JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Shittu OK, Lawal B, Abubakar NA, Berinyuy BE, Busari MB, Ibrahim AO. **Toxicological Implications of Methanol Extract from Nigerian Bee *Propolis* On Some Selected Rat Tissues.** *J Pharm Biomed Sci* 2015; 05(07):524-531.

Journal of Pharmaceutical and Biomedical Sciences (***J Pharm Biomed Sci.***), **Member journal. Committee on Publication ethics (COPE) and Journal donation project (JDP).**

PBMS
Journal of Pharmaceutical and Biomedical Sciences

ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

Original article

Toxicological Implications of Methanol Extract From Nigerian Bee Propolis On Some Selected Rat Tissues

Oluwatosin K. Shittu[1], Bashir Lawal[1,*], Asmau N. Abubakar[1], Eustace B. Berinyuy[2], ,Busari B. Musa[3], Ibrahim A Olalekan[4].

Affiliation:
[1]Tropical Disease Research Unit, Department of Biochemistry, Federal University of Technology, P.M.B.65, Minna, Nigeria
[2]Faculty of medicine and Biomedical Science, University of Yaounde 1, Cameroon
[3]Centre for Genetic Engineering and Biotechnology, Global Institute for Bioexploration Unit, Federal University of Technology Minna, Nigeria.
[4]Forestry Technology Department Federal College of Wild Life Management, New Bussa, Niger State, Nigeria

The name of the department(s) and institution(s) to which the work should be attributed:
1.Tropical Disease Research Unit, Department of Biochemistry, Federal University of Technology, P.M.B.65, Minna, Nigeria
2.Faculty of medicine and Biomedical Science, University of Yaounde 1, Cameroon
3. Centre for Genetic Engineering and Biotechnology, Global Institute for Bioexploration Unit, Federal University of Technology Minna, Nigeria.
4. Forestry Technology Department Federal College of Wild Life Management, New Bussa, Niger State, Nigeria

Address reprint requests to
*** Bashir Lawal.**
Department of Biochemistry, Tropical Disease Research Unit, Federal University of Technology, P.M.B. 65, Minna, Nigeria or at bashirlawal12@gmail.com

Article citation: Shittu OK, Lawal B, Abubakar AN, Berinyuy BE, Busari MB, Ibrahim AO. **Toxicological implications of methanol extract from Nigerian Bee propolis on some selected rat tissues**. *J Pharm Biomed Sci.* 2015; 05(07):524-531. Available at www.jpbms.info

ABSTRACT: **Objectives:** The present study investigates the effects of Nigerian bee propolis on some biochemical indices in some selected rat tissues.
Methods: A total of Fifteen wistar rats were grouped into 3(A-C) of 5 rats each. Group A rats serve as control groups and received 0.5ml of DMSO, while groups B and C received 300 and 600 ml/kg of methanol extract of honey bee propolis respectively, for 21 days through oral route.
Results: The extract significantly increased ($p<0.05$) the Spleen and small intestine ALT activities, kidney and small intestine AST activities, kidney ALP activities as well as the level of total protein in Liver and spleen of bee propolis treated rats. In contrast, the Liver and spleen AST activities, and liver ALP activities decrease significantly ($p<0.05$) in bee propolis treated rats when compared with their corresponding controls. However, the spleen and small intestine ALP activities, level of kidney and small intestine total protein as well as ALT activities in the kidney of bee propolis treated rats compared well ($p>0.05$) with the normal value at the extract doses studied. The computed liver, kidney, and small intestine body weight ratios of rats treated with the extract does not differ($p>0.05$) with those of the control rats, however, increase in spleen/body weight in propolis treated rats than the control group was observe.
Conclusion: The chronic administration of methanol extracts of bee propolis altered the normal values of some biochemical parameters in rat organs. Clinical application of the bee propolis should therefore be carefully controlled as it chronic administration may be relatively unsafe for the integrity of organs.

KEYWORDS: Propolis; Liver; Kidney; Spleen; Small intestine; Biomarker enzyme; Total proteins.

INTRODUCTION

Nature has presented to humanity the gift of biological and cultural diversity of natural product for healing practices[1]. According to the world health organization, about 80% of the populations in many third world countries still use traditional materia medica (medicinal plants and other materials) for their primary health care needs[2]. This is as a result of the high cost of

PBMS
ournal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

Western pharmaceuticals and health care, or because the traditional medicines are more acceptable from a cultural and spiritual perspective[1]. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from natural origin. Natural products also benefit from containing many specific molecular principles in their natural state, which possess a variety of influences on human physiological and biochemical systems, as opposed to purified synthetic drugs which are based on a single molecular substance derived from the natural product[3].

However administration of herbal/natural products for therapeutic purpose without dose specification and proper scientific validation of its safety has raised concern on their toxicity[4]. In the study of toxicity of plants materials, animals are majorly used to investigate the potential risk it may pose to human's health due to unpleasant properties of some chemical constituents of the plants[5]. These effects may alter the concentration and activities of biomolecules such as enzymes and metabolite, adequate functioning and normal organs histomorphology[6].

One of the natural products commonly used in traditional medicine without proper consideration of its safety is bee propolis. Propolis (bee glue) is the generic name of the resinous product which is collected by bees from various plant sources[7]. The composition of propolis varies with the source; generally, it is composed by 50% resin and vegetable balsam, 30% wax, 10% essential oils and aromatics, 5% pollen, and 5% other substances[8]. In the last 50 years, numerous studies have revealed versatile biological activities of propolis: antibacterial, antifungal, antiviral, cytotoxic, antioxidant, anti-inflammatory, immunomodulatory among others[9]. It is also used as the active substance of some medicinal products for external use in the treatment of wounds, burns and frostbite, but also as an ingredient in dietary supplements and cosmetics[10]. With all the uses and claims of efficacy of propolis derivatives in treatment of the numerous ailments, it is necessary to study the effects on vital organs, like spleen, small intestine, liver and kidneys which are the most commonly affected organs following ingestion of xenobiotics. Chemical studies on the essential oils and volatiles of propolis also revealed that propolis composition is complex and very much variable in different regions[11]. The numerous and widespread availability of organic compounds in bee propolis would present different chemical properties and influence their biological and toxicological effects.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

A total of fifteen (15) adult Wister rats, 170.00±5.70 mean weight were bought from the Small Animal Holding Unit of the Department of Biochemistry, Federal University of Technology Minna. The rats were kept in clean plastic cages and maintained under standard laboratory conditions (temperature: 22±3ºC; 12h natural light and 12h dark; humidity: 40-45%).The animals were maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review[12], were duly observed.

SOURCE OF PROPOLIS

Propolis material was collected from an apiary in Akure, Ondo State, Nigeria. The identity of the Propolis was authenticated by an Entomologist in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria, where a voucher specimen was deposited. The Propolis material was chopped in to small pieces and air dried in the Shade at room temperature for two weeks.

PREPARATION OF PROPOLIS EXTRACT:

Preparation of the extract of Propolis material followed standard procedures[13]. Two hundrad grams of Propolis pellets were percolated in 1600 mL of absolute methanol and subsequently allowed to stand in the shade for 48h before filtration, using Whatman No. 1 filter paper. The extract concentrate obtained was stored in air-tight vials in the refrigerator at 4°C, until needed for bio-assay.

ANIMAL GROUPING AND EXTRACT ADMINISTRATION

The animals were grouped into three of five (5) rats each. Group A (control) were given oral 0.5 ml of DMSO (Vehicle for extracts administration) for 21 days while Groups B and C were treated like the control except that they

PBMS
Journal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

received 300 and 600 mg/kg body weight of the propolis extract. The extract and DMSO were administered daily between 1000 – 1030 h using metal oropharyngeal cannula.

PREPARATION OF TISSUE HOMOGENATES

Collection and Preparation of of tissue homogenates was as previously described[14]. The animals were anaesthetized in ether vapour. After loosing their consciousness, the jugular veins were revealed by clearing the fur and skin area of the neck. The veins were sharply cut with sterile blade and the bloods were collected. The rats were dissected and the organs (spleen, liver, small intestines and kidney) were removed, cleaned, weighed and then stored in ice-cold 0.25 M sucrose solution. Homogenization of the organs was done in 0.25 M sucrose solution at ratio 1:4 w/v using ice-cold ceramic mortar and pestle. The homogenates were centrifuged at 1398 x g for 20 min and the resulting supernatant stored frozen before taken for analysis.

DETERMINATION OF BIOCHEMICAL PARAMETERS

The biochemical analyses were determined for alkaline phosphatase (ALP) based on methods of Tietz[15], Aspartate transaminase (AST) and alanine transaminase (ALT) as described by Reitman and Frankel[16]. The serum total protein concentration was estimated by biuret method as described by Gornall et al.[17].

DETERMINATION OF RELATIVE ORGAN WEIGHT

The body weights and organs weight of the rats were determined on day 22 of the experiment and relative organ weights were computed by expressing the absolute weight of the organs to the animal's body weight as described below[18].

Relative organ weight= organ weight (g)/body weight (g) x 100

STATISTICAL ANALYSIS

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SEM [19]. Comparisons between different groups were done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $p<0.05$ were considered as statistically significant.

RESULTS

BIOMARKER ENZYME

The extract significantly increased ($p<0.05$) the activities of ALT in the Spleen and small intestine. The activity of the enzyme in the kidney compared ($p>0.05$) well with the normal value at the extract doses studied. In contrast, the activity of ALT in the Liver of the animals treated with 300mg/kg was significantly ($p<0.05$) lowered than the control value, however those treated with 600mg/kg compared well ($p>0.05$) with their controls (Fig. 1).

The extract produce dose dependent and significant increased ($p<0.05$) in the activities of AST in the Kidney and small intestine. In contrast, the activity of AST decrease significantly ($p<0.05$) in the Liver and spleen of the animals treated at all the doses investigated when compare with the control value (Fig. 2).

The extract significantly increased ($p<0.05$) the ALP activities in the kidney but decrease the activities significantly ($p<0.05$) in the liver when compare with their control value, however those treated with 600mg/kg compared well ($p>0.05$) with their controls. The extract however, did not significantly ($p>0.05$) alter the spleen and Small intestine ALP activities (Fig. 3)

TOTAL PROTEINS

The extract did not significantly ($p>0.05$) alter the level of total protein in the kidney and small intestine of the animals at all dose investigated. While the level of total protein in liver and spleen was significantly ($p<0.05$) raised in a dose dependent fashion than the control value (Fig. 4).

RELATIVE ORGANS WEIGHT

The computed liver, kidney, and small intestine body weight ratios of the rats were not significantly ($p>0.05$) different from those of their control at the two doses of the extract investigated. However the computed spleen body weight ratio significantly ($p<0.05$) increase in the extract treated group than the control group (Table 1)

PBMS
ournal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

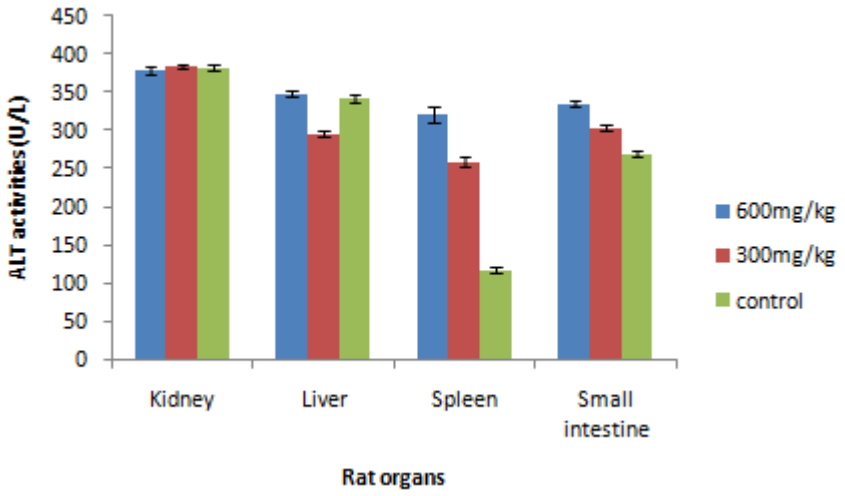

Figure 1. Alanine transaminase activities in rats organs following 21 days administration of bee propolis. Values are mean ± SEM of 5 determinations.

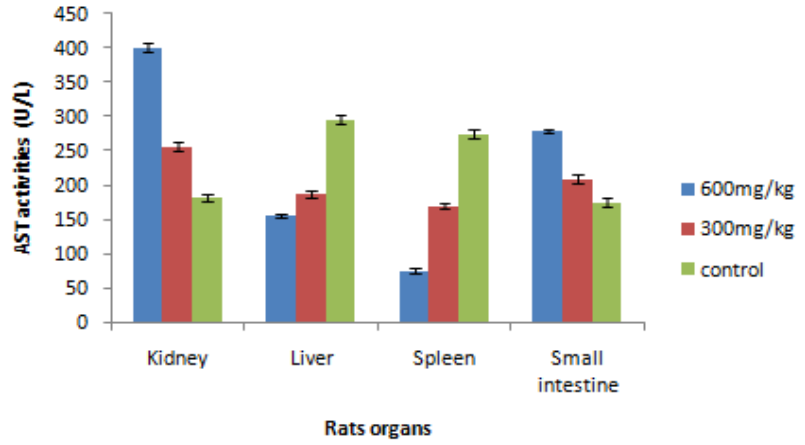

Figure 2. Aspartate transaminase activities in rat's organs following 21 days administration of bee propolis. Values are mean ± SEM of 5 determinations.

PBMS
ournal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

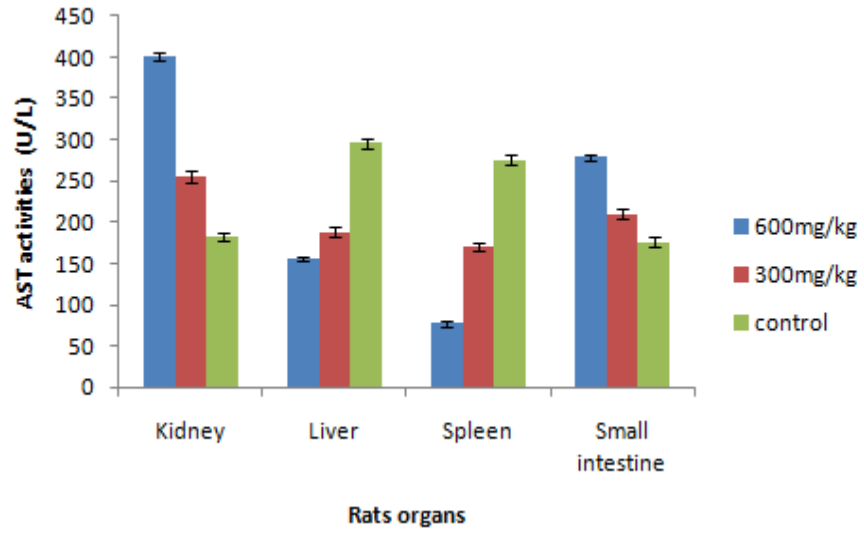

Figure 3. Alkaline phosphatase activities in rat's organs following 21 days administration of bee propolis. Values are mean ± SEM of 5 determinations.

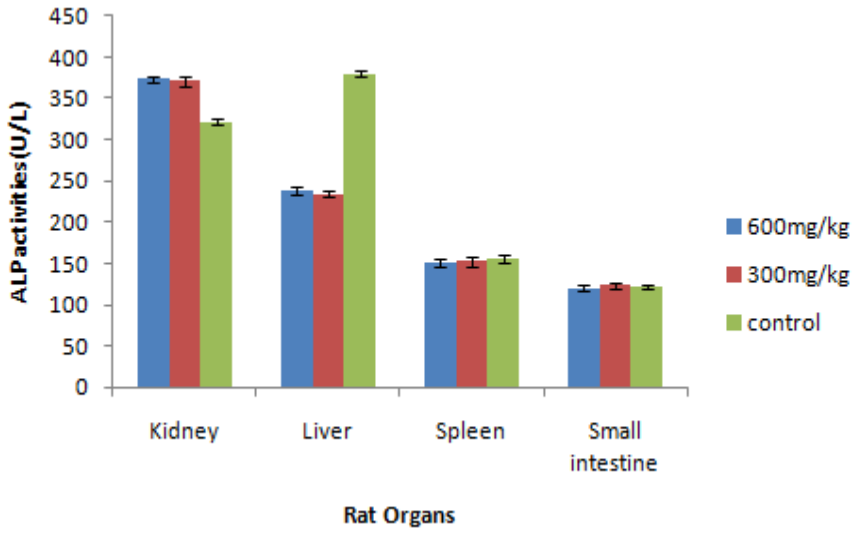

Figure 4. Total protein in rat's organs following 21 days administration of bee propolis. Values are mean ± SEM of 5 determinations.

Table 1. Relative organ weight of rats administered methanol extracts of bee propolis.

Parameters	Rat dose with 600 ml / kg Bee propolis	Rat dose with 300 ml / kg Bee propolis	Control rats
Kidney	0.037±0.001[a]	0.035±0.001[a]	0.037±0.001[a]
Liver	0.0071±0.001[a]	0.0069±0.000[a]	0.0068±0.005[a]
Spleen	0.0072±0.002[b]	0.0077±0.002[b]	0.0041±0.000[a]
Small intestine	0.028±0.003[a]	0.023±0.001[a]	0.024±0.002[a]

Values are mean ± SEM of 5 determinations. the values along the same row with different superscripts are significantly different ($p < 0.05$).

PBMS
Journal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

DISCUSSION

Despite the toxic attribute of natural products, more than 75% of the world population still relies on natural product for their health care needs. However, despite widespread use, few scientific studies have been undertaken to ascertain the safety or toxicity risks of many natural remedies[18]. The various biochemical indices investigated in this study are useful indices that can be employed to assess the toxic potentials of natural product extracts/botanicals in living systems. Toxicity assessment is significant to risk valuation as alterations in the biochemical system have higher prognostic value for human toxicity, when results are translated from animal studies[20]. The activities of ALP in organs and body fluid are widely accepted as a bioindicator of compromised integrity of the plasma membrane and endoplasmic reticulum. The results of kidney and Liver ALP activity from the present study, suggest that the 21 days administration of the propolis disrupted the integrity of plasma membrane in the kidney and liver. The reduction in ALP activities in liver may obviously be attributed to either loss of membrane component (including alkaline phosphatase) into the blood of the animal[21], or inhibition of the enzyme molecule by the propolis extract or inactivation of the enzyme molecule in situ[22]. Reduction in ALP activities could also result from disruption of the ordered lipid bilayer of membrane structure, leading to the release of detectable quantities of ALP out of the cell. This disruption hinders adequate transportation of required ions or molecules across cell membrane[4]. Similarly, the elevated levels of ALP in kidney of rats administered the extract could constitute threat to the life of cells which depends on a array of phosphate esters to function since these cells will be deprived of adequate energy due to unsystematic phosphate ester hydrolysis[23]. These changes will have their negative effects on normal functioning of the enzymes in the animals. However, the lack of an effect on this enzyme activity in small intestine and Spleen suggest that the normal functions of plasma membranes of these organs were not disrupted at these doses.

The aminotransferases (ALT and AST) normally localized within the cells of the liver, heart, kidney, muscles and other organs. They are well known transaminases that play important roles in amino acids metabolism and providing necessary intermediates for gluconeogenesis. These transaminases are therefore used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST[5]. The reduction in the activity of the aspartate transaminase from the liver and spleen could be translated to the leakages of the enzyme from these organs into the serum, it could also be due to inhibition of the enzyme activity by components of the extract, inactivation of the enzyme molecule in situ[6], or depletion of important molecules required by the enzyme for maximum activity[6]. The loss in the activity of these enzymes in the liver will adversely affect carbohydrate and amino acid metabolism, thereby affecting energy production. While the significant increase in the activities of AST in the Kidney and small intestine suggest that the administration of bee propolis increased the functional activity of AST that probably led to de novo synthesis of the enzyme molecules *in situ*. Such hyper-activity of AST, might lead to autolysis and consequently hemolysis.

The overall trends in organ ALT activities of rat administered propolis extracts for 21 days suggest a dose dependent and selective toxicity of bee propolis on rats organs, such selective toxicity effect is widely known properties of natural product which could however impaired the biosynthesis of some crucial macromolecules in cells, and leads to the failure of organs involve.

The concentrations of total proteins, is also an important indicator of normal or impaired functions of liver and Kidney. The observed dosed dependent increase in the total proteins in Liver and spleen of treated rat suggests a compromise of the synthetic ability of the liver arising from the administration of the extract. The propolis might have increased the functional activity of the liver by interfering with the equilibrium in the rate of synthesis and destruction, removal or clearance of total protein, from the system of the animals[24]. Such increase in total protein could, however, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals. However, the fact that the extract did not significantly ($p>0.05$) alter the level of total protein in the kidney and small intestine of the animals at all dose investigated suggested that the integrity of the kidney was not effected as far as the excreation of this biomolecule is concern.

According to Lawal et al.[18], Alteration in Organ-body weight ratio could reflects impairment in the normal functioning of the organs, an increase in ratio of organ to body weight indicate hypertrophy or inflammation of the organs, while

ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

a reduction in the same parameter can be adduced to cellular constriction or atrophy. Therefore, the absence of an effect on the computed liver/body, kidney/body and small intestine /body weight ratios suggest that the extract did not cause any form of swelling, atrophy and hypertrophy on the organs. Although, some alterations in some of the biochemical parameters on these organs were observed, it is possible that the alteration were not sufficient enough to produce atrophy or organ constriction. This alteration could therefore be considered as an earlier event preceding gross morphological changes in the organs. However, the increase spleen-body weight ratio could be an indication of hypertrophy. These speculations however required a histopathological backup.

CONCLUSION

The chronic administration of methanol extracts of bee propolis altered the normal values of some biochemical parameters in rat organs. Clinical application of the bee propolis should therefore be carefully controlled as it chronic administration may be relatively unsafe for the integrity of organ.

ACKNOWLEDGEMENTS

We acknowledge Mal. Shuaibu. Ma'aji, Mal. Sani Sakpe and Mr. Prince C. Ossai, laboratory Unit of Department of Biochemistry federal University of Technology. Minna.

REFERENCES

1. Bashir L, Shittu OK, Sani S, Busari MB, Adeniyi KA. African Natural Products with Potential Antitrypanosoma Properties: A Review. International Journal of Biochemistry Research and Review 2015a; 7(2): 45-79.
2.Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 2006; 27:1–93.
3.Taylor JLS, Rabe T. McGaw LJ, Jäger AK, van Staden J. Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation. 2001,34:23–37.
4.Oyewo EB, Fakunle PB. Safety Concerns Of The Repeated Administration Of Yoyo Bitters In Male Wistar Rats. International Journal of Pharmacy and Pharmaceutical Sciences 2013.5(3); 323-332.
5.Salau AK, AkanjiMA, Yakubu, MT.Safety Evaluation of Aqueous Extract of Crateva adansonii Leaves on Selected Tissues of Rats. Fountain J of Nat Appl Scie 2013; 2(1): 17 – 28.
6.Anofi OTA, Latifat OO,, Musa TY.Toxicity profile of ethanolic extract of Azadirachta indica stem bark in male Wistar rats. Asian Pac J Trop Biomed 2012; 2(10): 811-817.
7.Sforcin JM, Bankova V: Propolis: is there a potential for the development of new drugs? J Ethnopharmacol 2011;133:253–260.
8.Sforcin J.M. Propolis and the Immune System: a review, Journal of Ethnopharmacology 2007;113:1-14.
9.Watanabe MA, Amarante MK, Conti BJ, Sforcin JM. Cytotoxic constituents of propolisinducing anticancer effects: a review.Journal of Pharmacy and Pharmacology 2011; 63(11):1378-1386.
10.Guimarães NS, Mello JC, Paiva JS, Bueno PC, Berretta AA, Torquato RJ, Nantes IL, Rodrigues T. Baccharis dracunculifolia, the main source of green propolis, exhibits potent antioxidant activity and prevents oxidative mitochondrial damage. Food Chemistry Toxicology 2012; 50 (3-4):1091-1097.
11.Basista-Sołtys K, Allergy to Propolis in Beekeepers-A Literature Review. Occup Med Health Aff, 2013;1(1):1-3
12.Institute of laboratory animal resources, commission on life sciences, National Research Council. Occupational Health and Safety in the Care and Use of Research Animals, Washington, DC: National Academy Press; 1997.
13.Shittu OK, Olayemi IK, Omalu ICJ, Adeniyi AK. Antiplasmdial properties of methanolic extract of Muscadomestica maggot on P. berghei infected mice.International journal of biology pharmacy and allied science 2013; 2(5): 1064-1070.
14.Yakubu MT, Musa IF.Liver and Kidney Functional Indices of Pregnant Rats Following the Administration of the Crude Alkaloids from Sennaalata (Linn. Roxb) Leaves. Irani Jour Toxicol 2012;6:16, 615-625.
15.Tietz NW. Clinical Guide to Laboratory Tests. 3rd Edition, 1995, Pp. 286 – 288.
16.Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American J of Clin Pathol 1957; 28:56-63.
17.Gornall AC, Bardawill CJ, David MM. Determination of serum protein by means of biuret reaction. Journal of Biol Chem 1949; 177:751-756.
18. Bashir L, Shittu O.K , Busari M.B, Sani S. and Aisha MI. Safety Evaluation of Giant African land Snails (Archachatinamarginata) Haemolymph on Hematological and Biochemical Parameters of Albino Rats. J. Adva. Med. Pharm.Sci 2015b,3(3):122-130.
19.Statistical Package for Social Sciences for Windows (SPSS) 14.0 .Computer Program, MS. For Windows, Version 14.00, SPSS Inc., Chicago. 2005 Release 14.0. http://www.Spss.com.
20.Ashafa AOT, Sunmonu TO, Afolayan AJ.Effects of leaf and berry extracts of Phytolacca dioica L. on haematological and weight parameters of Wistar rats. African Journal of Pharmacy and Pharmacology 20115(2):150-154.
21.Olaoluwa TA, Odutola O, Olugbenga OA, Funmilayo DO, Sunday OO, Afolayan AJ.Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Activities in Selected Tissues of Rats Fed on Processed Atlantic Horse Mackerel (Trachurus trachurus) Advances in Bioscience and Biotechnology 2015; 6:139-152.
22.Lawal B, Shittu OK, Ossai PC, Abubakar AN, Ibrahim AM. Antioxidant Activities of Giant African Snail

ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

(Achachatina maginata) Haemolymph against CCl4-Induced Hepatotoxicity in Albino Rats. B. j.pharmal res 2015c.6(3): 141-154.
23.Oyewo EB, Akanji MA, Iniaghe MO and Fakunle PB. Toxicological Implications of Aqueous Leaf Extract of Andrographis paniculata in Wistar Rat. Nature and Science 2012; 10(2):91-108.
24.Adeyemi OS, Fambegbe M, Daniyan OR and Nwajei I. Yoyo Bitters, a polyherbal formulation influenced some biochemical parameters in Wistar rats. J Basic Clin Physiol Pharmacol 2012;12;0 (0):1-4.

Statement of Originality of work: The manuscript has been read and approved by all the authors, the requirements for authorship have been met, and that each author believes that the manuscript represents honest and original work.

Source of funding: None

Competing interest / Conflict of interest: The author(s) have no competing interests for financial support, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

Disclaimer: Any views expressed in this paper are those of the authors and do not reflect the official policy or position of the Department of Defense.
Majority of the information gathered are from media sources which don't reflect the author's own opinion.