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Acute Oral Toxicityeffects of Hermon Tea Aqueous Extract Inwistar Rats.

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Abstract
Aim: *The study investigated acute oral toxicity effect of Hermon tea aqueous extract in Wistar rats*
Material and Method: *Hermon tea was obtained from herbal medicine practitioner and prepared in accordance to recommended therapeutic label for acute oral toxicity evaluation.Wistar rats weighing between 120g – 150g were used for the study and treated orallywith a fixed 300mg/kg Hermon tea aqueous extract for 7 days andrat body weight was recorded daily throughout the treatment. At the end of 7 days ,the rats were sacrificed and blood were obtained through cardiac puncture and collected into EDTA bottle and HEPARIN bottle for Haematological and Biochemical analyses. Their vital organs were harvested and stored in 10% formalin for histopathological examination.*
Result*: Hermon tea aqueous extract shows significant ($p<0.05$) increase in the body weight value in the treated rats and significant ($p<0.05$) decrease in lungs weight value and non - significant decrease were observed in heart, kidney and liver. Meanwhile significant ($p<0.05$) increase in the mean red blood cell (RBC) value andsignificant ($p<0.05$) decrease in the mean haemoglobin value in the treated rats were observed compared with the control. Also, non-significant ($p>0.05$)decrease in the white blood cell(WBC), packed cell volume(PCT) and platelets(PLT) were noticed in the treated rats compared to control rat. More so, there was non-significant increase in the creatinine, alanine amino transferase (ALT), aminoasphatase (AST) values and decrease in alanine amino phosphatase value compared with the control. Further, histopathological examination revealed normal structure and no significant adverse effects observed on the kidney, heart, liver and lung.*
Conclusion: *the present result shows that Hermon tea aqueous extract does not cause any apparent acute oral toxicity in animal model. Also, histology examination revealed no changes in the architecture of the internal organs. Hence, Hermon tea at a treated dose 300mg/kg is safe and could be used as a medicinal agent.*
Key words: *Hermon tea, acute oral toxicity, Haematology, Biochemistry and histopathology*

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I. Introduction

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the years as this type of medicine is easily accessible in some regions (Humber, 2002). Plant-derived foods, particularly vegetables and fruits, are generally considered to be highly beneficial components of the human diet. They contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. In general, natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Newman et al., 2003). There has been increasing demand for the use of plant products due to low cost, easy accessibility, availability and lesser side effects, therefore plant materials are continuously scrutinized and explored for their medicinal properties. There are numerous traditional medicinal plants reported to have medicinal properties such as*Allium sativum* (Garlic),*Vernonia amygdalinas,Panax – ginseng, Dioscorea dumentorum,Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Trigonella foenum* (Fenugreek), *Momordica charantia* (Bitter ground), *Ocimum santum*(Grover et al, 2002) and their prescription as natural remedies are commonly employed and practiced in developing countries for the treatment of various diseases as alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora 1989; Zhu, 2002).

Hermon tea is a polyherbal formulation used for various ethno medicinal purposes. Ethno medicinally, Hermon tea is taken daily for general body debility as contained in the marketing label. . Moreover, botanicals are enjoying widespread use of plants for treatment of several ailments, but still little known about their toxicity and safety issue which are always a concern. Investigations on functional plants provide evidence for the presence of substances that are offer potential human health benefits. However, it should be a vital requirement to determine the toxic effects of some of the substances contained in the plants (Bellini et al., 2008). Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being (Asante- Duah 2002).The present study aimed to investigate the acute toxicity ofaqueous extract of Hermon tea in animal models (Joshi et al., 2007)

II. Materials And Methods

Preparation of Hermon Tea

Hermon tea was prepared by weighing **5g** of the sample and dissolved in 100ml of distilled water and gave a **50mg/ml** as a stock solution. The solution was filtered using what man England filter paper and the extract was kept in the toxicology laboratory at the room temperature 25^{oc} – 28^{oc} prior the study and the residues were discarded.

Acute oral toxicity study

The acute oral toxicity study was conducted according to the procedure as described in Organisation for Economic Cooperation and Development (OECD) guideline.

Animals

Wistar rats weighing between **120 – 150g** were used for the study. The rats were supplied by the animal facility centre of Nigeria Natural Medicine Development Agency (NNMDA) they were fed with standard feed and had free access to water adlibitum. They were also maintained under standard conditions of humidity, temperature and 12h light /darkness cycle. The animals were allowed to acclimatize for one week before the commencement of the study. For the study, a standard laboratory protocol was drawn up in accordance to the good laboratory practice (GLP) Regulations of the WHO (1998).The principles of laboratory animal care (NIH Publication 85 -23, 1985) were followed in this study.

Experimental protocol

A total of 12 male rats (6 rats/group), were randomly selected and marked for individual identification. The test groups included a control group (received 0.9% saline) and treatment groups with dosages received 300 mg/kg body weight of Hermon tea extract respectively. The animals were observed and monitored daily for mortality, behaviour and appearance with priority given to the first 4 hours after administration of the Hermon tea extract. Food consumption and water intake were measured daily. However body weight gains of the rats were recorded weekly for 7 days.

Haematological analysis

On the 7th day of the study, the animals were anesthetized with diethyl ether and their blood were drawn through cardiac puncture and collected into Ethylene – di – aminetetra acetic acid (EDTA) anticoagulant tubes and their vital organs were harvested, weighed.. The blood were analysed for several parameters which included red blood cells (RBC) count, white blood cells (WBC) counts and haemoglobin (Hb) content using standard laboratory methods as described by Satyavati and Sharma (1989) and packed cell volume (PCT) content as described byBiswas and Ghosh 1973)

Biochemical analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate and creatinine were measured by using standard kits.

Histopathological study

The vital organs isolated from sacrificed rat were fixed in 10% formalin, then after processing embedded in paraffin wax. Paraffin sections were made at 5 mm and stained with haematoxylin and eosin. The slides were studied under a light microscope and captured the magnified images of tissues structure for study.

Statistical analysis

Results were presented as mean ± SEM and analysed by unpaired‘t’ test followed by Turkey’s multiple comparison test. $P \leq 0.05$ was considered as statistically significan

III. Result

At the end of 7 days oral administration of fixed dose of Hermon tea extract in rats, their body weight and Organ weight were determined. While the rats blood were obtained by cardiac puncture and collected into EDTA and heparin bottles for haematological and biochemical analyses. Their organs were harvested for histo - pathological studies. Results obtained showed significant ($p<0.05$) increase in the body weight (fig1) and significant increase lungs weight (fig2), but non – significant ($p>0.05$) increase in the mean liver weight (Fig 3) and non-significant decrease in the mean heart weight (fig 4) and mean kidney weight(fig 5) in rats treated with Hermon tea compared with the control rats. While haematological and biochemical analysis indicate non - significant ($P> 0.05$) decrease in the mean White blood (WBC) value (fig 6) but significant ($p<0.05$) increase in the mean red blood cell (RBC) value (fig7) and mean haemoglobin (HGB) value(fig 8) in treated rat compared with the control rats. While non - significant decrease in the mean packed cell volume (PCT) value (fig 9) and platelets(PLT) value(fig 10) were observed in rats treated with Hermon tea compared with the control rats.There was non-significant increase in the mean creatinine (fig 11)value in the treated rats compared with the control rats and non - significant increase in the mean alanine amino transferase (ALT) value (fig 12) and amino asphatatase transaminase(AST) value(fig 13) and non – significant decreasealanine amino phosphatase (ALP) values (fig 14) in all the treated rat compared with the control rats. Photo micrographic plate of 300mg/kg Hermon tea extract (fig 15) show normal heart but congested vessels with red blood cell was observed in kidney also edema with aggregate of red blood cell in the liver, but normal lung was observed

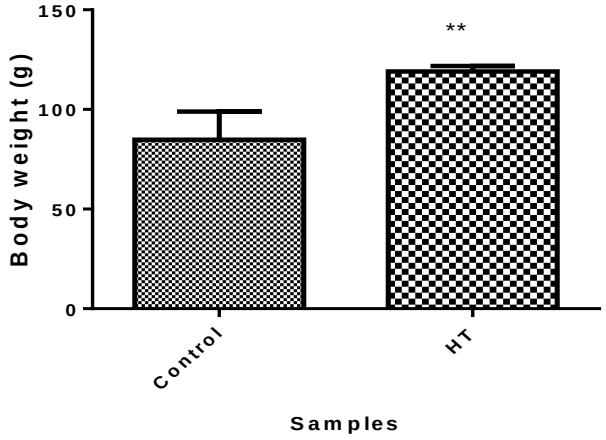

Fig 1: Shows mean body weight values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired‘t’ tests, significant*$p<0.05$ compared with control

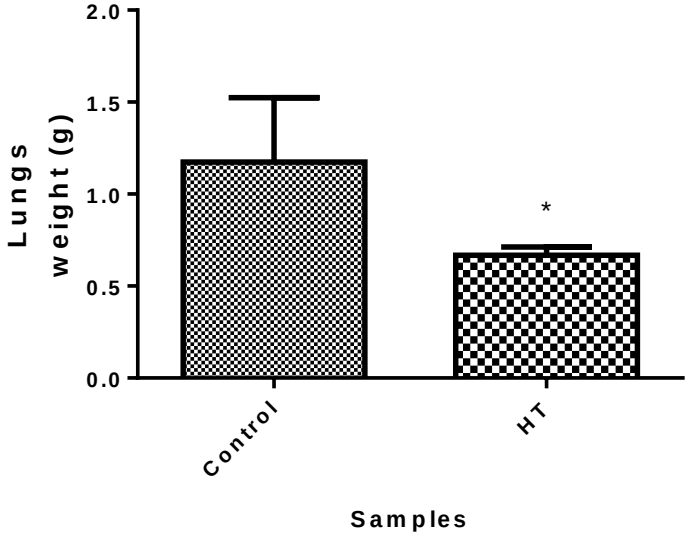

Fig 2: Shows mean lungs weight values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired ‘t’ tests, significant*$p<0.05$ compared with control

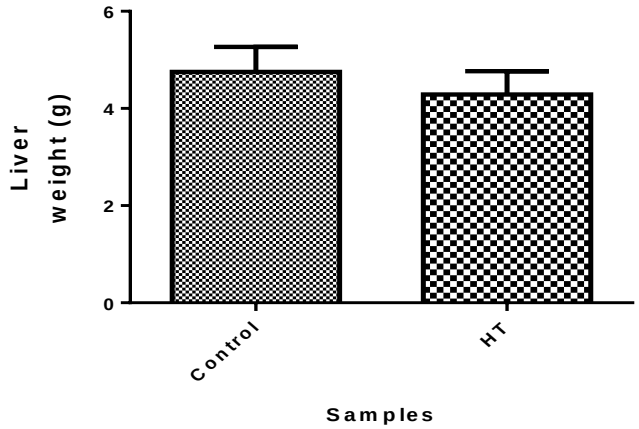

Fig 3: Shows mean liver weight values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired't' tests, significant*p<0.05 compared with control

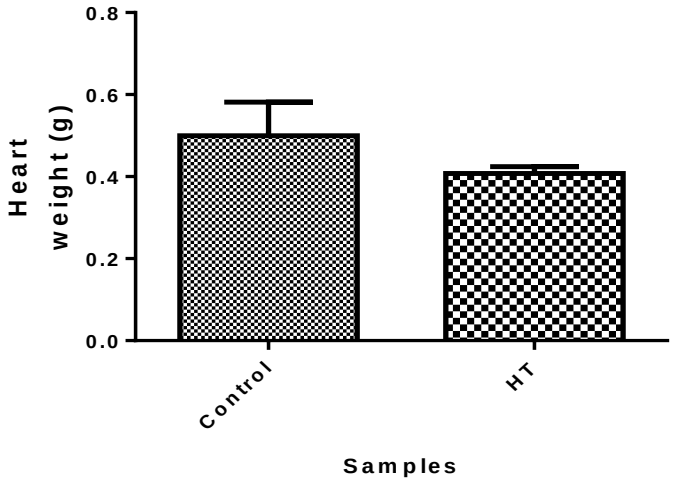

Fig 4: Shows mean heart weight values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. unpaired 't' tests, significant*p<0.05 compared with control

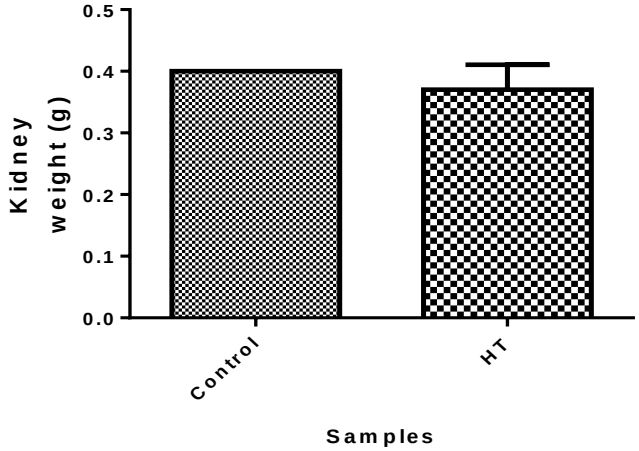

Fig 5: Shows mean kidney weight values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired't' tests, significant*p<0.05 compared with control

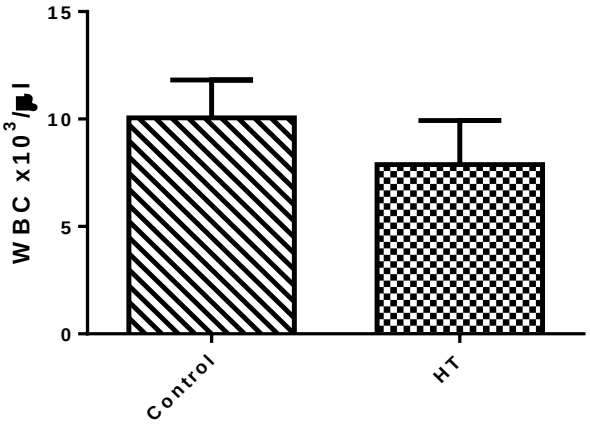

Fig 6: Shows mean white blood cell(WBC) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired 't' tests, significant*p<0.05 compared with control

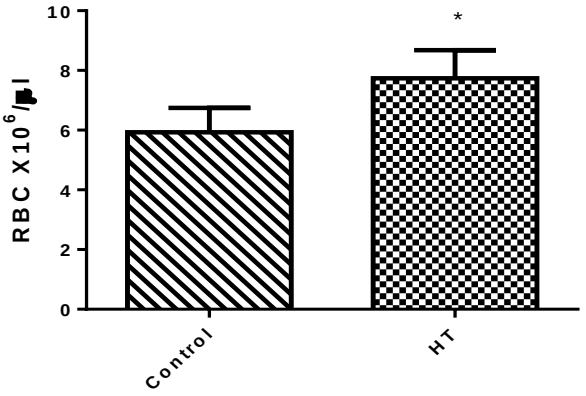

Fig 7: Shows mean white blood cell(RBC) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. unpaired't' tests, significant*p<0.05 compared with control

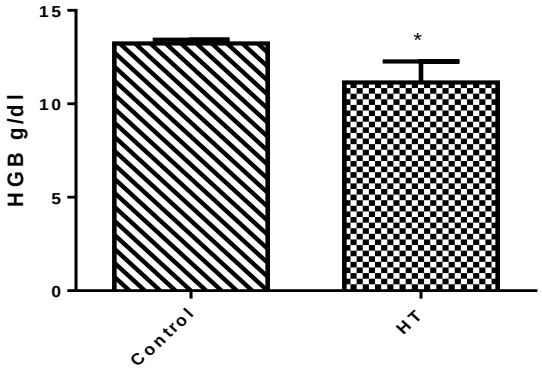

Fig 8: Shows mean white blood cell (HGB) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6.unpaired 't' tests, significant*p<0.05 compared with control

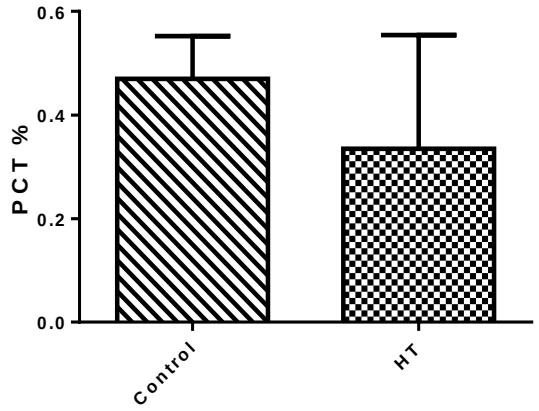

Fig 9: Shows mean white blood cell (PCT) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. unpaired 't' tests, significant*p<0.05 compared with control

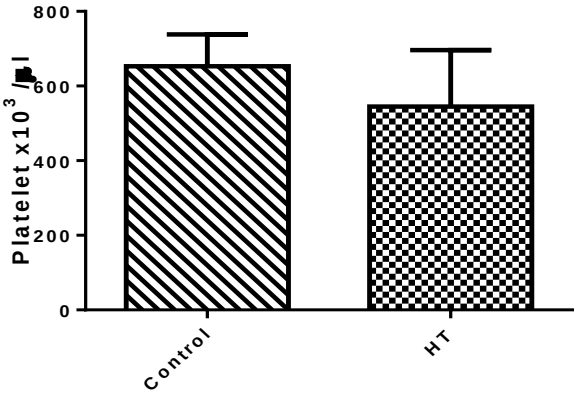

Fig 10: Shows mean white blood cell(Platelets) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. unpaired 't' tests, significant*p<0.05 compared with control

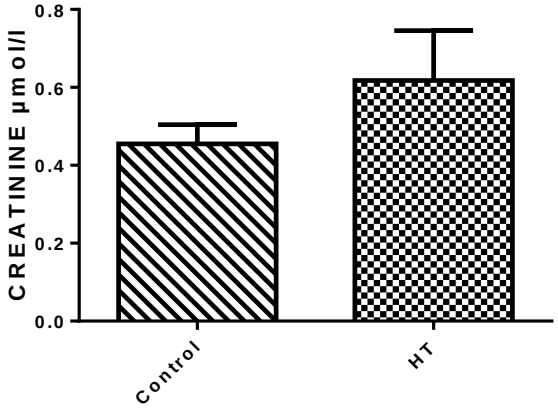

Fig 11: Shows mean on biochemical indices (Creatinine) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired 't' tests, significant*p<0.05 compared with control

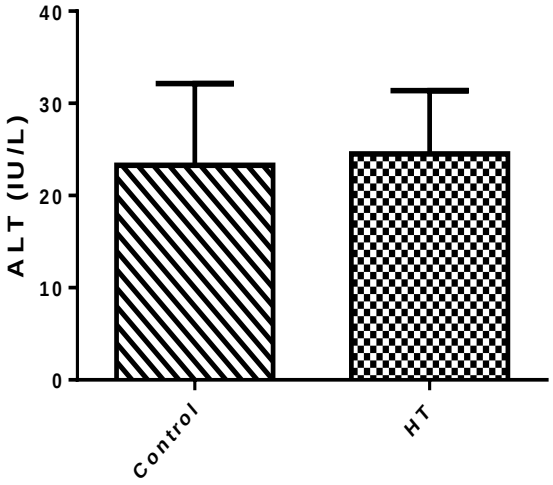

Fig 12: Shows mean on amino alanine transaminase (ALT) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. Unpaired 't' tests, significant*p<0.05 compared with control

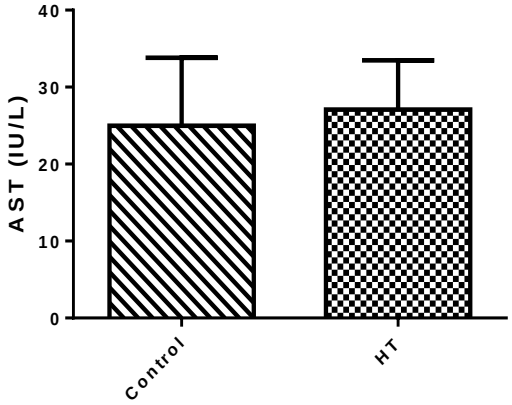

Fig 13: Shows mean amino asphatate transaminase (AST) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6.unpaired 't' tests, significant*p<0.05 compared with control

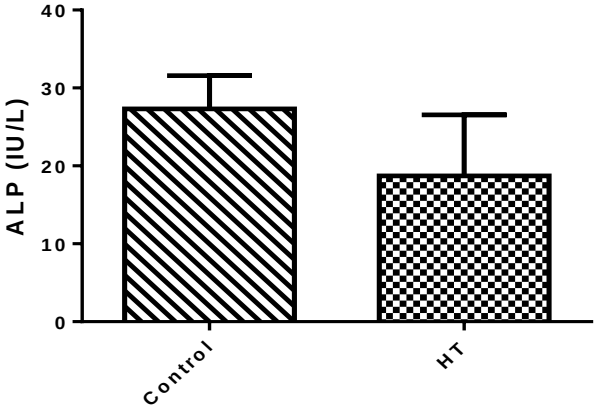

Fig 14; Shows mean alkaline phosphatase (ALP) values of Rats in acute treatment with Hermon tea(HT).Values are represented by mean ± SE for n = 6. unpaired 't' tests, significant*p<0.05 compared with control

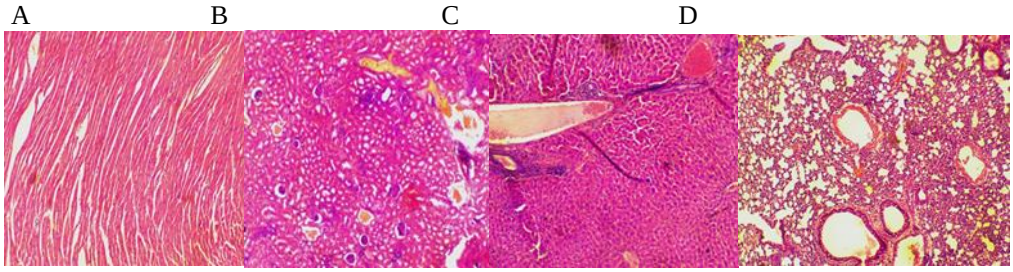

Fig 15: (A –D): show photomicrograph section of the organs of rats treated with a dose (300mg/kg) of Hermon tea extract for 7days in acute oral toxicity study. **(A)**Histologic sections of **HEART** muscle H & E stains x100 show interlacing fascicles of cardiac myocytes/ myocardial cells. No abnormalities are seen **(B**) H & E stains x100 sections of **KIDNEY** tissue show norm cellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels are seen. **(C)** H & E stains x100 sections of **LIVER**tissue show parallel radially arranged plates of hepatocytes, with the portal space and periportal zone filled with a smooth to slightly floccular pink fluid material common with edema and congested aggregates of red blood cells**(D)** H & E stains x100 sections of **LUNG** tissue showing some alveolar filled air spaces no abnormalities seen.

IV. Discussion

Phytotherapeutic products from medicinal plants have become universally popular in primary health care, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source. Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self-medication(Vaghasiya, et al.,2011). However, there is a lack of proven scientific studies on the toxicity and adverse effect of these remedies. Therefore, further acute oral toxicity study is vitally needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation (Rang and Dale 2001).Toxicity results from animals will be crucial in definitively judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products (Moshi, 2007).As use of medicinal plants increases, experimental screening of the toxicity of these plants is crucial to assure the safety and effectiveness of those natural sources. Hermon tea is a poly herbal tea formulated for the purpose of general body maintenance and other associated ailment. Acute oral toxicity was evaluated on Hermon tea extract in rat's accordance to OECD (2001) 423 guideline. Rats were chosen for the study because they are readily available and their bodily metabolisms. Result obtained(fig1) showedsignificant ($p<005$) increase in the rat body weightwhich may be due to the ability of certain phytonutrients in Hermon tea that increases their appetite. In the available literatures on phytochemistry of some medicinal plants, it has shown that plants possesses certain bioactive compounds and phytonutrientsthat are beneficial to the body.Also, significant ($p<0.05$) decreased in the lungs weight asobserved (fig 2) is an indication that the Hermon tea did not have any deleterious effects on the lungs when taking at a tested dose(300mg/kg). However, Organ weight is an important index of physiological and pathological status in animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, and lungs are the primary organs affected by metabolic reaction caused by toxicant (Dybing et al., 2002). Histological analysis fig 15 was done to further confirm the alteration in cell structure of the organs. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organ according to OECD guidelines. In general, the histopathology analysis collaborated with the results of body weight and organ weight. Hermon tea at tested dose (300mg/kg)did not cause toxicity towards the organs as there was no structural damage in the treated organs. Furthermore, since there is relationship between RBC and HGB in blood formation, analysis of blood parameters is relevant in toxicity evaluation. Their result indicate significant increase in red blood cell (RBC) figure 7 and significant decrease in haemoglobin (HGB) values figure 8 , it therefore show that Hermon tea has potential to stimulate erythropoietin released in the kidney for blood production.

V. Conclusion

In conclusion, the present result shows that Hermon tea aqueous extract does not cause any apparent acute oral toxicity in animal model. Also, histology examination revealed no changes in the architecture of the internal organs. Hence, Hermon tea at a treated dose 300mg/kg is safe and could be used as a medicinal agent.

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