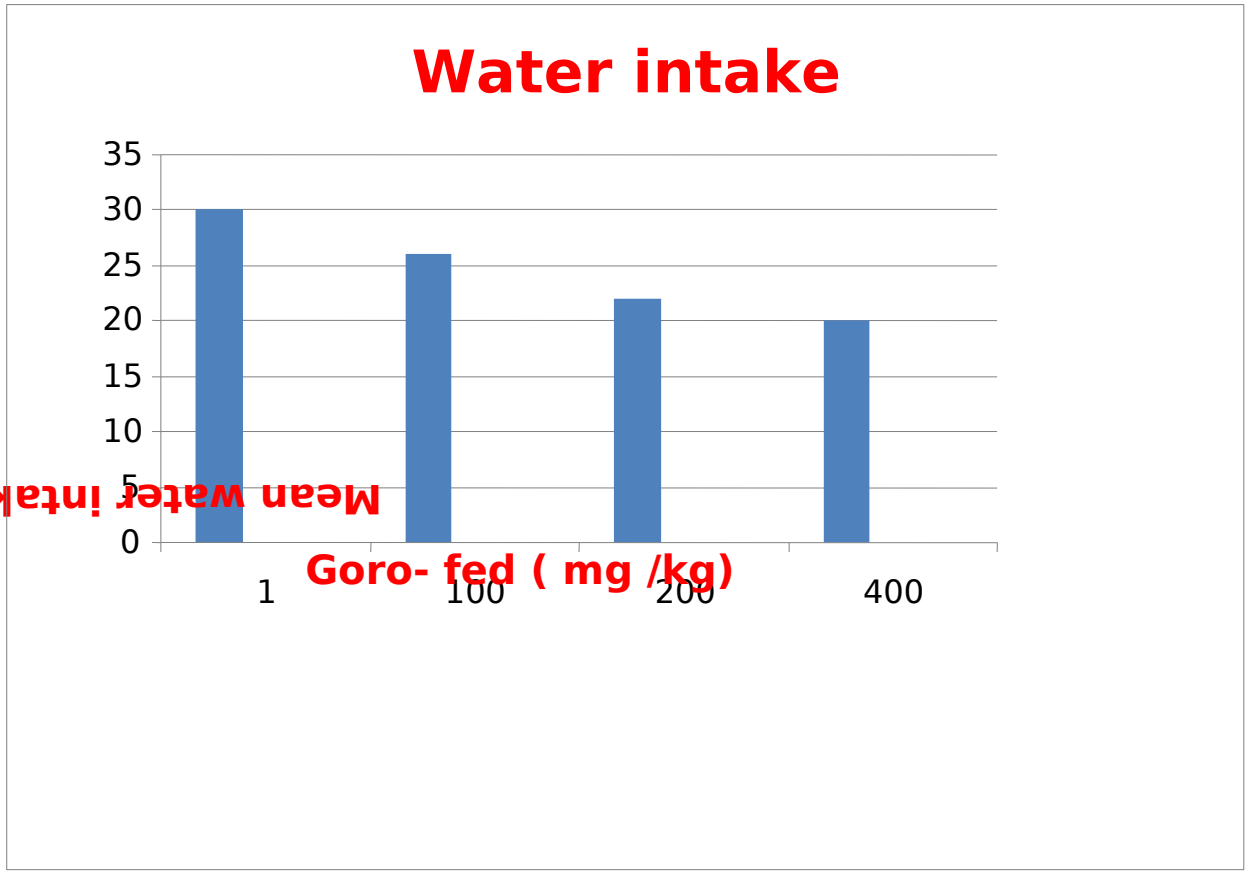


Fig1. Daily water intake for goro- fed groups of rats as compared to control group.

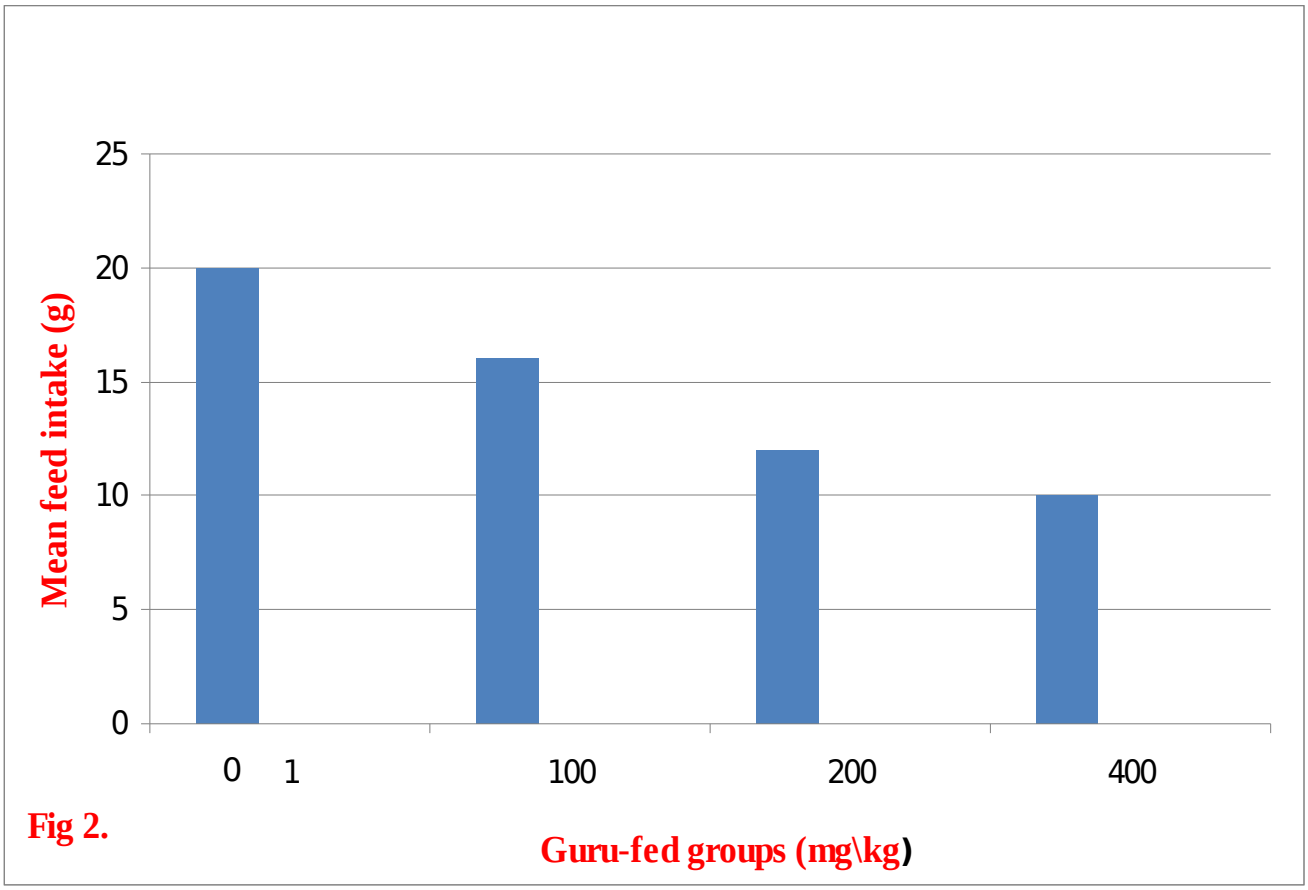


Fig 2. Daily food intake for goro- fed groups as compared to control group

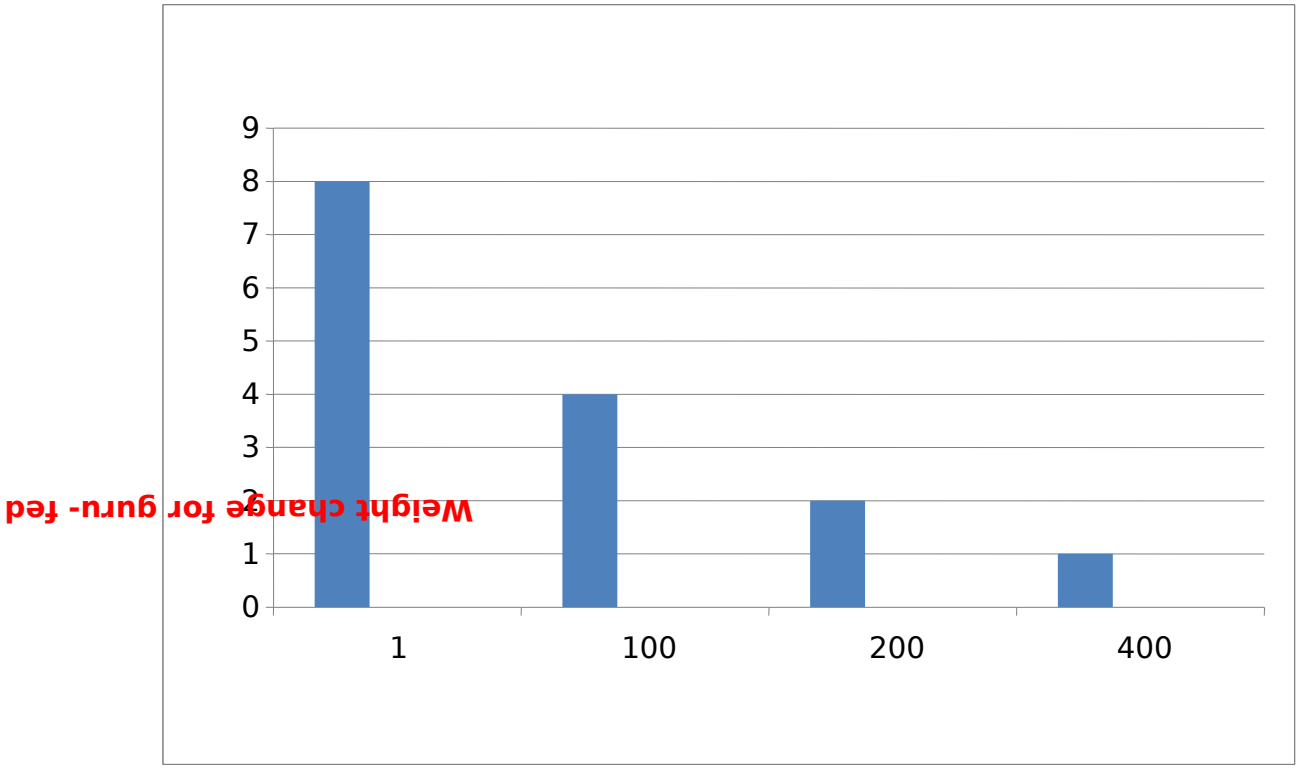


Fig 3. Mean body weight change for goro – fed groups as compared to control groups.

3.3 Effects of goro on hematology of rats:-

There was a non significant increase in total white cell count as shown in Table 1. The changes in the mean cell hemoglobin concentration and haematocrit were also not statistically significant ($P>0.05$). The results are as shown in Tables, 2, and 3 respectively. The post administration results of platelet count showed a significantly decreased value when compared with both the pre administration and the control result ($P<0.05$). the blood film for pre administration blood samples for test and controls shows normocytic/normochromic red blood cells, leucocytes and platelets appeared normal. The blood film for post administration blood samples for test shows normocytic/ normochromic red blood cells, platelets appeared inadequate.

Table 1.Mean total leukocyte count (x10^{o}/L) ± SD

Group	Pre-Administration	Post Administration	P-Values
1	12.3±0.30	12.0±0.41	P>0.05
2	10.9±0.25	9.5±0.93	P>0.05
3	11.0±0.30	10.4±0.61	P>0.05
4	12.7±0.30	10.0±0.58	P>0.05

Table 2. Mean haematocrit values (%) ± SD

Group	pre-administration	post administration	P-values
1	39±1.80	40±1.87	P<0.05
2	39±0.39	43±1.87	P>0.05
3	40±1.87	39±1.22	P>0.05
4	39.5±1.87	37±1.41	P>0.05

Table 3. Mean cell haemoglobin concentration (MCHC) values (%) ± SD

Group	Pre-Administration	Post Administration	P-Values
1	39±1.80	40±1.87	P<0.05
2	39±0.39	43±1.87	P>0.05
3	40±1.87	39±1.22	P>0.05
4	39.5±1.87	35±1.41	P>0.05

Table 4. Mean platelet count (x10^{o}/L) ± SD

Group	pre-administration	post administration	P-values
1	353±15.30	350±11.37	P>0.05
2	242±4.63	120±3.74	P<0.05
3	313±17.16	170±6.16	P<0.05
4	336±11.57	190±3.08	P<0.05

3.4 Effects of goro on biochemical variable in rats

The biochemical changes in serum of rats-fed goro are shown in Table 5. Goro at a dose of 100mg/kg has significantly increased serum protein in groups 2 animals increased serum protein in group 2 animals compared. However it has decreased (P<0.05) protein. When given at a dose of 200mg/kg and 400 mg/kg in groups 3 and 4 respectively. Creatine and urea concentrations and ALT and AST

activity were not affected in animals of group 2 (100mg/kg goro), but their concentration was significantly (P<0.05) increased in animals groups 3 and 4.

Table .5 Biochemical changes in serum of rats-fed goro

Parameter	Group1 (Control)	Group 2 (100mg/kg guru)	Group 3 (200mg/kg guru)	Group 4 (400mg/kg guru)
Total protein (g/dL)	7.15±0.13	8.22±0.14	6.21±0.11	6.06±0.11
Urea (mg/dL)	36.3±1.9	35.9±1.8	47.1±3.1	53.2±4.1
Creatinine (mg/dL)	0.69±0.05	0.70±0.05	1.1±0.12	1.6±0.14
AST (1U)	36.91±0.33	38.0±0.34	44.31±0.45	50.1±0.55
ALT (IU)	21.9±0.38	22.1±0.39	38.1±0.37	41.2±0.41

3.5 Effects of goro on locomotor behaviour

The frequency of line cross (Fig 4) in goro-fed rats of group 2 and 3 were 60 and 62 per 5 minutes session. This was significantly P<0.05 higher than the frequency in the control group. However the frequency of line cross in goro-fed rats of groups 4 (400mg/kg) was significantly (P<0.05) lower than the control group

The frequency of rearing (Fig 5) was significantly (P<0.05) higher in goro fed rats of group 2 (100mg/kg) and group 3 (200mg/kg) but lower (P<0.05) in group 4 (400mg/kg) as compared to controls. Likewise, the frequency of grooming (Fig 6) was significantly (P<0.05) higher in goro-fed rats of group 2 and 3 but lower (P<0.05) in group 4 as compared to controls.

The mean frequency of light and dark transition (Fig 7) was significantly (P<0.05) higher in goro-fed rats of group 2 (100mg/kg) but, lower (P<0.05) in group 3 (400mg/kg) as compared to controls.

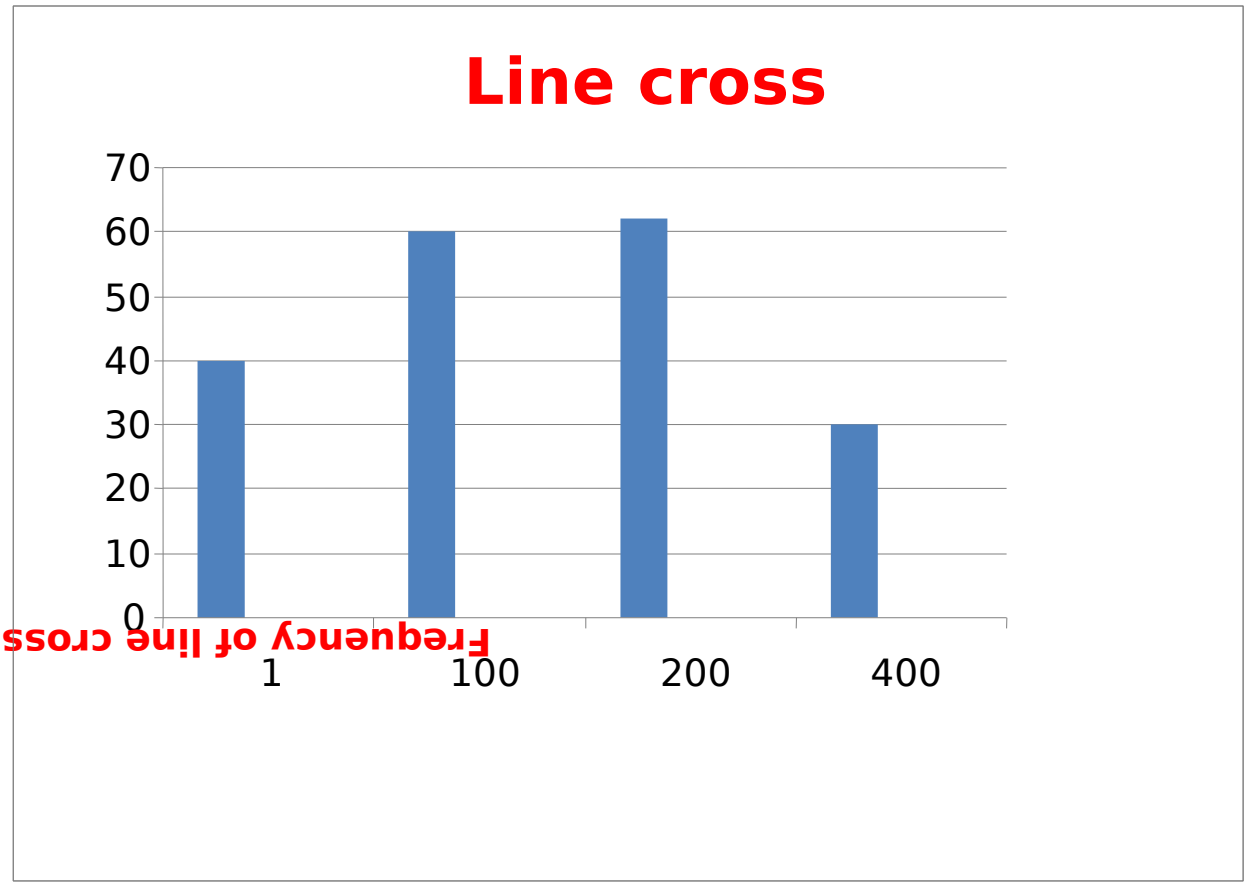


Fig 4. Mean frequency of Line cross in the open field apparatus for goro- fed rats as compared to controls.

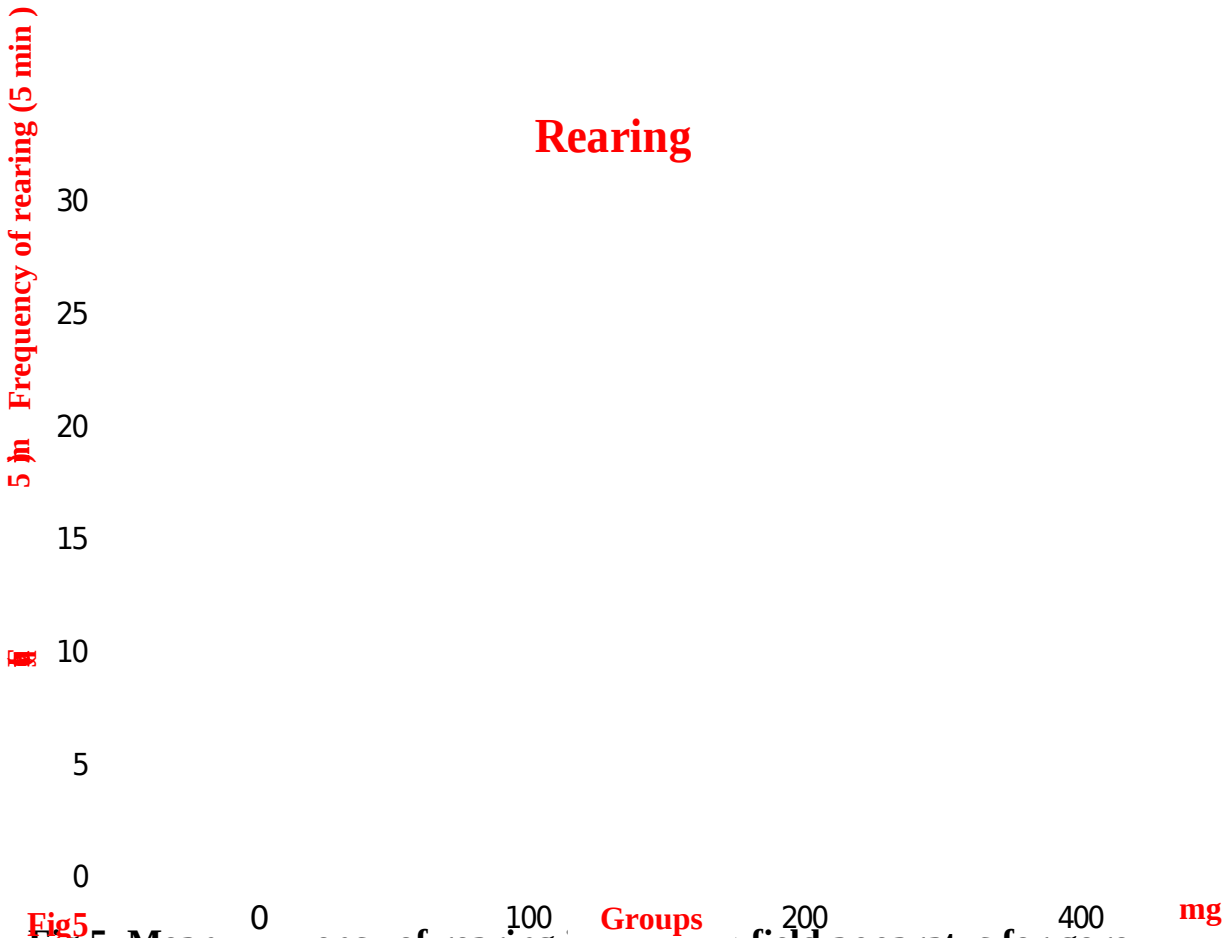


Fig5

Fig 5. Mean frequency of rearing in the open field apparatus for goro- ted rats as compared to controls.

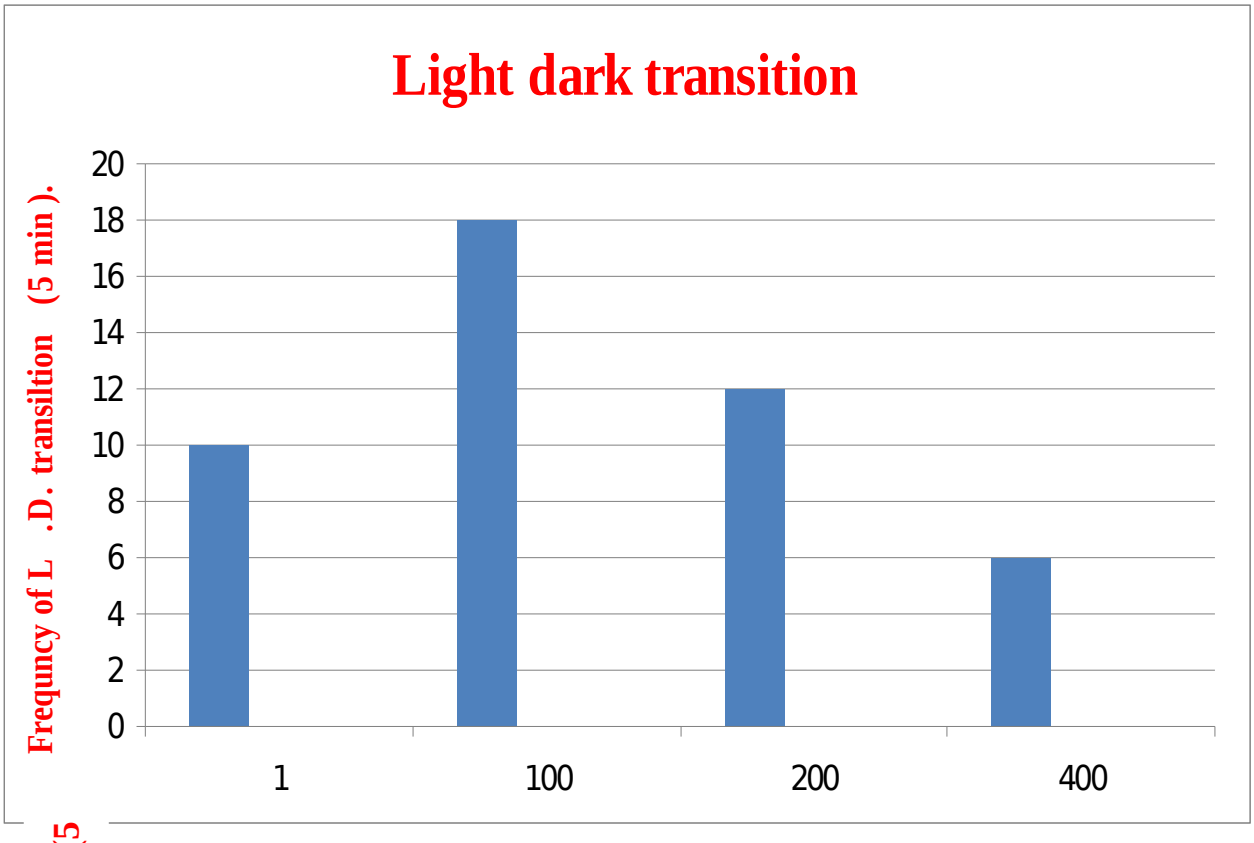


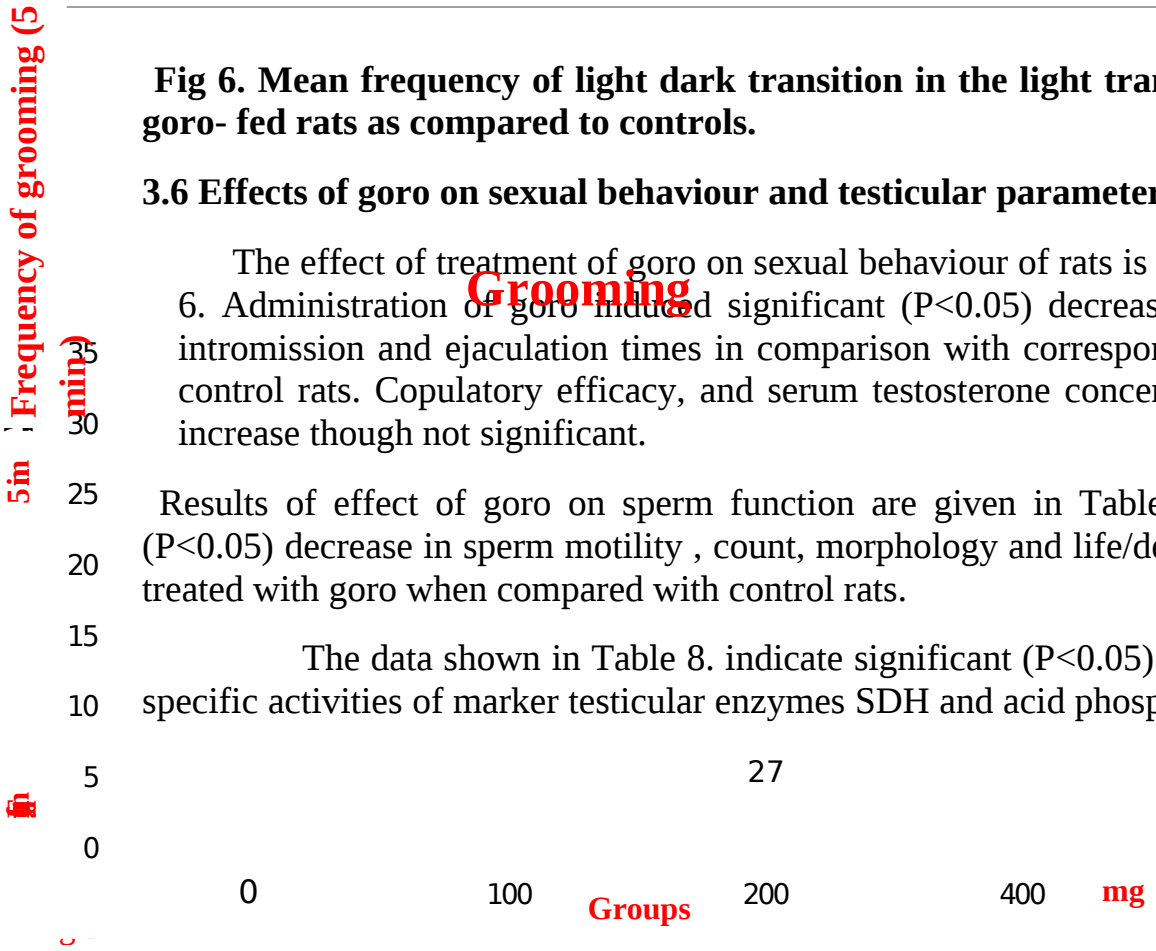
Fig 6. Mean frequency of light dark transition in the light transition box for goro- fed rats as compared to controls.

3.6 Effects of goro on sexual behaviour and testicular parameters.

The effect of treatment of goro on sexual behaviour of rats is shown in Table 6. Administration of goro induced significant (P<0.05) decrease in mounting, intromission and ejaculation times in comparison with corresponding values of control rats. Copulatory efficacy, and serum testosterone concentration tend to increase though not significant.

Results of effect of goro on sperm function are given in Table 7. Significant (P<0.05) decrease in sperm motility , count, morphology and life/dead ratio of rats treated with goro when compared with control rats.

The data shown in Table 8. indicate significant (P<0.05) decrease in the specific activities of marker testicular enzymes SDH and acid phosphatase, but the



activities of LDH, -GT and beta-glucuronidase were significantly (P<0.05) increased .

Fig 7. Mean frequency of grooming in the open field apparatus for goro- fed rats as compared to controls.

Table 6:- Effect of oral administration of goro at a dose of 200mg/kg body weight on the sexual behaviour of adult male rats.

Parameter	Saline treated	Guru treated
Mount Latency (Sec)	81.22±12.1	69.1±6.6*
Mount Frequency	5.72±2.1	3.4±1.1*
Intromission Latency (Sec)	282±22.5	202±16.1*
Intromission frequency	15.34±2.6	16.1±2.5
Ejaculatory Latency (Sec)	1036±96.3	902±89.2
Post ejaculatory interval Sec)	460±38.1	470±031.2
Copulatory efficacy	0.812±0.05	0.86±0.06
Inter-intromission interval (Sec)	72.3±12.9	78.3±11.1
Serum testosterone concentration (ng/ml)	3.1±0.33	3.2±0.36

* Significantly different from saline treated rats at P<0.05

Table 7:- Effect of goro on sperm function.

Parameter	Saline treated	Guru treated
Sperm Motility (%)	72.4±2.1	66.3±2.1
Sperm count per epididymis (millions)	25.2±4.1	18.1±1.9
Sperm morphology (%)	78.7±2.3	62.1±1.8
Life/ Dead (%)	96.1±1.63	78.1±2.2

* Significantly different from saline treated rats at P<0.05

Table 8:- Effect of treatment with goro on the marker testicular enzymes of rats treated for 28 days

Group	SDH?	Acid Phosphate?	LDH?	-GT?	-Glucuronidase?
Control	6.10±0.48	285.0±4.11	50.87±8.5	34.2±4.2	20.2±0.45
Guru treated (200mg/kg)	3.20±0.18*	200.0±6.25*	80.3±2.0*	81.3±3.0*	36.3±1.82*

a Values are means ± SD of six rats per group.

b nmol NADH oxidized min^{1} mg^{1} protein.

c nmol p-nitrophenol formed min^{1} mg^{1} protein.

d nmol p-nitrophenol liberated min^{1} mg^{1} protein.

e nmol phenolphthalein liberated min^{1} mg^{1} protein

* p<0.05.

Table 9:- The effect of 200mg single oral dose of goro given at 10.00 14.00h and 18.00h on the number of ova. (Group 1 rats)

Time of administration	Number of Ova
Control	Treated
10.00h 9.3±4.6	2.6±1.4*
14.00h 9.3±4.6	2.6±0.3*
18.00h 9.3±4.6	6.6±2.1*

* P<0.05

Table 10:- Effect of administration of 200mg/kg of goro on the normal phases of oestrous cycle in control and treated rats (Group 2 rats).

Oestrous Phase	Control (n=6)	Treated (n=6) 2 weeks (3 cycles)	Treated (n=6) 4 weeks (6 cycles)
Normal	99.2%	35.8%	90.9%
Irregular	00.8%	64.2%	09.1%
Proestrous	16.9%	12.9%	20.3%
Estrous	20.0%	11.5%	21.8%
Metestrous	36.9%	34.6%	33.9%
Diestrous	26.2%	41.0%	24.0%

Table 11:- The effects of goro on foetal parameters.

Foetal Parameters	Control (n=5)	Days 1-5 (n=5)	Days 7-9 (n=5)	Day 14-15 (n=5)
Foetal Number	6.60±0.55	6.30±0.57	*4.33±1.15	6.66±0.57
Foetal Weight	4.78±0.42g	*3.98±0.10g	*3.84±0.05g	4.00±0.44g
Foetal Morphology (Abnormal Limb)		2.00±0.00		

*= P<0.05

3.7 Effects of goro on female rat reproduction

Ovulation:- The number of ova in the oviduct of treated rats was significantly reduced after commencement of treatment (P<0.05) when compared with the control (Table 9).

3.7.1 Oestrus cycle:- The normal pattern of estrous cycle was significantly altered (99.2 to 35.8%) in the treated rats after two weeks (three cycles) of goroadministration but returned later to normal (90.9%) (Table 2). It was observed that cycles in the last four weeks were similar to control values (six cycles). The duration of dioestrus phase was increased from 26.29% to41.0% in the treated rats after two weeks while the duration of proestrous and oestrous phases were reduced from 16.9% and 20.0% to 11.5% respectively.

3.7.2 Gestational parameters and morphologic Defects:-

All dams on study survived to their scheduled termination day. There were no abortions, no early deliveries and no death of animal during the study. Data on rat foetal weight are presented in Table 11. The weights of foetuses produced by pregnant rats, which received goro were significantly reduced ($p<0.05$). Among the experimental groups 7% of the foetuses from pregnant rats fed on the first five days of gestation showed morphological anomalies (truncated limbs). Parameters for growth (crown-rump, placenta weight) were not effected. There were no resorption and no post-implantation sites.

3.8 Effects of goro on the activity of drug metabolizing Enzymes

Effect of goro on microsomal protein concentration and on the activity of drug metabolizing enzymes are presented in Table 12. Goro at a dose of 100mg/g significantly ($P<0.05$) increases protein concentration in whole homogenate, cytosolic and microsomal fractions in animals of group 2, but significantly $P<0.05$ decreased protein in animals of group 3 and 4. The activity of cytochrome P-450, aminopyrine-N-demethlase, aniline-4-hydroxylase, were significantly ($P<0.05$) increased in group 2, but decreased by goro in group 3 and 4. No effect was seen on the activity of UDP-glucuronyltransferase and glutathione-S-transferase.

Table 12:- Effect of goro Mean ± SD concentration of protein and values of activity of drug metabolizing enzymes in microsomal protein homogenate of liver of rats.

Protein (mg g^{-1})	Group1 (control)	Group 2 goro treated (100mg/kg)	Group 3 goro treated (200mg/kg)	Group 4 goro treated (400mg/kg)
Whole homogenate	190.31±20.45	215±21.1*	108±2.5*	96±2.46*
Cytosolic fraction	112.31±10.30	125±9.5*	86±3.0*	80±2.91*
Microsomal fraction	30.91±2.11	4.01±3.1*	22±1.61*	20±1.31*
Enzyme activity of microsomal protein (nmol g^{-1})				
Cytochrome PP-450	0.222±0.012	0.402±0.022*	0.131±0.012*	0.121±0.03*
Aminopyrine-N-demethrylase	11.70±1.31	16.3±1.6*	8.31±0.061*	7.11±0.063*
Aniline-4-Irydroxylase	0.281±0.02	0.402±0.03*	0.081±0.011*	0.061±0.012*
UDP-glucurony ltransferase	1.103±0.051	1.113±0.050	1.133±0.050	1.061±0.41
Glutathione-S-tranferase	172±12	170±13	168±13	173±12

*Significant ($P<0.05$) different from control group

Chapter four

Discussion

4.1 Discussion

Goro is the seed-pods of various evergreen trees that are native to Africa. In West Africa and Sudan, are popular matiscatory (Russel, 1955). Animals, too are sometime exposed to it due to its evergreen nature. They are important various social and religious customs and may also be used to counteract hunger and thirst. In Nigeria for instance the rate of consumption of goro especially by students is very high as a principal stimulant to keep awake and withstand fatigue (Purgesleve, 1977).

The results of the present study showed that chronic goro consumption administration of goro to rats produced no effect on haemotological parameters expect platelet count. Goro also decreased food intake, water intake and body weight when compared to the control group of rats. Goro and its active constituent, caffeine may possess appetite-suppressant effects and this effect produces weight loss in habitual users (Jessen et al 2003; 2005). So, the decrease in body weight following chronic consumption of goro may de due to the suppression of appetite in animals. These results however, do not support the reports of Lopez-Garcia et al. (2006) who found an association between weights regain and habitual coffee consumption.

The platelet count showed statistically significant decreased value in post administration rats when compared with the control. These reductions in platelets count could be due to the DNA strand breakages in these cells or could have been bone marrow suppression with selective megakaryocyte depression (Hoffbrand et al 2004).

In this study, goro at doses of 200 and 400mg/kg and not 100mg/kg body weight produced an increase in the activity of alanine and aspartate amino transferase (ALT and AST), resulting in hepatotxcity due to inflammation of the cytoplasm, and a resultant leakage of cytoplasmic enzymes into the blood stream. These enzymes acted to block the transcription and translation steps of the genetic code, resulting in decreased processes of protein biosynthesis. Therefore serum protein was decreased as a consequence of this insult.

ALT catalyze a reversible amino group transfer reaction in the Krebs (Tricarboxylic acid) cycle necessary for tissue energy production while AST catalyzes transfer of the nitrogenous portion of an amino acid of the amino acid residue. ALT is released from the hepatocellular cytoplasm into the blood stream when there is acute hepatocellular damage; AST is found in the cytoplasm and mitochondria of many cells such as liver, heart and is released into serum in

proportion to cellular congestion due to heart failure (Rodwell, 1966). The increase in serum urea and creatinine would indicate a nephrotoxic effect of goro.

In order to assess the comparative effects of chronic (28 days) consumption of goro administration on locomotor behaviour in rats, the open field apparatus and the light and dark transition box (LD) were employed. This method is in line with Brown et al (1999), Archer (1973), Rodgers (1997) and Streng (1974), who used the open field apparatus to assess the locomotory and exploratory behaviour of animals in a novel environment. The locomotor behaviours scored in this study included line crossing, rearing, grooming and frequency of light-Dark transitions in the L/D transition box. The frequency of line crossing and light/dark transition were significantly increased in 100 and 200mg/kg dose compared to controls. This typical stimulatory effect of caffeine has been reported elsewhere. Cola nut has been show to mediate some physiological effects such as CNS stimulation (Carrillo and Bennitez 2000) caffeine can improve memory consolidation when administered after training for a habituation task in rats, inhibitory avoidance in mice and rats also active avoidance in rats (Angelucci et al., 2002). On the other hand, some studies reveal that long-term consumption of caffeine could inhibit hippocampus-dependent learning and memory (Han et al., 2007). Indeed in this study goro at higher doses (400mg/kg) produced a decrease in frequency of line crossing, rearing, grooming and Light/dark transition.

The decrease in locomotor activity following chronic consumption of caffeine is in consonance with reports of Neil (1978) which showed that chronic consumption of caffeine caused mixed depressive states in psychiatric patients. In support, the work of Greden et al (1978) also reported depressive syndrome following chronic consumption of caffeine. The depressive state is likely to lead to a decrease in locomotor and exploratory behaviour as shown by the results of this study. Reduced locomotor behaviour was shown by reduced rearing and grooming frequencies following chronic goro consumption. Although caffeine is a stimulant, chronic consumption of caffeine leads to the development of tolerance (Griffiths et al., 1988). Since the amount of caffeine consumed was not increased to maintain its stimulant action, it is conceivable that the rats were depressed after chronic consumption of same amount of caffeine as shown by the decrease in locomotion. The results of this study are also in consonance with the report of Sudakov et al (2003) who reported a decrease in locomotor activity in the open-field test for fisher-344 rats following chronic caffeine consumption.

Goro has been used in folk medicine as an aphrodisiac. Therefore, in this study it is attempted to elucidate this point. Goro has significantly decreased mounting, intromission and ejaculation times in comparison to controls.

These parameters are considered to sexual motivation and libido (Beach, 1956). In addition, the Copulatory efficacy is also increased but not significant in these animals due to goro treatment. Copulatory efficacy represents the efficiency of erection and penile orientation and is considered an indication to sexual potency and performance (Agmo,1997). Furthermore, goro has produced decreased effect on sperm motility, count, morphology and life/death ratio. Similar effect of cola nitida extract has been produced in rats (Adisa et al 2010). In other studies it has been shown that Cola nitida extract did not show any significant effect on semen parameters (i.e, motility, morphology, sperm count and semen volume). This is in agreement with the findings of Lopez and Alvarino (2000), who reported that caffeine, a plant alkaloid, and the main chemical component of kola nut extract, has no effect on sperm motility, sperm morphology and sperm count.

These finding on semen quality agrees with the report by Nan et al. (1992), who reported that there is no association between sperm quality, smoking habits, coffee drinking, moderate alcohol intake, exposure to heat (Sauna, hot baths, type of underwear, sedentary activities) or physical activities in man. Thus, it could be said that goro has some, impact on semen quality and therefore may be associated with infertility problem in males. This assertion support the report that caffeine impaired semen quality (Marshburn et al., 1989) and increased motility (Barkay et al., 1977, Harrison 1978, Aitken et al., 1983, Hammit et al., 1989).

Administration of goro to rats for 28 days has produced biochemical changes in testes. Biochemical studies have associated the activities of some testicular enzymes with specific cell types of testes. The activities of SDH, LDH and acid phosphatase are reported to be associated with different stages of maturing germ cells. An increase in the activity of LDH and a decrease in the activities of SDH and acid phosphatase in goro treated animals suggests damage to germ cells. A significant increase in the activity of sertoli cell marker enzymes (ȣ-GT and beta glucuronidase) was also noticed. This particular pattern in the activity of SDH, acid phosphatase, LDH, ȣ-GT and beta-glucuronidase is characterstic of testicular atrophy associated with damage to germ cells and sertoli cells by many xenobiotics in the literature (Nebbia et al., 1987; Srivastava et al., 1992; Pant et al., 1995).

Aphrodesiac effect of goro was further explored in the female. Goro was shown to block ovulation, alter estrous cycle and produced gestational and morphologic problems in the female rat.

Epidemiological studies have been advocated as the best approach to exploring any link between caffeine and reproductive outcome (Olsen, 1991). Caffeine has been implicated as a risk factor for delayed conception (Wilcox et al., 1988; willams et al., 1990; Hatch and Bracken, Jensen et al., 1988).

Caffeine consumption has also been implicated as a risk factor for spontaneous abortions (Sirsuphan and Bracken, 1986; Fenster et al., 1991; Armstrong et al., 1992; Infante-Rivard et al., 1993). Caffeine has also known to decrese birth weight (Santos et al 1988) and increase the percentage of dead sperm (Marshburn et al 1989).

Feeding of goro at a dose of 100mg/kg bodyweight to rats increased protein concentration of liver homogenate and activity of phase-I metabolizing enzymes such as cytochrome p 450, aminopyrine-N-demethylase and aniline-4-hydroxylase. Similar effects have been produced by caffeine in rats (Mitoma et al 1969, Govindwar et al 1988), suggesting that goro at low doses may induce activation of microsomal mixed oxidaze system. However goro at doses of 200 and 400mg/kg has inhibitor effect on metabolizing enzymes suggesting that goro at these doses may produce toxic effect on the enzymes. Goro failed to produce any effect on phase-2 drug metabolizing enzymes represented by UDP-glucuronly transferase and glutathione. These enzymes were found to be resistant to hepatoxin induced liver injury (Gergus et al 1982; El-Sheikh et al 1991). This is probably due to deep location of these enzymes within endoplasmic reticulum close to inner surface of the membrane (Gergus et al 1982). Consumption of goro which is capable of modulating activity of drug metabolizing enzymes may result in unpredictable pharmcodynamic and toxicologic effects of drugs and xenobiotics co-administered with goro and therefore human and animals should not be allowed to take the plant and drugs con-comittantly.

4.2 Conclusion and recommendations

This Study concludes that:-

1. Goro at dose of 100mg/kg body weight produces pharmacological effects where as at 200mg/kg and higher produces toxicity
2. Goro produces decrease in water and food intake and body weight gain.
3. The plant increases locomotor activity when give at 100mg/kg body weight but decrease the activity at higher doses.
4. The plant at the dose of 200mg and higher produces hepatotoxic and nephrotoxic effects and inhibits durg metabolizing enzymes.
5. Goro is aphrodisiac in male but decreases testicular function and sperm quality. In the female the plant inhibits ovulation, alter estrous cycle and may produce teratogenic effect.

It is recommended that:-

1) In the light of some toxicities of goro the understanding of the traditional medical use of goro should be reconsidered.
2) Due to effect of goro on drug metabolizing enzymes, goro is not adviced to be co-administered with other drugs.
3) Further research should focus on toxicity of goro in humans and animals and isolation of pharmacological and toxicological active moiety.

4.3 REFERENCES

1. Agmo, A.,(1997). Male rat sexual behaviour. Brain Res. Protocols, 1:203-209.

2. Aitken, R.J., Best F., Richardson D.W , Schats.R and.Simm.G (1983). Influence of caffeince on movement characterstics, fertilizing capacity and ability to penetrate cervical mucus of humans spermatozoa. J. Reprod. Fertil., 67, 19-27.

3. Amori N and Ashira H (1994) Biosynthesis of theobromine and caffeine in developing leaves of coffee Arabica. Phytochemistry 36, 1359-1361.

4. Angelucci, M.E., C. Cesario, R.H. Hiroi, P.L. Rosalen and C.Da Cunha, (2002). Effects of caffeine on learning and memory in rats tested in the morris water maze. Braz. J. Med. Biol. Res., 35, 1201-1208.

5. Archer, J. (1974). Test for emotionality in rats and mice: A Review: Animal Behaviour, 21, 205-235.

6. Armstrong LE, Casa DJ, Maresh CM, Ganio MS (2007). “Caffeine, fluid-electrolyte balance, temperature regulations, and excersie-heat tolerance”. Exerc Sport Sci Rev 35 ,135-40.

7. Armstrong LE, Pumerantz AC, Roti MW, Judelson DA, Watson G, Dias JC, Sokmen B, Casa DJ, Maresh CM, Lieberman H, Kellogg M (2005). “ Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption”. Int J Sport Nutr Exerc Metab 15, 252-65.

8. Armstrong, B.G., A.D. McDonald and M.Sloan, (1992). Cigratte, Alcohol, and coffee consumption and spontaneous abortion. Am. J. Publ. Health. 82,85,87.

9. Arnaud M j (1987). The pharmacology of caffeine. Prof Drug Res. 31, 273-313.

10. Arnaud, MJ (2011). Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handbook of experimental pharmacology. Handbook of experimental pharmacology 200 ,33-91.

11. Astorino TA, Roberson DW (2010). Efficacy of acute caffeine ingestion for short-term high-intensity excersie performance: a systematic review” . J Strength Cond Res 24 : 257-65.

12. Bahtnager, R. and Misra, U.K. (1988). Mixed function oxidases in response to quality and quantity of dietary protein. Biochemistry International 17, 1-8.

13. Bakhiet Amel.0. and Adam S.E.I. (1995) Therapeutic utility, constituents and toxicity of some medicinal plants: A review. Vet. Human toxical. 37, 255-258.

14. Barkay, J., H, Zuckerman, D. Sklan and S.Gordon, (1997). Effect of caffeine on increasing the motility of frozen human sperm. Fertil. Steril., 28:175-177.

15. Beach., F.A., (1956). Characterstics of Masculine, Sex Drive. In: Nebraska Symposium of motivation, Jones, M.R. (Ed) Press Lincoln, University of Nerbraska, pp: 1-31.

16. Bertolini, A., poggioli, R. Bernardi, M. Genedani, S., and Ferraro, W. (1980). Pharmacological interferences on the protein synthesis during the total and neonatal period in rats: behavioral outcome in the adult hood. Pharmacological resource communication 12(3), 227-232.

17. Bishop D (2010). Dietary supplements and team-sport performance. Sports Med 40 : 995-1017.

18. Bolumar, F., J. Olsem, M, Rebagliato and L. Bisanti, (1997). Caffeine intake and delayed conception: a europeon multicenter study on infertility and subfecundity. Am. J. Epidemiol., 145: 324-334.

19. Brown, R.E, Corey, S.C. and Moore A.K. (1999). Difference in measures of exploration and fear in MHC-Cogenic C57BL6J and B6H-2K mice. Behaviour and Genetics, 29, 263-271.

20. Carrillo, J.A. and Bennitez, J. (2000). Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clin pharmacokinet., 39 ,127-153.

21. Chukwu, L.U., W.O. Odiete and L.S. Briggs, (2006). Basal metabolic responses and rhythmic activity of mammalian hearts to aqueous kola nut extracts. Afr. J. biotechnol., 5(5): 484-486.

22. Cucinnell, S.A. Conney, A.H. and Burns, J.J (1965). Application of drug metabolism to durg toxicity studies. Chemical pharmacology theory 6, 420-422.

23. Danbolt, N. C. (2001). Glutamate uptake. Journal of Neurobiology, 65, 100-105.

24. Davis, J.M., Z. Zhao, H.S. Stock, K.A. Mehl, J. Buggy and G.A. Hand (2003). Central nervous system effects of caffeine and adenosine on fatigue. Am. J. Physiol. Regul. Integr. Comp. Physiol., 284, R399-404.

25. Dutton G.J. and Storey, I.D.E (1962) Glucuronide forming enzymes. In: Method in enzymology, colowick SP and Kaplan NO (Eds). Academic press, New York. Pp: 159-164.

26. Effect of aqueous kola nut (cola nitida rubra) extract on sperm quality in male-spraque-dawley rats. Asian . J. Med. Sciz, 77-80.

27. Elinav, E., G. Pinsker, R. Safadi, O. Pappo and M. Bromberg et al., (2007). Association between consumption of herbalife nutritional supplements and actue hepatoxicity. J. Hepatol., 47: 514-520.

28. El-Sheikh H.A., Ali, B.H. Homeida, A.M. Lufti A.A.A and Hapke, H.J. (1992). The effects of fascioliasis on the activities of some drug metaboilizing enzymes in desert sheep liver. British Vet. J., 148, 249-257.

29. El-Sheikh H.A., B.H. Ali, A.M. Homeida, T. Hassan, O.F. Idris and H.J. Hapke, (1991) the activities of drug metabolizing enzymes in goats treated orally with latex of calatropis procera and the influence of dieldrin pretreatment. J. Comperative Pathol., 104, 257-268.

30. Esimone, C.O., M.U. Adikwu, C.S. Nworu, F.B.C. Okoye and D.C. Odimegwu (2007). Adaptogenic potentials of camellia sinesis leaves, Garcinia kola and kola nitida seeds. Sci.res. Essay., 2: 232-237.

31. Eteng, M.U., Eyong, E.U., Akpanyong, E. O. Againg, M.A. and Aremu, C.Y. (1997). Recent advances in caffeine and Theobromine Toxicities: A Review. Plant food for human nutrition, 51, 231-243.

32. Fredholm, B.B., K. Lindstorm and A. Wallman-Johansson, (1994). Propentofylline and other adenosine transport inhibitors increase the efflux of adenosine following electrical or metabolic stimulation of rat hippocampal slices. J. Neurochem., 62, 563-73.

33. Funk GD (2009). Losing sleep over the caffeination of prematurity". J. Physiol. (Lond) 587 ,5299-300.

34. Cheeke 1998 , Toxins in grasses, herbs shrubs and trees throughout the world

35. D'NeLLo 1997 , classes of toxins-alkaloids, terpenoids, tannins, cyanogenic glycosides.

36. Ekam, 2001; Obochi, 2006, Caffeine acts to increases alertness, anxiety and hallucination due to it its blocking of adenosine receptors which normally inhibits glutamate release

37. Fredholm et al 1999, caffeine intake appear to have only minor negative consequence on health.

38. Garrett, B.E. and R.R. Griffiths, (1997). The role of dopamine in the behavioral effects of caffeine in animals and humans. Pharmaco. Biochem. Behave., 57, 533-541.

39. Gawin F. H and Ellinwood E.H (1988) Cocaine and other stimulants. N.Engl.J.Med. 318, 1173-1182.

40. Govindwar S.P, Kachole M.S and Pawar. S.S (1988). Effect of caffeine on the hepatic microsomal mixed function oxidase system during Phenobarbital and benzo [a] pyrene treatment in rats. Toxicology letters 42, 109-115.

41. Greden, J.F., Fountaine, P., Lubestky, M. and chamberlin, K. (1978). Anxiety and depression associated with caffeinsim among psychiatric inpatients. Am. J. Psychiatry, 135, 963-966.

42. Gregus, Z., Wathins, J.B. Thompson T.N. and Klaassen, C.D. (1982) Resistance of some phase II biotransformation pathways to hepatoxins . J. Pharmacology and experimental Therapeutics, 222, 471-479.

43. Griffiths, R.R, and Woodson, P.P. (1988). Caffeine physical dependence: a review of human and laboratory animal studies. Psychopharmacol. 94, 437-451.

44. Habig W.H., Pabst M.J and Jakoby, W.B (1974) Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249, 7130-7139.

45. Hackett PH (2010). Caffeine at high altitude: java at base CAMP. High Alt. Med. Biol. 11 : 13-7.

46. Hammitt, D.G., E. Bedja, P.R. Rogers, C.H. Syrop J.F. Donovan and R.A. Williamson, (1989) Comparison of motility stimulants for cryopreserved human semen. Fertil. Steril., 52,495-502.

47. Han, ME., K.H. Park, S.Y. Baek, B.S. Kim, J.B. Kim, H.J. Kim et al., (2007). Inhibitory effects of caffeine on hippocampal neruogenesis and function. Biochem. Biophys. Res. Commun, 356, 976-980.

48. Harrison, R.F., (1978) insemination of husbands semen with and without the addition of caffeine. Fertile. Steril., 291, 532-534.

49. Hatch, E.E and M.B. Barcken, (1993). Association of delayed conception with caffeine consumption. Am. J. Epidemiol., 138, 1082-1092.

50. Haustein, A., (1971). Cola nut: Custom and rituals of some of ethais rating Ivore. Anthropos, 69: 457-493 (in French).

51. Hoffbrand et al 2004, The reductions in platelets count could be due to the DNA strand breakages in these cells or could have been bone marrow suppression with selective megakaryocyte depression.

52. Howell et al., 1997, higher doses of caffeine can suppress behavioral activity and even performances associated with learning and memory.

53. Ibu, J.O., A.C. Lyam, C.T. ljije, D.A. Ishmeal and S.N. Ibeshi, (1986). Effect of colacuminata and cola nitida on gastric acid secretion. J. Gastroenterol., 129:39-45.

54. Infante-Rivard, C., A. Fernandez, R. Gauthier, M. David and G.E. Rivard, (1993). Fetal loss associated with caffeine intake before and during pregnancy. J. Am. Med. Assoc., 270,2904-2943.

55. Jensen, T.K., T.B. Henriksen, N.H. Hjollen, T. Scheike, H. Kolstad, A. Giwercman E. Ernst, J.P. Bonde, N.E. Skakkbaek and J.Olsen, (1998). Caffeine intake and fecundability: a follow-up study among 430, Danish couples planning their first pregnancy. Reprod. Toxicol., 12,289-295.

56. Jessen, A, Buemann B., Toubro S., Skovgaard, I.M. and Astrup, A. (2005). The appetite-supperssant effect of nicotine is enhanced by caffeine. Diabetes Obes. Metab. 7, 327-333.

57. Jessen, A.B., Toubro S. and Astrup A. (2003). Effect of chewing gum containing nicotine and caffeine on energy expenditure and substrate utilization in men.Am.J.Clin. 77, 1442-1447.

58. Jinka TR, Toien O, Drew KL (2011). " Season Primes the brain in an arctic hibernator to facilitate entrance into torpor mediated by adenosine A(I) receptors". J. Neurosci. 31, 10752-8.

59. Leon, L. and Mitoma C., (1970). Effect of caffeine on hepatic microsomal cytochrome P450. Biochemical pharmacology 19, 2317-2321.

60. Lopez and Alvero, (2000). Assessment of motility, acrosomal integrity and viability of giant panda (Ailuropoda melanoleuca) sperm following short term storagpe at 4°C. Zoo Biol., 22, 529-544.

61. Lopez-Garcia, E. Van Dam, R.M., Rajpathak, S., Willett, W.C., Manson, J.E. and Hu, F.B. (2006). Changes in caffeine intake and long-term weight change in men and women. Am. J. Clin. Nutr 83, 674-680.

62. Lowry, O.H., Rosenburg N.J. and Farr, A.L. (1951) protein measurement with the folin regent.J.Biol. Chem, 193, 265-275.

63. Marshburn, P.B., C.S. Sloan and M.G.H. Hammond, (1989). Semen quality and association with coffee drinking, cigratte smoking, and ethanol consumption. Fertile. Steril., 52,162-165.

64. Maughan RJ, Griffin J (2003). Caffeine ingestion and fluid balance: a review". J Hum Nutr Diet 16, 411-20.

65. Mazel, P., (1976) Experiments illustrating drug metabolism in vitro. In: fundamental of drug metabolism and drug disposition, La Cu BN, Mandel HG and way El (Eds). Williams and Wilkings, Baltimore, pp: 546-582.

66. Mitoma C., Lombrozo., La Valley S.E. and Dehn F. (1969). Nature of the effect of caffeine on the drug-metabolizing enzymes. Archives of biochemistry and biophysics, 134, 434-441.

67. Nan, B., H.R. Oldereid and P. Keneth, (1992). Life style of men in barren couples and their relationship to sperm quality. Eur. J. Obstet. Gyn. 43, 51-57.

68. Nebbia C., Brando C., Burdino E., Rasero R., Valenza F., Arisio R. and Ugazio G., (1987). Effects of the chronic administration of sodium selenite on rats tests. Res. Commun, Chem, Pathol, Pharmacol. 58: 183-197.

69. Nehlig A. Daval J.L Derby G. (1992) Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects. Brain Res. Dev. 17, 139-170.

70. Neil, J.F. (1978): caffeinism complicating hypersonic depressive episodes. Comp pshychiarty. 19, 337.

71. Newall, C.A., L.A. Anderson and J.D. Philipson, (1996). Herbal medicine: A Guide for health care professionals London, the pharmarceutical press, pp: 199-200.

72. Newton R, Broughton LJ, Lind MJ, Morrison PJ, Rogers HJ, Bradbrook ID (1981). Plasma and salivary pharmacokinetics of caffeine in man . Eur. J. Clin. Pharmacol. 21, 45-52.

73. Obochi, G. O. (2006). Effect of alcohol-kolanut interaction on biochemical indices of neuronal function and gene expression in wistar albino rats. A PhD Thesis submitted to the Graduate School, University of Calabar, Nigeria.

74. Olsen, J., (1991) Cigarette smoking, tea and coffee drinking, and subfecundity. Am. J. Epidemiol., 133: 734-739.

75. Omura T. and Sato, R. (1964) The carbon monoxide binding pigment of liver microsomes 1. Evidence for its hemoprotein nature. J. Biol. Chem., 239, 2370-2378.

76. Pant N., Prasad A.K., Srivastava S.C., Shanker R., Srivastqava S.P. (1995). Effects of oral administration of carbofuran on reproductive system of rats. Hum. Exp. Toxicol. 14:889-894.

77. Peters, Joseph M. (1967). Factors affecting caffeine toxicity: a review of the literature". The journal of clinical pharmacology and the journal of new drugs 7, 131-141.

78. Purgesleve, J.W., (1977) Tropical crops dicotyledons. Longmans London, pp: 564-612.

79. Rainyake W.M (2009)Contents of Cola

80. Rainyake W.M, Pelleties G. Hollywood R.Malcolm S. and Starvic B (1995) investigation of the effect of coffee on serum lipids and cholesterol in hamsters. Food chem. Toxicol. 33, 195-201.

81. Rodgers., R.J . (1997). Animal models of "anxiety" where next? Pharmacol. Biochem. Behave.: 8:477-496.

82. Rodwell, V.W. (1996). Enzymes: regulations of activities, in: Harpers Biochemistry (24th ed), Pp.98-108. Appleton & Lange, USA.

83. Russel, T.A., (1955). The kola nut of west Africa. World crop., 7:221-225.

84. Santon, C.K. and R.H. Gray, (1995). Effects of caffeine consumption on delayed conception. Am. J. Epidemiol., 142: 1322-1329.

85. Santos, I.S., C.G. Victora, S. Huttly and S.Morris, 1998a. caffeine intake and pregnancy outcomes: a meta-analytic review. Cadrenos de sude publica, 14: 523-530.

86. Schoepfer, A.M., A. Engel, K. Fattinger, U.A., Marbet and D. Criblez et al., (2007). Herbal does not mean innocuous: Ten cases of severe hepatotoxicity associated with dietary supplements from herbalife products. J. Hepatol., 47: 521-526.

87. Shaffiner, F. and Popper, H. (1969). The problem of hepatotoxicity. Procedure of Europeon society. Study of drug toxicity 7, 9-21.

88. Srisuphan, W. and M.B Bracken, (1986). Caffeine consumption during pregnancy and association with late spontaneous abortion. Am. J.Obstet. Gynecol. 154, 14-20

89. Stickel, F., (2007). Slimming at all costs: Herbalife-induced liver injury. J. Hepatol., 47: 444-446.

90. Streng, J. (1974). Exploration and learning behaviours in mice selectively breed from high and low levels of activity. Behave. Genetics 4, 191-204.

91. Sudakov, S.J., Rusakova, I.V. and Medvedeva, O.F. (2003). Effect of chronic caffeine consumption on changes in locomotor activity of WAG/G and fischer-344 rats induced by nicotine, ethanol, and morphine. Bull . exper. Boil. Med. 136, 563-565.

92. The Psychoactive encyclopedia (2008): the history usage, botanical and chemical aspects of psychoactive susbtanc, fungi and plants.

93. Trindall, R. (1997). Ethno botanical leaflets: the culture of cola: social and economic aspects of a west African domesticate carboondale: southern Illinois University Herbarium.

94. Verbeeck RK (2008). Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. Eur. J . Clin. Pharmacol. 64, 1147-61.

95. W. A. Adisa, B.O. Iranloye, C.C. Ezekunie, A.O. Nwaopara, C.U. Osifoh, E.N.S. Igbinovia and H.O. Otamere (2010).

96. Walsh, R.N. and Cummins, R.A. (1976). The open-field test: a critical review. Psycho. Bull. 83: 482-504.

97. Wilcox. A., C Weinberg and D. Baird (1988) caffeinated beverages and decreased fertility. Lancet, 2, 1453-1456.

98. Williams, M.A., R.R. Monson, M.B. Goldman, R. Mittendorf and K.J. Ryan, (1990). Coffee and delayed conception. Lancet, 335 , 1603.

99. Zhang, Z; Hu, G, Caballero, B, Appel, L, Chen, L (2011). Habitual coffee consumption and risk of hypertension: a systematic review and meta-analysis of prospective observational studies. The American Journal of clinical nutrition 93 : 121-9.