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The Effect of *Cymbopogon citratus* Essential Oil-Water Emulsion on Some Blood Parameters and Histopathology of Liver and Kidney in Mice

Abinet Gebremickael[a*], Girmai Gebru[b], Asfaw Debella[c], Getachew Addis[d], Eyasu Makonnen[e], Yonas Bekuretsion[f]

[a]*Department of Biomedical Sciences, School of Medicine, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia.*

[b]*Department of Anatomy, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.*

[c,d]*Traditional and Modern Medicine Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia.*

[e]*Department of Pharmacology, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.*

[f]*Department of Pathology, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.*

[a]*Email: abinetgebremickael@gmail.com/abinet.gebremickael@amu.edu.et*

[b]*Email: girmai.gebru4@gmail.com*

[c]*Email: asfawdebella@gmail.com /*

[d]*Email: assfawd@ehnri.gov.et*

[e]*Email: eyasumakonnen@yahoo.com*

[f]*Email: yonasbtsion@yahoo.com*

Abstract

This study was aimed to assess the possible toxic effects of *Cymbopogon citratus* essential oil-water emulsion after sub chronic oral administration in mice.

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* Corresponding author.

The essential oil extraction of the leaves of *C.citratus* was carried out through hydro-distillation technique. In this sub-chronic toxicity evaluation, two treatment groups of mice each containing twelve animals were used. Two test doses, 1.5 and 2.5ml/kg, were selected based on the earlier findings of acute toxicity study on similar emulsion in mice and administered to the treatment groups. The control group animals were received vehicle. At the end of 12 consecutive week sub-chronic administration, blood samples were taken by cardiac puncture and then, all animals were sacrificed and the vital organs were collected for gross and histopathological examinations. No death was recorded, and there was no significant change in the evaluated hematological and biochemical parameters after the sub-chronic oral administration in mice. Besides, there was no obvious change in sections of the liver and kidneys of mice treated at 1.5ml/kg dose. However, some pathological changes were observed in liver sections of mice treated at 2.5ml/kg dose. The emulsion at and below 2.5ml/kg body weight doses does not produce obvious toxic effects after sub chronic treatment in mice. Further toxicological investigation is however, needed to confirm this.

Keywords: Hydro-distillation; *Cymbopogon citratus;* Emulsion; Sub-chronic; Essential oil.

1. Introduction

Ectoparasitic infestations constitute an important health problem of ruminant livestock's in Ethiopia. Though several problems are associated with the use, there are now many alternatives of synthetic anti-ectoparasitic drugs. The problems associated with the use of synthetic drugs lead to research efforts to discover new plant derived natural compounds that are widely accepted as safe. *Cymbopogon citratus*, commonly called lemongrass, is an aromatic grass belonging to the family Gramineae. It is generally cultivated almost in all tropical and subtropical countries [1]. The essential oil obtained from leaves of this plant has antibacterial and antifungal activities [2, 3]. Its sedative and anticonvulsant properties as well as its use as an anxiolytic agent has been documented [4]. Hot water extract of its dried leaves is taken orally as a hypotensive, for catarrh and rheumatism in Cuba and as a renal antispasmodic and diuretic in Egypt [5]. Parallel with recent increasing interest in using herbal medicines, there is increasing concern about their safety. Literatures have documented severe toxic reactions from the use of herbs and spices for medication because of some toxic bioactive chemicals contained in some plants [6, 7]. Inappropriate formulation, dosage, and lack of understanding of acute and long term effects of the use of drugs or traditionally used medicines had led to adverse reactions that are sometimes life threatening [8]. Earlier findings of a collaborative work between the Ethiopian Public Health Institute (EPHI) and Addis Ababa University (AAU) / Faculty of Veterinary Medicine (FVM), Ethiopia, has reported that a 2.5%*Cymbopogon citratus* essential oil-water emulsion with *Jatropha curcas* oil, which is used as fixer, prepared for the control of major animal ectoparasites was found effective against damalinaand lenognatus species of lice [9, 10]. Beside the antiectoparasitic efficacy, investigators also reported that the emulsion was tested for skin sensitization potential in small ruminants and found safe. However, infested animals were aggressively licked and internalized the emulsion following topical treatment. Hence, it was found compulsory to carryout toxicological evaluation to ensure the safety of the emulsion when internalized. Accordingly, this study was aimed to assess the sub-chronic toxic effects of the emulsion after oral administration in mice.

2. Materials and Methods

2.1 Study setup

The study was chiefly conducted at Traditional and Modern Medicine Research Directorate (TMMRD) Laboratories of EPHI, Addis Ababa, Ethiopia. Tissue processing and photomicrography of tissue sections was conducted at the Histology laboratory of department of Anatomy, College of Health Science, AAU.

2.2 Plant material collection, extraction and preparation of an emulsion

The leaves of *Cymbopogon citratus* were collected from Wondogenet Agricultural Research Center (WARC) experimental plot, located in Southern Nations, Nationalities and Peoples' Region (SNNPR), 260 km south of Addis Ababa, the capital city of Ethiopia. A taxonomist in the TMMRD of EPHI did the botanical identification and authentication of the collected plant specimens. Freshly collected leaves of *C.citratus* were cleaned by using distilled water and air-dried at room temperature under shade. Then, chopped in to smaller pieces and the essential oil extraction was carried out by using hydro-distillation technique in a modified Clevenger-type apparatus [11]. The obtained oil was then filtered and then concentrated using rotary evaporator. An emulsion comprising 2.5% concentration of *C.citratus* essential oil in water by using 2% Tween 80 as emulsifier was prepared for subsequent experiment.

2.3 Experimental animal preparation

All animals used in this study were bred and reared at the animal house of the EPHI and transported to the TMMRD Laboratories. Experiments were conducted on healthy adult male and female albino Swiss mice aged 6-8 weeks weighing 23-30g. The male and female mice were kept in separate aluminum cages with bedding of clean paddy husk in well ventilated house at maintained temperature (22 ± 3^0C) and 12 hours light/dark cycles till the end of the experiment. All animals had a free access to standard pellet diets and drunken tap water *ad libitum*. The mice were acclimatized to laboratory conditions for a week prior to the experimental protocol to minimize any nonspecific stress [12].

2.4 Sub-chronic toxicity study

The sub-chronic toxicity study was carried out through twelve weeks (84 days). In this study, two doses (1.5ml/kg and 2.5ml/kg body weight) were selected based on the earlier findings of acute toxicity study on similar emulsion in mice. The control group animals were received vehicle. In accordance with the OECD guideline [13], the calculated doses of an emulsion were diluted by additional water to raise the volume administered at one time to 1ml/100g body weights of a mouse in 24 hours interval throughout the study period. For repeated dose sub chronic toxicity study, thirty six albino Swiss mice were used. Before dosing, they were assigned to three groups (G-I, G-II, and G-III) each consisting of twelve animals of both sex. The male and female mice were kept in separate aluminum cages and the cages were labeled appropriately. The control group (G-I) received vehicle while G-II and G-III were administered with 1.5 and 2.5 ml/kg body weight test doses of the emulsion respectively.

2.5 Hematological and biochemical analyses

24 hours after the last oral administration of the emulsion, blood samples were collected by cardiac puncture. Some of the blood samples obtained from each mouse were then collected in separate test tubes with Ethylene Diamine Tetra-acetic Acid (EDTA) and the other in plain test tubes. Blood samples from EDTA containing test tubes were immediately processed for hematological parameters to dermine hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLC), red blood cell count (RBC), White blood cell count (WBC) using Automated Hematological Analyzer, SYSMEX XT-1800i (SYSMEX CORPORATION, Japan). Blood samples in the plain test tubes were allowed to stand for 3 hours for complete clotting and then centrifuged at 5000 rpm for 15 minutes using a bench top centrifuge (HUMAX-K, HUMAN-GmbH, Germany) to obtain the sera. Biochemical analysis of the sera was done by using COBAS INTEGRA 400 plus Analyzer (ROCH DIAGNOSTICS, Japan) and, the concentrations of Alanine Transaminase (ALT), Aspartate Transaminase (AST), total bilirubin, urea, uric acid and creatinine were determined.

2.6 Histopathological studies

The liver and kidneys collected from all mice were processed through routine tissue processing technique and the specimens were stained using Hematoxylin and Eosin staining method for histopathological examination. Stained tissue sections were carefully examined under binocular compound light microscope (LEICA DM 750, Germany). After examination, photomicrograph of selected samples of liver and kidney sections of both treated and control group animals were taken under a magnification X200 and x400 by using (LEICA ICC50 HD, Germany) automated built-in digital photo camera.

2.7 Statistical analysis

All data were organized and analyzed by using Statistical Package for Social Sciences (SPSS) version-16 software packages. Values of the body weight changes, hematological and biochemical parameters were analyzed and the results were expressed as M ± SEM (Mean ± standard error of the mean). Differences between the treated and control groups were compared using one-way analysis of variance (ANOVA), followed by Dunnett's t-test to determine their level of significance. Differences at $p<0.05$ were considered statistically significant.

2.8 Ethical consideration

The study design and the use of the experimental animals were reviewed and approved by the Institutional Review Board (IRB) of the school of Medicine, College of Health Science, AAU. The handing of animals was conducted in accordance with international guidelines of the care and use of laboratory animals.

3. Results

The sub-chronic toxicity study was carried out to assess effect of the emulsion on some blood parameters, liver,

and kidney after prolonged oral administration in mice.

The 12 subsequent weeks daily treatment with the emulsion did not result in statistically significant ($p>0.05$) changes in any of the hematological parameters of the mice at both 1.5 and 2.5ml/kg body weight test doses as compared to the controls (Table 1).

Table 1: Comparison of the evaluated hematological parameters between the mice treated with both doses of *Cymbopogon citratus* essential oil-water emulsion and vehicle control

Hematological Parameters	Control	1.5ml/kg dose	2.5ml/kg dose
WBC ($x10^3$/µL)	4.53 ± 1	2.7 ± 0.11 (0.09)	2.6 ± 0.48 (0.09)
RBC ($x10^6$/µL)	9.6 ± 0.28	8.9 ± 0.25 (0.12)	8.9 ± 0.12 (0.15)
HGB (g/dL)	14.6 ± 0.43	13.4 ± 0.32 (0.87)	14.1 ± 0.17 (0.99)
HCT (%)	44.6 ± 0.9	40.6 ± 0.83 (0.81)	45.67 ± 1.77 (0.99)
MCV (fL)	46.5 ± 0.43	45.58 ± 0.93 (0.92)	48.1 ± 2.09 (0.14)
MCH (pg)	15.2 ± 0	15 ± 0.23 (0.98)	15.7 ± 0.25 (0.70)
MCHC (g/dL)	32.7 ± 0.33	33 ± 0.44 (0.99)	31 ± 0.8 (0.53)
PLT ($x10^3$/µL)	1077.3 ± 44.9	1037.4 ± 45 (0.99)	951.7 ± 208.2 (0.96)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control.

Treatment with both doses of the emulsion did not also bring statistically significant ($p>0.05$) changes in any of the tested serum biochemical parameters as compared to the control (Table 4).

Table 2: Comparison of the evaluated biochemical parameters between the mice treated with both doses of *Cymbopogon citratus* essential oil-water emulsion and vehicle control

Biochemical Parameters	Control	1.5ml/kg dos	2.5ml/kg dose
Uric Acid	4.88 ± 2.16	4.37 ± 0.44 (1.00)	15.24 ± 12.05 (0.44)
ALT (IU/L)	84.2 ± 8.52	78.14 ± 5.74 (0.98)	57.50 ± 8.83 (0.52)
AST(IU/L)	142.5 ± 29.47	288.3 ± 33.27 (0.99)	137.55 ± 20.30 (0.09)
Total Bilirubin	1.88 ± 0.41	4.29 ± 1.09 (0.50)	1.42 ± 0.39 (0.48)
Urea (mg/dL)	56.5 ± 6.6	50.4 ± 4.73 (0.95)	68.25 ± 17.85 (0.74)
Creatinine (mg/dL)	0.57 ± 0.12	0.46 ± 0.06 (0.91)	0.5100 ± 0.04 (0.99)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control.

There was no morbidity, noticeable change in the general behavior, and toxicity related death report of mice treated at both test doses throughout the sub-chronic study period. Moreover, there was no change in postmortem gross visceral examinations of treated mice at the end of the study.

Microscopic examinations of liver sections of control group mice (Figure 1A) and mice treated with 1.5ml/kg body weight dose of the emulsion (Figure 1B) showed normal general microscopic architecture of the liver. However, in some areas of the liver sections of mice treated at 2.5ml/kg body weight dose (Figure 1C), the general microscopic architecture of hepatic lobules was not maintained, hepatocytes appeared shrunken, hepatic sinusoids altered.

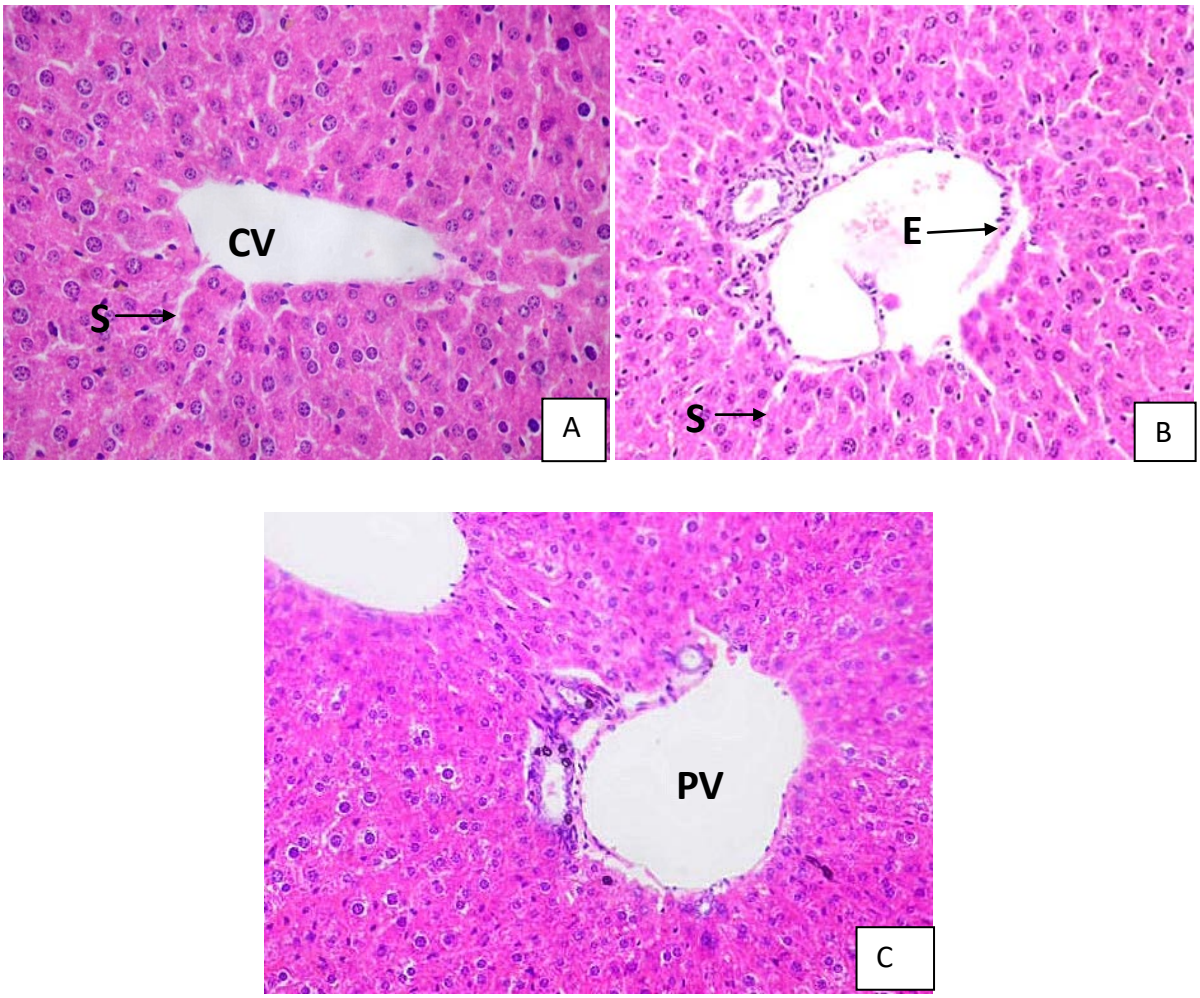

Figure 1: Photomicrographs of liver sections of control mice (A), mice administered with 1.5ml/kg body weight dose (B), mice administered with 2.5ml/kg body weight dose (C). CV = Central vein, PV = Portal vein, E = Endothelial cells, S = Hepatic Sinusoids. (Sections were stained with H&E, X200).

Sections obtained from kidneys of a control group animals (Figure 2A) revealed normal glomeruli and the tubular microscopic appearance. Similar to the control, Examination of kidney sections of mice treated with the emulsion at both 1.5ml/kg (Figure 2B) and 2.5ml/kg doses (Figure 2C) showed the normal histological structures of kidney, and no obvious pathological change were observed.

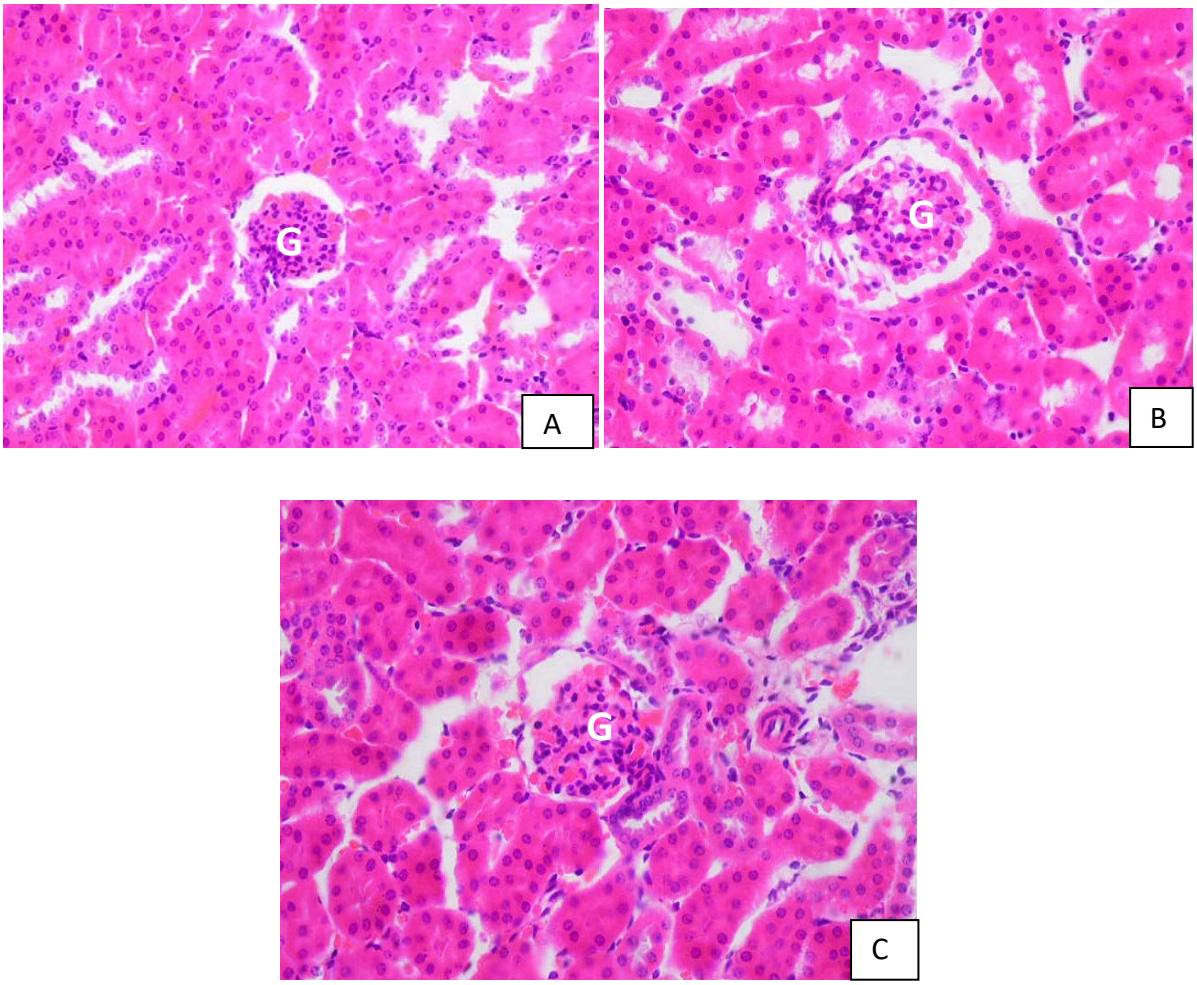

Figure 2: Photomicrographs of kidney sections of mice in the control group (A), mice treated with 1.5ml/kg dose (B), and 2.5ml/kg dose of the emulsion. G= Glomerulus. (Sections were stained with H & E, X200).

4. Discussion

The present study was designed to reveal the possible toxic of an emulsion comprising essential oil of *Cymbopogon citratus* after sub-chronic oral administration in mice.

The sub-chronic toxicity study was conducted with repeated daily administration of 1.5 and 2.5ml/kg body weight doses of the emulsion to investigate its long term effects on some selected parameters. The three months daily treatment with the emulsion at both doses did not show any toxicity related mortalities and changes in general health, behavior, and growth. Hence, it can be concluded that the emulsion at these test doses does not have effect on growth and normal functions of mice after prolonged administration [14].

The extent of toxic effect of drugs can be determined by assessment of haematological parameters, because blood profile usually provides important information on the response of the body to injury or lesion, deprivation and stress [15, 16]. In clinical pathology, the core haematology tests recommended include total WBC count, RBC count, platelet count (PLT), hemoglobin concentration (Hgb), HCT, MCV, MCH, and MCHC [17].

Red blood indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are the most useful indicators in the diagnosis of anemia in most animals [18, 19]. Effect of the emulsion on MCV, MCH, and MCHC was not significant ($p>0.05$) in both treated groups as compared to the control. These observations demonstrate that the emulsion at both 1.5 and 2.5ml/kg doses does not affect red blood indices in mice. The changes in RBC count, average haemoglobin (Hgb) and hematocrite (HCT) levels of treated group animals were also insignificant ($p>0.05$) as compared with the control. This result is in line with the finding of a related study conducted by Ademuyiwa and his colleagues [24]. The 12 weeks administration of both doses of the emulsion used in this study did not significantly change ($p>0.05$) the total WBC count as compared to the control. This result may indicate that the emulsion does not possess chemicals capable of inducing either leukocytosis or leucocytopenia. Besides, the platelets count indicated that the emulsion also does not induce thrombocytopenia or thrombocytosis at both doses after prolonged treatment [19].

Serum biochemical parameters have significant importance to evaluate changes produced by a toxicant. Elevated serum levels of enzymes produced by the liver or nitrogenous wastes to be excreted by the kidney might be indications to their spillage into the blood stream as a result of necrosis of the tissues [16, 20].

Liver function tests include several serum chemistries that reflect the state of liver function. The most commonly used serum liver chemistry tests include serum transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)). Injuries to liver cells allow the escape of these enzymes and raise their levels in the blood [21]. However, in Swiss albino mice treated with both doses of the emulsion tested in this study, there was no significant change on the serum level of ALT and AST as compared to the vehicle received control. This indicates that the emulsion at both doses does not cause significant hepatic damage after prolonged oral administration.

Kidney function test is a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning. Many conditions can affect the ability of kidneys to carry out their vital functions. A decline or failure of kidney function can result in a buildup of metabolic waste products in the blood. Therefore, the measurement of concentrations of various chemicals in the blood normally regulated by the kidneys can help to determine the cause and extent of kidney dysfunction. Accordingly, renal function can be assessed by measuring the levels of plasma creatinine, urea and uric acid concentrations [22]. The serum level of creatinine, urea and uric acid concentrations in mice treated at both doses of the emulsion did not show significant alteration after twelve weeks oral administration. This suggests that the emulsion does not cause significant alteration in the renal function at doses tested in the present sub chronic toxicity study in mice.

Histopathological examinations indicated that some of the liver sections obtained from mice treated with the emulsion at 2.5ml/kg body weight test dose showed distorted general hepatolobular architecture, including eradication of the hepatic sinusoids, as observed in other related studies conducted by Ebaid and his colleagues [23]. However, all these changes were not accompanied by significant change in any of the biochemical markers of liver injury tested in this study. This result may indicate that the changes were minor and insignificant. Conversely, liver sections obtained from mice treated at 1.5ml/kg body weight dose, and kidney sections of

mice treated at both doses of the emulsion maintained the normal microscopic appearances similar to the control.

5. Limitations of the study

In this study, the effect of emulsion on gastrointestinal tract and viscera's other than liver and kidneys was not considered. Besides, effect of the emulsion on higher experimental animals was not studied.

6. Conclusion and recommendations

It can be concluded from the findings of the present study that 2.5% *Cymbopogon citratus* essential oil-water emulsion does not produce significant toxic effect after sub chronic oral treatment at doses up to 2.5ml/kg body weight. However, further toxicological investigation is recommended on other vital internal organs as well as on non rodent species to confirm this result.

7. Author contribution

AG has contributed to conception and design of the study, conducted the experiment, drafted the manuscript, recorded, analyzed and interpreted the data; GG was involved in tissue processing, slide examination and photomicrography; AD and GA were involved in plant material collection, extraction and preparation of the emulsion, close follow up and supervision of the experiment; EM and YB were engaged in the pharmacological and pathological concerns of the study respectively; All the authors read the draft manuscript, improved and approved the final manuscript.

8. Conflict of interest

The authors report no conflicts of interest in this work.

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