Sub-acute toxicity and safety study of Mecca Myrrh *(Commiphora opobalsamum)* by experimental histological analysis.

ABSTRACT

Background: The native herbal medicinal products are vital source of the key medication; their excellent dogmatic safety study is of prime importance despite their strong validated evidence of efficacy.

Aim: To study the sub-acute toxicity of *Commiphora opobalsamum (CO)* by histological analysis of vital organs.

Method: Twenty-four male Wister rats of 10-12 weeks age were used. Oral toxicity studies with repeated dose were accomplished in four group of rats, six rats were included in each group. Three groups of rats were administered *CO* orally in the graded doses of 250,500, and 1000 mg/kg/day once daily for 14 days Vs normal control. Determination the relative weight of vital organs and their histological changes were performed at the end of the study. Subsequently, the digital images of the *CO* - administered groups were equated with the control group.

Results: Intriguingly, the relative weights of the vital organs, performed following 14 days of graded doses of CO given were equated with the control group, significant differences were not detected ($p>0.05$). Furthermore, histological examination of the brain demonstrated no changes in its cellular neuronal components Regarding the heart, *CO* extract did not produce any histological changes, insignificant changes were observed in liver parenchyma of rats, regarding the kidney, no degenerative changes were noticed in glomerular tuft capillaries or renal tubular epithelium, conversely trivial changes were observed in liver parenchyma. Finally, no minor

histological changes were observed in stomach (high dose) and spleen tissue of animals on comparison with the control group.

Conclusion: Seemingly, the impressive outcome of this fundamental histological scrutiny of methanolic extract of *CO* demonstrated an imperative facet of safety and correspondingly displayed the key feature of its nontoxic medicinal application for short term supplementation.

Key words: *Commiphora opobalsamum, sub-acute toxicity, histology, toxicological assessment, methanolic extract and experimental analysis.*

Introduction

There is a long history of traditional or ritualistic medicine with global utility for the enhancement of health. Thus, logistically these medicines are often envisioned as a source for the discovery of the novel drugs. The current era has observed that quite proliferation of the researchers are eagerly focusing on the herbal medicines universally to seek the evidence of their efficacy following prolong histories of folk utility[1-3].

Yet, active principles extracted from the herbal medicinal products exhibits adverse drug reactions, like aristolochic acid present in the plant of *Aristolochia* and Asarucamis quite renowned to produce renal failure and cancerous condition, this type of adverse drug reaction is a candid reflection of a matter of concern for the safety of herbal medicines [4] Furthermore, aristolochic acids are a group of acids found naturally in many types of plants known as Aristolochia (birthworts or pipevines) and some types of plants known as Asarum (wild ginger), which grow worldwide. [4, 5]

Moreover, several studies observed toxicities and harmful effects of herbal products after chronic use[6]. Hence, it is vital that herbal medicine needs to be critically studied and analyzed for the safety and therapeutic utilization in human being [7].

Realistically, corticosteroids are by far the most potent anti-inflammatory compound, yet their multiple adverse effects play an important obstructive role to substantiate their therapeutic benefit[8, 9].The essential rationale behind exploration of the pharmacological profile of naturally occurring flora is to determine their anti-inflammatory properties to overcome the adverse drug reactions of corticosteroids and NSAIDs.

We have laid relatively meticulous scientific experimental input in our current studies for the anti- inflammatory effect, toxicological profile and putative mechanism of anti-inflammatory effect Commiphora opobalsamum *CO* with the decisive target to comprehend a strong, prudent and safeguarding substitute derived from the natural herbal products to afford unique attributes of a perfect anti-inflammatory drug [1-3].In view of further reinforcing of our enlightened experimental studies of *CO*, the present work focused on explorative study of acute and sub - acute toxic effect of *CO* on different organs and histological changes in brain, heart, liver, stomach, spleen and kidneys in rats.

Material and Methods

Animals:

The experimental design of this study comprises of utilizing twenty-four male Wister rats of 10-12 weeks with average weight of (170-200gms), they were brought from the animal house, King Fahad Medical research center (KFMRC), Jeddah, Saudi Arabia. These rats were housed in a optimal laboratory environment at the average temperature of 25 ± 5^{0}C and relative humidity of 30-70% with 12 hours each light and dark cycles for a minimum duration of one week before start of the experimental study. Moreover, the rats were comfortably kept in plastic cages in a group of six rats in each cage with free access to food and water supply. It needs to be emphasized that the experiment protocol was followed strictly according to the guidelines of KFMRC and due permission of the ethical committee of institution was secured before the commencement of this experimental study.

Description of plant and its extraction

The collection of aerial components of the plant *CO* was accomplished from a village Al-zemah which is located midway between Al-Taif and Makkah Munnawara cities, Saudi Arabia, during the summer period, moreover its herbarium number is 21.618466,40.107040 and presumably the taxonomical acknowledgement was duly executed in the department of natural product and alternative medicine, college of pharmacy, King Abdulaziz University (KAU), KSA. Notably, extraction of the plant was duly performed by the procedure illustrated [10].

Afterwards the next step was the natural drying of the stem and aerial part of the plant and for this motive, they were stored very carefully in an appropriate well ventilated and dry place with controlled temperature of 30-40^0 C to get it dried naturally over a period of 5 days and this was followed by its mechanical grinding to the state of powder, approximately 900gms and then it was soaked in 99%w/v methanol at room temperature (controlled) of 25 -30^0 C for a duration of two days and subsequently the process of extraction was carried out four consecutive times. Furthermore, the extracted product was meticulously double filtered to ensure the removal of fine particles with help of cotton and filter papers. The next step was evaporation of the filtrate for the removal of minimum quantity of methanol; this was accomplished using a rotator evaporator (Buchi®Schweiz). The ultimate step consists of acquisition of the final production in a moist nature, and it was kept in a refrigerator at -80^0 C for about one hour, then it was undergone a rapid process of drying by utilization of a freeze vacuum dryer (Zirbus® German) at 85^{0}C. Remarkably, the methanolic extract of *CO* which is considered as the ultimate product of investigation in an approximate amount of 100gm was very cautiously stored in a container which provide protection against the natural light and kept in a refrigerator with a tight controlled temperature. Finally, whenever administration of extract was performed for the experimental work, a fresh preparation of suspension in 0.9%

saline solution was executed with the volume administered were meticulously adjusted to 10ml/kg body weight of the rats.[2, 11, 12]

Experimental design:

Observation of oral sub-acute toxicity by repetitive dose administration in rats The process of sub-acute toxicity study with the oral dose of *CO* extract was accomplished by the approaches of the former investigators, pioneered the evaluation of sub-acute toxicity by administration of the test compound every day for a duration of 14 days [11, 12], we followed this method by administration of *CO* extract orally in three graded doses (250,500 and 1000mg/kg/day) to the three groups of randomly selected rats (n = 6 in each group), ,we also took one additional group (normal control) and administered the vehicle (0.9% saline solution) in the same volume , care was taken to make the availability of food and water at all the time for all the animals.

It needs to be emphasized that selection of the graded dosed was accomplished based on our recent finding of the lethal dose as > 2000mg [2]. The duration of the observation period in this study comprises of 14 days, on the very first day it was every six hours and subsequently reduced to once daily. However, this specific duration of the experimental study a meticulous watch was kept on the rats to observe the warning symptoms of central nervous system toxic effects in the form of tremors and convulsions in addition to diminution of locomotor activity and their acuteness during the process of handling. The observation of rats by the cage side and their mortality.

During the experimental procedure, weight of each rat was measured daily prior to administration of the extract for the entire duration of the study. Finally, at the end of the study duration, rats were sacrificed with ether for the purpose of:

a) Precise measurement of the weight of all dissected out essential organs viz, the brain, heart, kidney, stomach and spleen. The organs then cleaned meticulously with normal saline, individually measured accurately and ultimately preserved in 10% formalin for histological observation.

b) Elaborated microscopic examination of all the selected important organs were done conspicuously, to accomplish this important task, part of each organ was first fixed in 10% solution of neutral buffered formalin followed by its embedding in paraffin blocks to enable them to be cut into ribbons precisely of 5 µm and ultimately mounted on the glass slides, for exact identification the slides were coded then suitably stained with H&E and scrutinized for histopathological examination under light microscope.[13] The final step comprised of comparative analysis of digital images of treated groups with the control group.[11, 12]

Statistical analysis of this study:

The results of this study were statistically accomplished by statistical social science software (SSPS) version16(IBM®USA). Furthermore, one way analysis of variance, (ANOVA), repeated one way ANOVA and Tukey's *post adhoc* test were utilized for the execution of multiple evaluations. However, for the purpose calculation of statistically difference between the mean values were quantified at p value of less than 0.05($p<0.05$) and 0.001 ($p<0.001$). In addition, the values illustrated in the text and tables were considered as ± SEM. Finally, the preparations of the graphs were carried out by the software of GraphPad Prism, Version 5

Results:

Comparative weight of vital organs:

Table 1. Demonstrated significant differences in the comparative weights of vital organs - brain, heart, kidney, liver and spleen at the end of 14 days of oral treatment with *CO* in graded doses 250, 500 and 1000 mg/kg/day compared with control group (p>0.05) with means values ± SEM of 1.07±0.03, 1.07±0.01, 1.05±0.02 for brain, 0.39±0.02, 0.39±0.04, 0.45±0.02 for heart, 0.58±0.07, 0.55±0.09, 0.53±0.06 for kidney, 4.77±0.69, 4.76±0.71, 5.13±0.40 for liver and 0.52±0.06, 0.56±0.10, 0.58±0.09 for spleen, respectively.

Group n = 6	Relative organ weight (%)				
	Brain	**Heart**	**Kidney**	**Liver**	**Spleen**
Control (0.9% saline)	0.92±0.11	0.41±0.04	0.50±0.06	4.68±0.61	0.56± 0.03
CO (250 mg/kg /day)	1.07± 0.03	0.39±0.02	0.58±0.07	4.77± 0.69	0.52±0.06
CO (500 mg/kg /day)	1.07±0.01	0.39±0.04	0.55±0.09	4.76±0.71	0.56±0.10
CO (1000 mg/kg /day)	1.05±0.02	0.45±0.02	0.53±0.06	5.13±0.40	0.58±0.09

Table 1. Relative weights of vital organs (per 100 g body weight) of rats at the end of 14 days of oral treatment with *CO* extract in the graded doses – 250mg,500mg and 1000mg/kg/day.

Histological examination of vital organs:

The effects of *CO* extract on the histological inspection of the brain, heart, kidney, liver, spleen and stomach tissues were performed at the end of 14 days of oral treatment at doses of 250, 500 and 1000mg/kg/day are revealed in the Figure 1 - Figure 6.

Histological examination after oral administration of *CO* extract at different doses for 14 consecutive days (Figure 1.) was found to be safe and did not produce any changes in cerebellar folia or its cellular neuronal components (white arrows) . No signs of meningeal irritation (black arrows), vascular congestion or neuronal degeneration were observed.

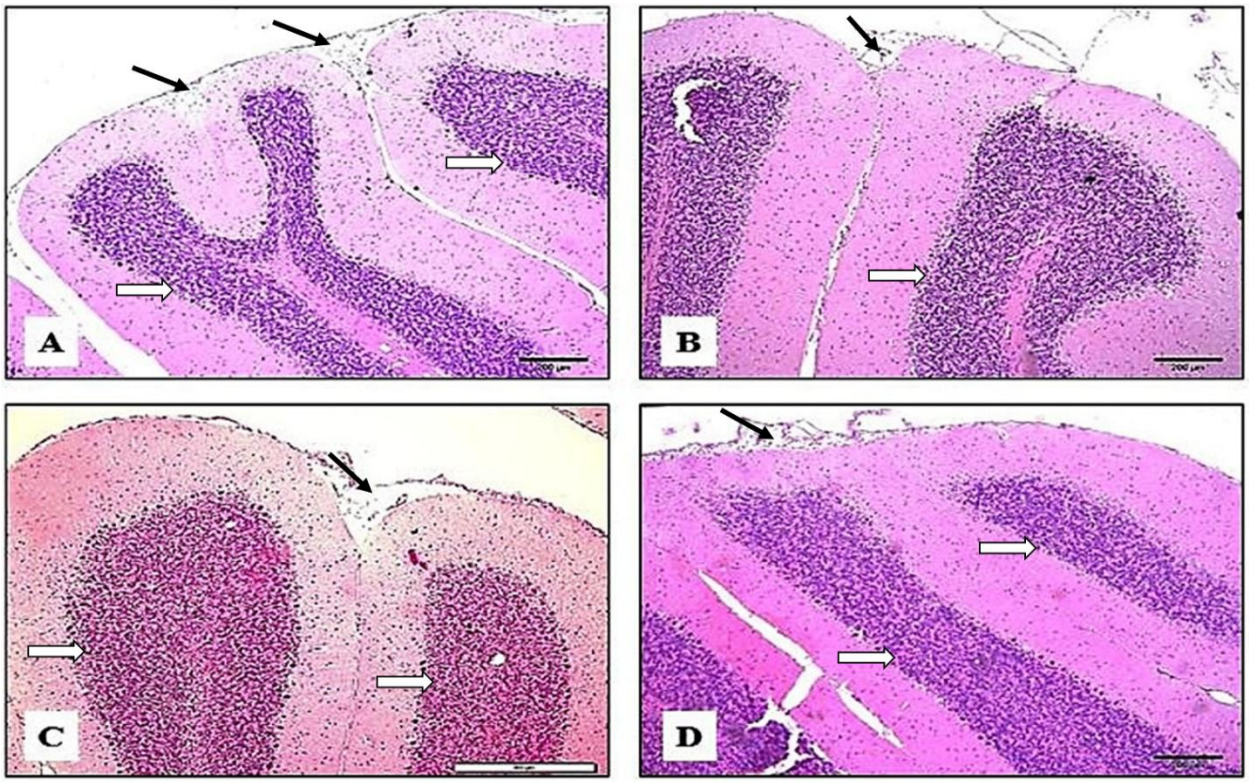


Figure1. Microscopic digital image of H&E section of the brain of rats after 14 days of oral treatment with A: Control- 0.9% saline; B:CO (250 mg/kg/day); C:CO (500 mg/kg/day); D:CO (1000 mg/kg/day). Treatment with CO extract revealed normal histology. Observation was made at 10 X magnification

Furthermore, cardiac muscle of control heart mice appeared as elongated cylindrical fibers that branch and anastomose together. (Figure2.) Their nuclei are oval and central (Blue thin arrows) thin-walled blood capillaries were seen among the fibers (white arrows). Administration of *CO* extract orally at different doses for 14 consecutive days did not produce any histological changes in cardiac tissue. However, Focal areas of Coagulative necrosis (stars) of the heart and infiltrated by leukocytes (B& C) could be observed (thick black arrows) in some samples. furthermore, even at doses (500 and 1000 mg/kg/day) cardiac fiber looked more regular with less fibroblasts (dotted arrows) among the fibers.

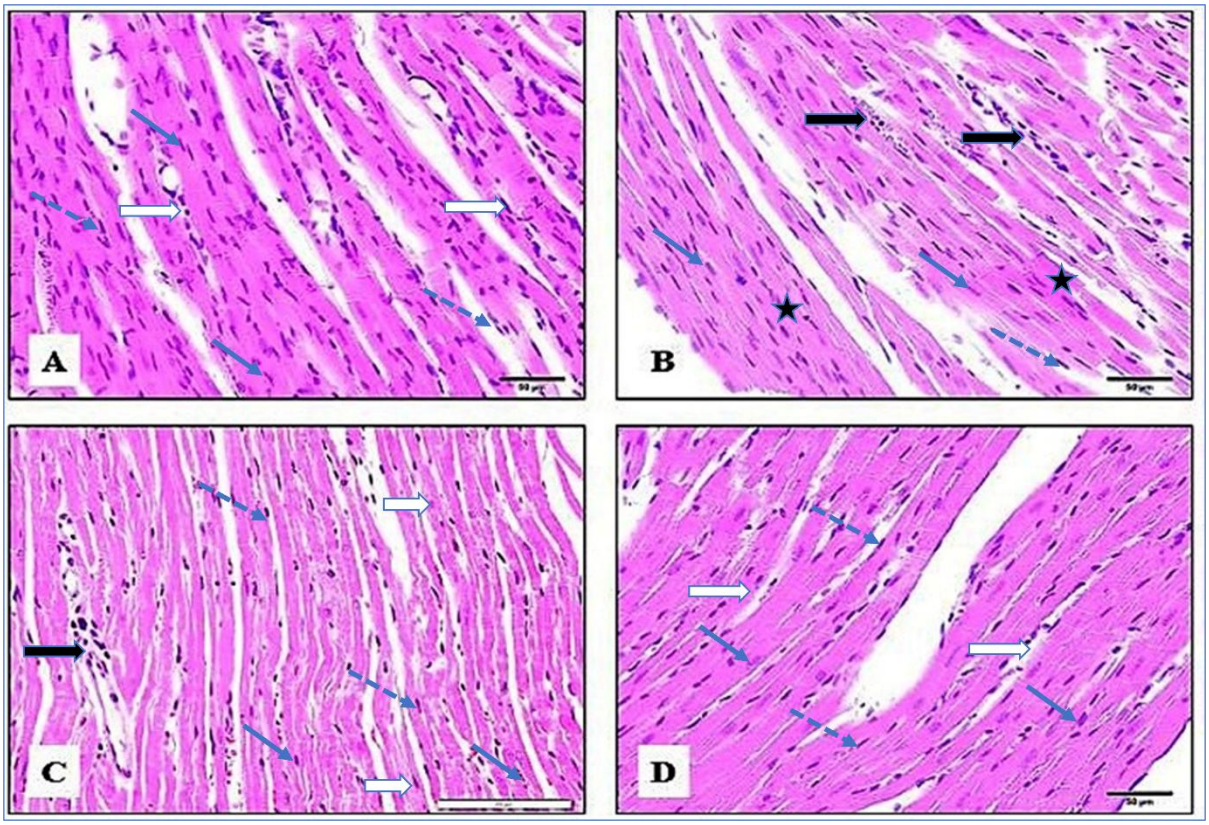


Figure2. Microscopic digital image(x 400 Bar= 50µm) of H&E section of the heart of rats after 14 days of oral treatment with A: Control - 0.9% saline; B:CO (250 mg/kg/day); C:CO (500 mg/kg/day); D:CO (1000 mg/kg/day). Treatment with CO extract revealed normal histology. Observation was made at 40x magnification.

Moreover, the kidney tissue (Figure 3) of control mice showed normal renal corpuscles including the thin-walled bowman capsule (black arrows(and glomerular capillaries (red stars). Renal tubules either proximal or distal showed normal lining epithelium (black arrows). Peri-tubular capillaries were thin and compressed between the tubules. Oral Administration of different dose of *CO* extract for 14 consecutive days did not produce any degenerated changes in glomerular capillaries or tubular epithelium (black arrows). No congestion or tubular casts were observed at

250 and 500mg/kg/day. Slight hypertrophy of glomeruli and tubules were observed at 1000mg/kg/day. In high dose (D) , few regions of tubular degeneration and leucocytic infiltration could be observed (dotted black arrow).

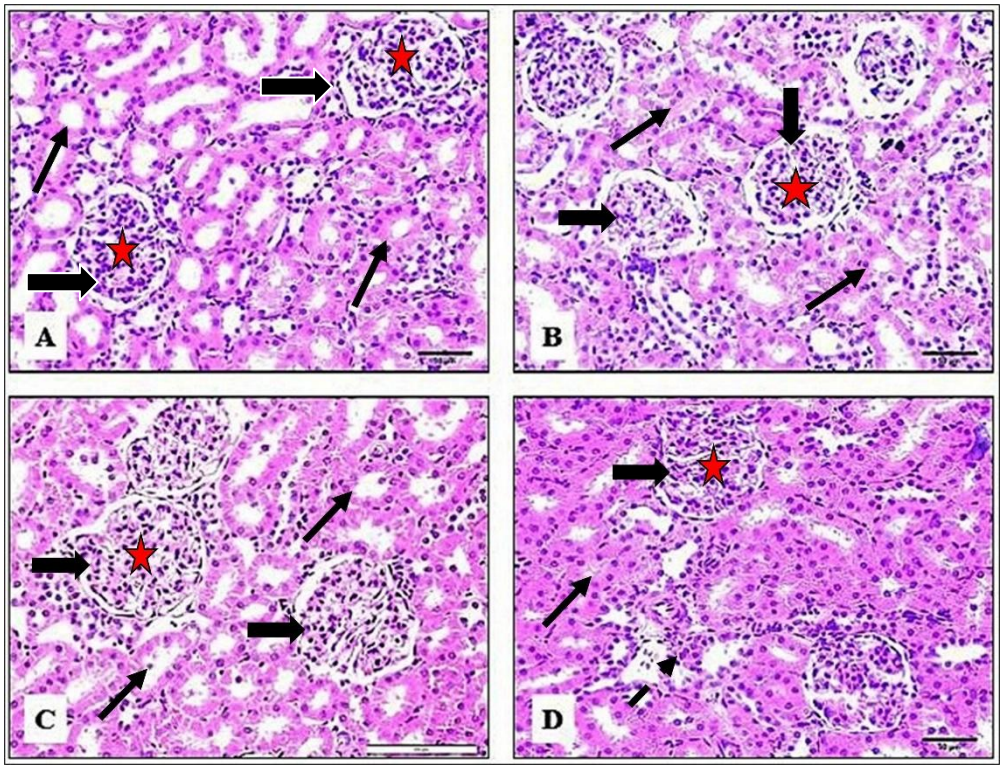
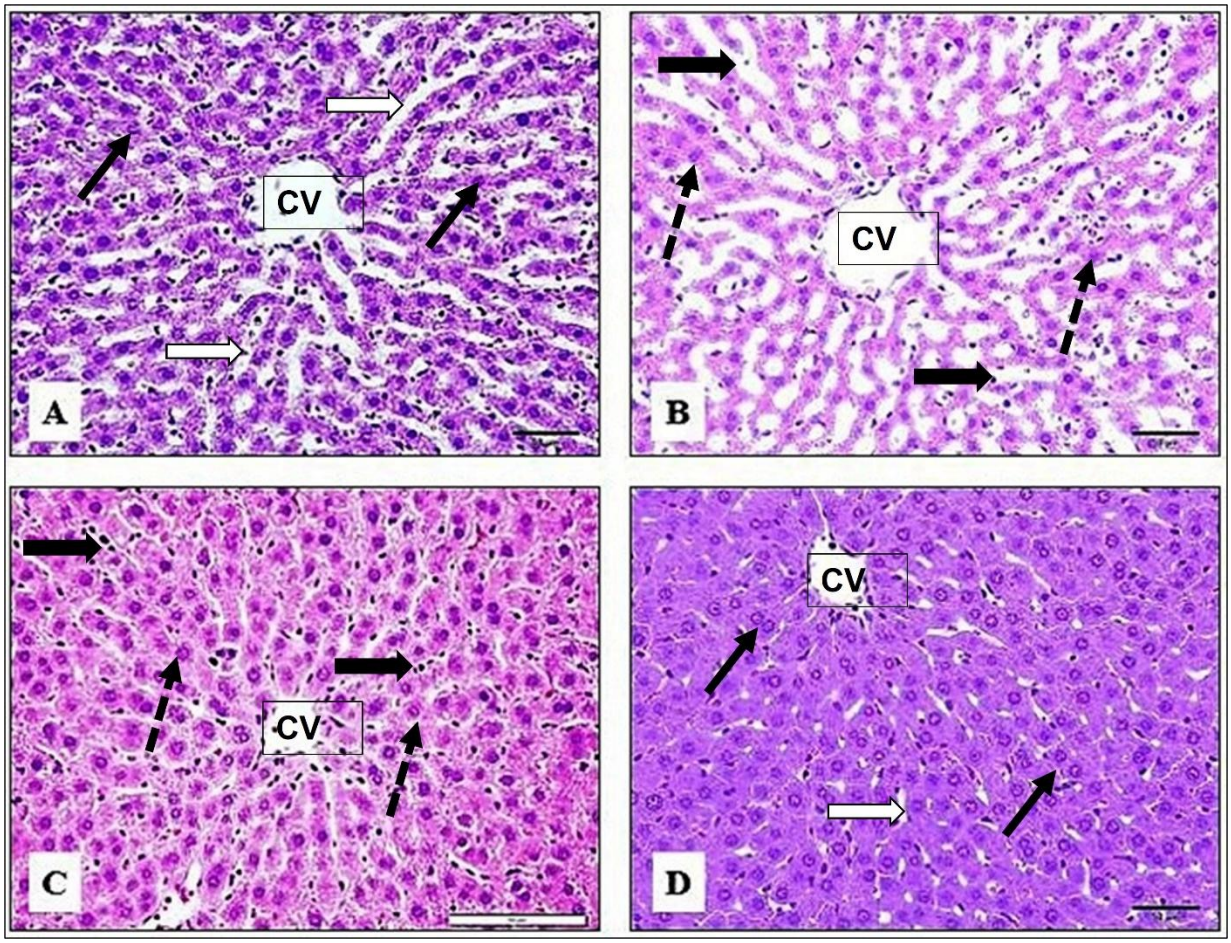


Figure3. Microscopic digital image (x400 Bar= 50µm) of H&E section of the kidney of rats after14 days of oral treatment with A: Control - 0.9% saline; B: CO (250 mg/kg/day); C: CO (500 mg/kg/day); D: CO (1000 mg/kg/day). Treatment with CO extract revealed degenerative as well as necrotic changes in the renal tubular epithelial cells ,glomerular hypercellularity (A-D) with periglomerular (interstitial) leukocytic cells infiltration Observation was made at 40x magnification

Notably, in the control mice liver (Figure 4.) hepatocytes were observed to radiate from the central vein (CV) as branched plates or cords (thin black arrows), separated by thin-walled blood sinusoids lined by endothelium cells (white arrows). Insignificant changes were observed in liver parenchyma of mice administered *CO* extract (250 mg/kg/day). Activation of hepatocytes with increase cell size and nuclei were observed (dotted arrows) . Slight infiltration of sinusoids

with lymphocytes was also evident at the dose of (500 mg/kg/day) of *CO* extract. While at the dose level of 1000 mg/kg/day), hepatocytes looked hypertrophied compressing blood sinusoids. They have active vesicular nuclei (black arrows)

Figure4. Microscopic digital image of H&E section of the liver of rats after 14 days of oral treatment with A: Control - 0.9% saline; B:*CO* (250 mg/kg/day); C:*CO* (500 mg/kg/day); D:*CO* (1000mg/kg/day). Treatment with *CO* extract revealed normal histology. Observation was made at 40 x magnification. Mild degeneration and necrosis in the hepatocytes (B)



The spleen of control mice consists mainly of well-defined white pulp (WP) identified by central arteriole(CA) and consists of densely populated lymphocytes and red pulp (RP) contains blood sinusoids and less dense populated lymphocytes. (Figure5.) Oral Administration of different dose

of *CO* extract for 14 consecutive days was found to increase white pulp follicular germinal center (GC) width Some Samples showed sinusoids congestion especially at (500 &1000mg/kg/day).

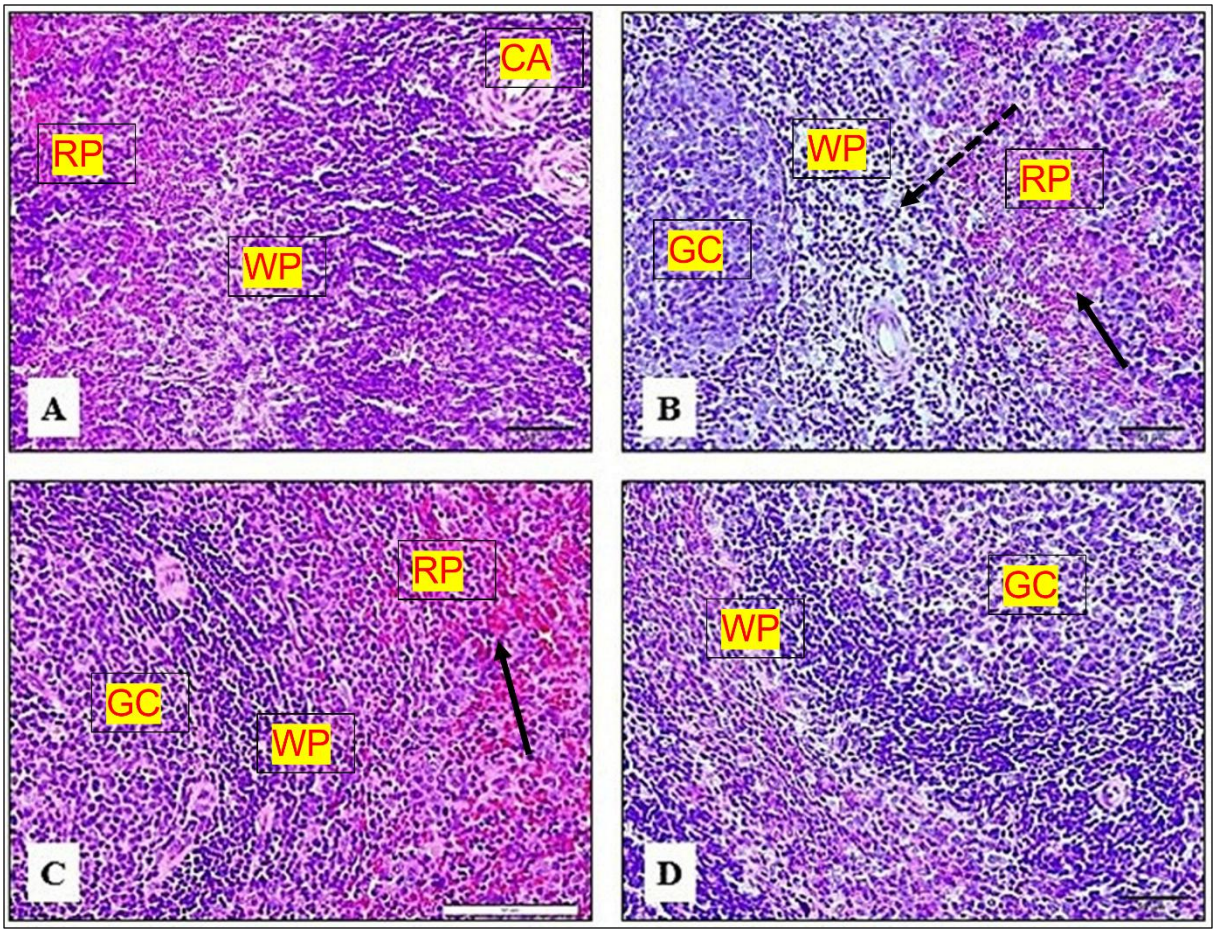


Figure5. Microscopic digital image of H&E section of the spleen of rats after 14 days of oral treatment with A: Control - 0.9% saline; B:*CO* (250 mg/kg/day); C:*CO* (500 mg/kg/day); D:*CO* (1000 mg/kg/day). Treatment with *CO* extract revealed normal histology. Observation was made at 40x magnification. Lymphoid element depletion (interstitial)(B) with foci in hemorrhage (C)

Finally, stomach of control rats showing normal surface mucous cells (black arrows) and underlying glands (white arrows). Furthermore, *CO* in the dose of 250 mg/kg/day revealed more intact healthy epithelium and glands, *CO* (500 mg/kg/day) showing intact surface epithelium with slight epithelial atrophy(thin black arrows) and glands degeneration (thick back arrows). *CO* (1000 mg/kg/day) revealed slight changes in some animals in the form of focal desquamation of surface epithelium (thin black arrows) and atrophy of glands (thick black arrows) in few animals.

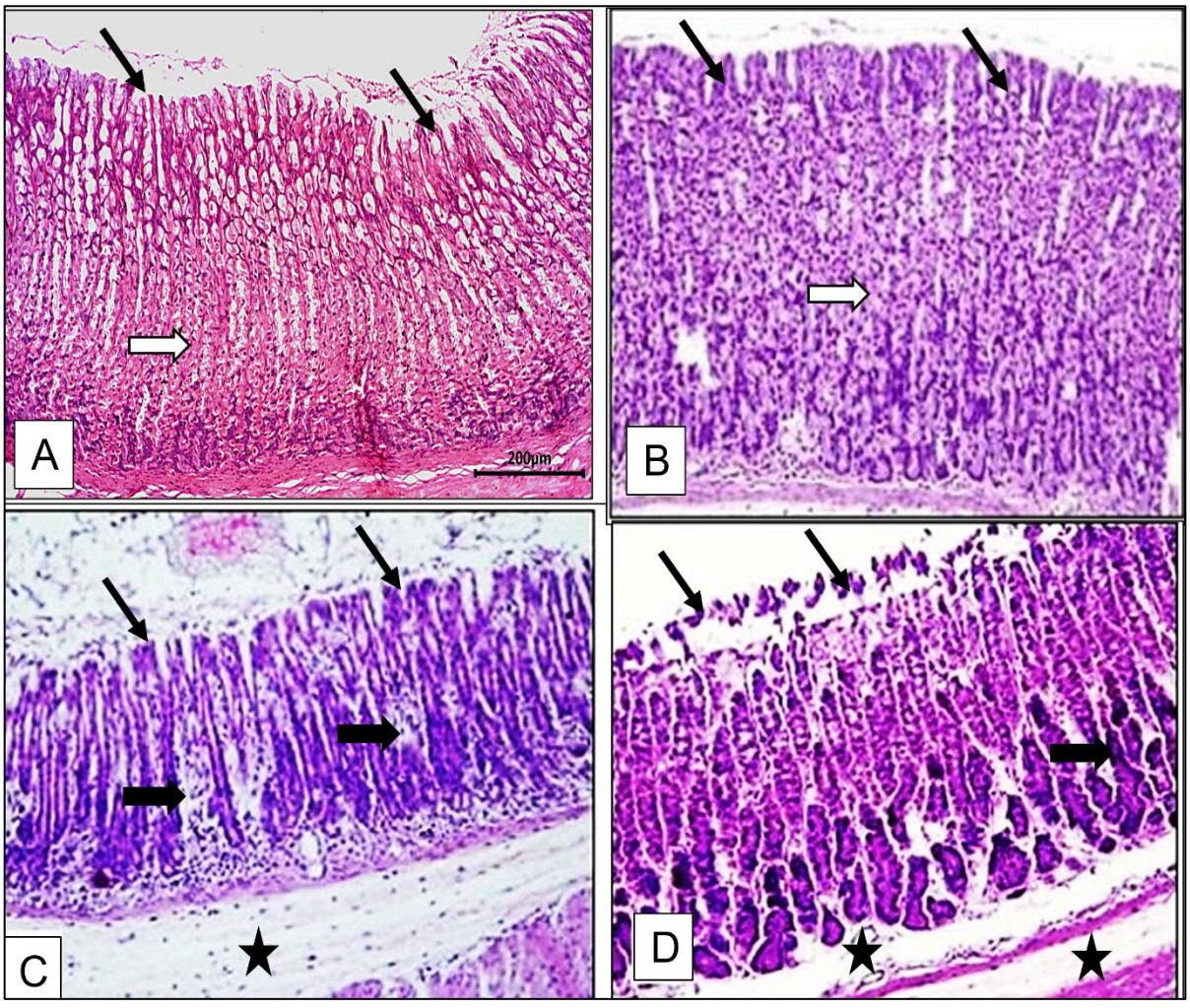


Figure 6. Microscopic digital image of H&E section of the stomach of rats after 14 days of oral treatment with A: Control - 0.9% saline; B:*CO* (250 mg/kg/day); C:*CO* (500 mg/kg/day); D:*CO* (1000 mg/kg/day). Treatment with the *CO* extract revealed normal histology. Gastric epithelial cells were degenerated, necrotic and sloughed with edema in the submucosa. (B&D) Observation was made at 40x magnification.

Discussion

The fundamental essence of conducting safety profile of a medicinal plant is to establish the acute and sub-acute toxic effect on the vital organs of the body in experimental animals[14].Yet, during performance **of** the sub-acute study of toxicity alteration in weight of the organs are highly sensitive marker and of paramount importance for the toxicity and target organ injury[15].Remarkably, hypertrophy of an organ is characterized by weight gain, in contrast reduction of weight is suggestive of necrosis [16]**.**

Interestingly, therapeutic administration of a compound in a chronic disease necessitate s repetitive sub-acute toxicity study since daily use likely leads to deposition in the body compartments with significant steady effect on the vital tissues and the organs[17, 18].

Consequently, in this experimental study of sub-acute toxicity scrutiny of methanolic extract of *CO* was executed in the rats. utilizing histological parameter while hematological and biochemical findings were reported in our earlier studied[2, 3].

Concerning the sub-acute toxicity of *CO* extract, the extract was administered daily in the present study to the healthy rats over 14 days at doses levels of 250, 500and1000mg/kg/day. By the end of the repeat dose oral toxicity assay, rats were assessed for changes in body weight, and histological changes of selected organs. The repeated dose oral toxicity assay demonstrated no significant difference in the body weight of the rats at the end of 14 days of daily therapy of CO extract in the doses of 250,500 and 1000mg/kg/day in comparison with the control group.

Interestingly, these outcomes have significant similarities revealed in other analogous studies[19, 20].It is worth mentioning that the histological evaluation of the tissues and organs are of prime importance for the assessment of drug therapy related to their pathological changes[21].

It needs to be highlighted that histological effect after oral administration of *CO* extract at different doses for 14 consecutive days (Figure 1.) demonstrated to be quite innocuous and was neither accompanied with alteration in the neuronal cellular constituent nor any transformation in cerebral folia, furthermore, the extract of *CO* fails to produce any noteworthy vascular congestion and degenerative changes in the neurons.

The basic hallmark of excellent physiological function of the liver and kidneys revealed their perfect roles in the metabolism and excretion and their exposure to the exogenous chemicals and their metabolites is most likely to results in the toxicity or cellular damage to these organs[22].

Moreover, it needs to be emphasized that the whenever there is exposure to the foreign compounds in rats, irrespective of being hepatotoxic or not, the main target organ to develop acute toxicity is undoubtedly the liver [22, 23].

Remarkably, in this experimental study basic architecture of the liver, the portrait of hepatocytes, portal triads, central vein and the hepatic sinusoids (Figure 4) were observed to be normal as compared with controls. In addition, neither any inflammatory response nor necrosis was demonstrated. Consequently, it can be suggested that methanolic extract of *CO* have no basic interaction with the cellular structures and no alteration in the biological systems of the animals.

Nevertheless, the microscopic observation of kidney in the rats exposed to the graded doses of the *CO* extract didn’t divulge any significant differences in comparison with the control(Figure 3).The in-depth histological analysis of the kidneys of the rats treated with the graded doses of

CO extract illustrated entirely normal morphological configuration of the kidney and absolutely typical façade of tubules and glomeruli, in addition to the well-integrated appearance of the proximal convoluted tubules, distal convoluted tubules and the macula densa.

Correspondingly, architecture of cardiac tissue and spleen section could not be affected by analogous oral treatment of *CO* extract at different doses for 14 consecutive days (Figure.2 and Figure.5 respectively), cardiac fiber seems to be consistent in regularity with fewer fibroblast and the spleen section revealed typical size and cellularity in the white and red pulp.

While, succeeding ahead to observe the toxicological effect of *CO* extract, stomach seems to be additional distinctive organ which commonly bears the direct brunt of toxic effect of any chemical compound and it was also selected for this sub-acute toxicity study, predictably the results of methanolic extract of *CO* fails to produce any significant damaging effect on the surface mucosal cells, epithelial cells and the glands were also remained unaffected, and no atrophic changes were revealed. Strikingly, this present finding is consistent with the past studies of Commiphora molmol conferring protective effect against the indomethacin induced mucosal damage[24] and a study utilizing orally administered balsam extracts protection vis-a vis ethanol induced gastric ulcer[25].

It needs to be stressed that our histological study of the vital organ like brain, heart, liver, kidney, spleen and the stomach has invariably demonstrated an unremarkable architecture of the rats treated with the graded dose of CO extract in comparison with the control group indicating no adverse effect of CO extract on the morphological configuration of the vital organs, a candid and precise reflection of similar findings observed by the preceding analogous studies[24, 25]

Seemingly, it is highly imperative that well-designed studies of chronic toxicity is necessitated for the lucid illustrate of the safety of CO extract prior to its development as a safe as health product.

Conclusion:
Seemingly, the impressive outcome of this fundamental histological scrutiny of methanolic extract of CO demonstrated an imperative facet of safety and correspondingly displayed the key feature of its nontoxic medicinal application for short term supplementation.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Disclosures: None

Conflict of interest: Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

Author contribution: LMK: Conception of the work, editing & proof-reading methodology, **AAS:** Acquisition, experimental work, analysis, and script writing & Statistical analysis. **SSA:** interpretation of data for the work and all histological work. **MAASA:** supervision, validation and software. “All authors have read and agreed to the published version of the manuscript.”

References:

1. Al-Salmi, A.A., M.A. Sattar, L.M. Khan, and S.E. Al-Harthi, *Comparative study of analgesic and anti-inflammatory effects of Commiphora opobalsamum with diclofenac in rodents.* African Journal of Pharmacy and Pharmacology, 2015. **9**(32): p. 806-817.
2. Ahmad, M..A.A.SA., A.A. Al-Salmi, F.O. Kamel, and L.M. Khan, *Evaluation of the toxicological profile of commiphora opobalsamum in wister rats for its safety and rational use.* Journal of Pharmaceutical Research International, 2021: p. 31-42.
3. Al–Salmi, A.A., M.A.A.A.S. Ahmad, and L.M. Khan, *An Experimental Exploratory Study for the Mechanism of Anti-Inflammatory Action of Mecca Myrrh (Commiphora opobalsamum).* Journal of Pharmaceutical Research International, 2021: p. 152-165.
4. Zhao, Y.-L., M. Su, J.-H. Shang, X. Wang, G.S.S. Njateng, G.-L. Bao, J. Ma, Q.-D. Sun, F. Yuan, and J.-K. Wang, *Acute and chronic toxicity of indole alkaloids from leaves of Alstonia scholaris (L.) R. Br. in mice and rats.* Natural products and bioprospecting, 2020. **10**(2): p. 77-88.
5. Ang, L.P., P.W. Ng, Y.L. Lean, V. Kotra, N. Kifli, H.P. Goh, K.S. Lee, M.M.R. Sarker, Y.M. Al-Worafi, and L.C. Ming, *Herbal products containing aristolochic acids: A call to revisit the context of safety.* Journal of Herbal Medicine, 2021. **28**: p. 100447.

6. Han, H.-Y., J.-I. Huh, S.-R. Han, M.-G. Kang, S. Yoon, J.-S. Han, B.-S. Lee, J.A. Kim, and B.-S. Min, *Assessing the safety of an Ephedrae Herba aqueous extract in rats: A repeat dose toxicity study.* Regulatory Toxicology and Pharmacology, 2018. **94**: p. 144-151.

7. Langrand, J., H. Regnault, X. Cachet, C. Bouzidi, A. Villa, L. Serfaty, R. Garnier, and S. Michel, *Toxic hepatitis induced by a herbal medicine: Tinospora crispa.* Phytomedicine, 2014. **21**(8-9): p. 1120-1123.

8. Schlansky, B. and J.H. Hwang, *Prevention of nonsteroidal anti-inflammatory drug-induced gastropathy.* Journal of gastroenterology, 2009. **44**(19): p. 44-52.

9. Kidd, B.L., *Osteoarthritis and joint pain.* Pain, 2006. **123**(1): p. 6-9.

10. Sawant, S.B., G. Bihani, S. Mohod, and S. Bodhankar, *Evaluation of analgesic and anti-inflammatory activity of methanolic extract of curcuma caesia roxb. rhizomes in laboratory animals.* Int J Pharm Pharm Sci, 2014. **6**(2): p. 243-247.

11. Adeneye, A., O. Ajagbonna, T. Adeleke, and S. Bello, *Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats.* Journal of ethnopharmacology, 2006. **105**(3): p. 374-379.

12. Ramírez, J.H., M. Palacios, O. Tamayo, R. Jaramillo, and O. Gutiérrez, *Acute and subacute toxicity of Salvia scutellarioides in mice and rats.* Journal of ethnopharmacology, 2007. **109**(2): p. 348-353.

13. Suvarna, K.S., C. Layton, and J.D. Bancroft, *Bancroft's theory and practice of histological techniques E-Book.* 2018: Elsevier health sciences.

14. Ibrahim, M.B., A.A. Sowemimo, M.O. Sofidiya, K.B. Badmos, M.S. Fageyinbo, F.B. Abdulkareem, and O.A. Odukoya, *Sub-acute and chronic toxicity profiles of Markhamia*

tomentosa ethanolic leaf extract in rats. Journal of ethnopharmacology, 2016. **193**: p. 68-75.

15. Michael, B., B. Yano, R.S. Sellers, R. Perry, D. Morton, N. Roome, J.K. Johnson, and K. Schafer, *Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices.* Toxicologic pathology, 2007. **35**(5): p. 742-750.
16. Teo, S., D. Stirling, S. Thomas, A. Hoberman, A. Kiorpes, and V. Khetani, *A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Sprague–Dawley rats.* Toxicology, 2002. **179**(3): p. 183-196.
17. Abotsi, W., G.K. Ainooson, E.B. Gyasi, and W.K.M. Abotsi, *Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of Hilleria latifolia (Lam.) H. Walt.(Phytolaccaceae) in rodents.* West African Journal of Pharmacy, 2011. **22**(1): p. 27-35.
18. Bariweni, M.W., O.I. Yibala, and R.I. Ozolua, *Toxicological studies on the aqueous leaf extract of Pavetta crassipes (K. Schum) in rodents.* Journal of Pharmacy & Pharmacognosy Research, 2018. **6**(1): p. 1-16.
19. Abdallah, E.M., H.E. Khalid, and K.S. Al-Khalifa, *Toxicological assessment of the oleo-gum resins of Commiphora molmol and Boswellia papyrifera in rats.* Journal of Medicinal Plants Research, 2009. **3**(7): p. 526-532.
20. Donkor, K., L.N. Okine, W.K. Abotsi, and E. Woode, *Acute and Sub-Chronic Toxicity Studies of aqueous extract of root bark of Cassia sieberiana DC in Rodents.* 2014.
21. Loha, M., A. Mulu, S.M. Abay, W. Ergete, and B. Geleta, *Acute and subacute toxicity of methanol extract of Syzygium guineense leaves on the histology of the liver and kidney*

and biochemical compositions of blood in rats. Evidence-Based Complementary and Alternative Medicine, 2019. **2019**.

22. Marcela, E., B. Silvia, C. Jaime, and P. Pilar, *Effect of Solanun nudum extracts on the liver of mice infected with Plasmodium berghei.* The American journal of Chinese medicine, 2001. **29**(03n04): p. 477-484.

23. Debelo, N., M. Afework, A. Debella, E. Makonnen, W. Ergete, and B. Geleta, *Assessment of hematological, biochemical and histopathological effects of acute and sub-chronic administration of the aqueous leaves extract of thymus schimperi in rats.* Journal of Clinical Toxicology, 2016. **6**(286): p. 2161-0495.

24. Al-Harbi, M., S. Qureshi, M. Raza, M. Ahmed, M. Afzal, and A. Shah, *Gastric antiulcer and cytoprotective effect of Commiphora molmol in rats.* Journal of Ethnopharmacology, 1997. **55**(2): p. 141-150.

25. Al-Howiriny, T., M. Al-Sohaibani, M. Al-Said, M. Al-Yahya, K. El-Tahir, and S. Rafatullah, *Effect of Commiphora opobalsamum (L.) Engl.(Balessan) on experimental gastric ulcers and secretion in rats.* Journal of ethnopharmacology, 2005. **98**(3): p. 287-294.