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Evaluation of the Toxicological Profile of *Commiphora opobalsamum* in Wister Rats for Its Safety and Rational Use

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MAAASA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AAAS performed the experimental studies and statistical analysis. Author FOK managed the literature searches and author LMK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To study acute and chronic toxicity profile of *Commiphora opobalsamum* (CO) in rats.
Methods: Acute oral lethal effect and single oral dose toxicity of CO was determined in rats by comparison with standard control. In contrast repeated dose oral toxicity in rats were performed in four groups of rats, CO was orally administered to three groups of rats in graded doses (250, 500 and 1000 mg/kg/day) once daily for 14 daysVs standard control. The sub-acute toxicity of CO was observed on hematological, coagulation and biochemical parameters.
Results: Acute oral lethal dose of CO in rats was demonstrated to be safe and higher than the tested dose i.e., 2000 mg/kg, moreover, single oral dose toxicity revealed no symptoms of toxicity. Furthermore, sub-acute toxicological effect on hematological analysis of CO rats groups treated

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withgraded dosed after 14 days treatment revealed insignificant decrease in complete blood count (CBC)in contrast with the control group ($p>0.05$) additionally the biochemical parameters includedanalysis of urea, creatinine, glucose; lipid profile and thyroid function markers fairly demonstrated insignificant differences utilizing the same methodology.

Conclusion: Our current explorative study strongly substantiates that the CO extract have no significantoral toxicity in rats and even the results of 14 days oral treatment indicated that there were no obvious toxic effects at the dosage of 1000 mg/kg/day. The comprehensive sub-acute toxicological effect of CO extracts on hematological and coagulation parameters and biochemical parameters included analysis of urea, creatinine, glucose; lipid profile and thyroid function markers fairly demonstrated no significant harmful outcome even after 14 days treatment with CO.

Keywords: Commiphora opobalsamum; acute toxicity; chronic toxicity; lethal dose and toxic dose.

1. INTRODUCTION

All drugs with a future plan to accomplish the task of therapeutic benefits and eventually to enhance the quality of life are likely to have some adverse effects varying from minor effect to potentially very serious and infrequently lethal effect [1]. However, it is pertinent to observe that sometime the products or active ingredients of herbal compounds are not devoid of major adverse effects [2]. Interestingly, it is observed that some of the active ingredient of many herbal compounds was found to have some major adverse effects.The gloomy scenario created by such observation of adverse effect and toxicities, aroused an alarm about the safety of herbal medications [3]. Moreover, several animal experimental studies revealed significant harmful effects as well as toxicities of herbal medicines after prolonged therapeutic use. Hence, it is of vital importance to perform comprehensive critical assessment of the medicinal herbs in view of finding their safety profile in the needy patients [4]. *Commiphora opobalsamum* (CO) is one of commonly utilized plants in folk medicine for treating injuries, pain and inflammatory disorders. CO belongs to a member ofhuge general and regular consumed family of plant recognized as Burseraceae, it possesses a distinguishing and illustrious odor and this is well distinguished and designated as "Al-Besham", “balsam”, “Al-Balessan” and "balsam of Mecca" in our culture. Basically, CO is a shrub, usually detected in large amount on hilly areas of sacred places of Makkah, Madina in Saudi Arabia and Al-Quds in Palestine [5-7]. As the CO is commonly used among people in our culture, since they believe the myth that stated, "all products with natural source are safe." It is pertinent to observe that quite restricted studies of CO are performed by the researchers, yet a constrained exploration was executed on the toxicological effect of this plant. Remarkably, development of any new herbal medication from a plant often necessitates extensive information regarding its toxicity. Consequently, the basic objective to perform this study is investigate the methanolic extract of CO in the experimental animals to evaluate its toxicological effects through estimation of its oral lethal dose (LD50), evaluate its acute toxicological effect and evaluates its sub-acute toxicological effect in rats to assess its safety for rational drug use.

2.MATERIALS AND METHODS

2.1 Animals

This experimental work was performed by usingmale Wister rats of 10-12 weeks age, weighing 170-200 gm, the animals were purchased from the King Fahad Research Center, King Abdulaziz University, Jeddah, Saudi Arabia, The animals were kept under the optimum laboratory environment (at the temperature 25±5°C, relative humidity of 30-70% and 12/12h light and dark automated cycles) for a minimum period of one week prior to the commencement of experiments. The experimental animals were housed in transparent plastic cages (six rats in each cage) with water and food supply ad libitum. The experimental procedures was approved by KFMRC and conducted according to their guidelines, and permission of the institutional ethical committee were acquired prior to the initiation of the experimental study.

2.2 Plant Material and Extraction

The aerial parts of CO were collected from Az-zemah, KSA (from a village located between the city of Taif and Makkah, KSA) (Herbarium number: 21.618466, 40.107040) during the summer of 2015, its taxonomicalrecognition was performed in thedepartment of natural product &

alternative medicine, college of pharmacy, KAU, KSA. Extraction of the plant was executed by the procedure illustrated by Sawant S. B. et al 2014 [8]. Subsequently, the stem of the plant and its aerial part were stored carefully in a suitableventilated dry place under controlled temperature of 30-40^{o}C for a period of 5 days to allow natural drying and then mechanically grinded to powder form (900 gm) this was followed by subjecting it to soak in 99% w/v methanol at controlled room temperature (25±30C) for a period of 48 hours followed by extraction four times. Then it was subjected to double filtration to remove the fine particles using cotton and filter papers. Additionally, by the utilization of rotator evaporator (Buchi®, Schweiz) evaporation of the filtrate was done, in order to get rid of even minor amount of methanol. Summarily, the final product is acquired which is moist in natureand it was kept in the refrigerator at -80^{o}C approximately for one hour, then it was subjected to overnight drying in a freeze vacuum dryer (Zirbus®German) at 85^{o}C.The ultimate product of approximately 100gm was stored carefully in a light protective container, kept in a refrigerator under controlled temperature. The administration of the extract was done by preparing a suspension in 0.9% saline solution immediately prior to administration and the volumesadministered were adjusted to 10 ml/kg body weight of the rats.

2.3 Experimental Methods

Acute oral lethal effect and single oral dose toxicity of CO was determined in rats by comparison with standard control. In contrast repeated dose oral toxicity in rats were performed in four groups of rats, each group comprises of six rats. CO was orally administered to three groups of rats in graded doses (250, 500 and 1000 mg/kg/day) once daily for 14 days Vs standard control. The sub acute toxicity of CO was observed on hematological, coagulation and biochemical parameters.

2.4 Oral Lethal dose in Rats

The oral lethal dose of CO extract was estimated using method of the "limit dose test" by following the guidelines of "Organization of Economic Co-operation and Development (OECD)."In earlier studies of instructions for guidelines for examination of chemicals in animals recommended a maximum (limiting) dose of 2000 mg/kg body weight by oral administration. Furthermore, the limit test uses a maximum of 5 animals [9,10]. Random selection of five male rats was done and subsequently placed individually for 5 days prior to the administration of dose of the drug with food and water available all the time. Furthermore, rats were subjected to overnight fasting with free access to water, before calculation of the dose, body weight of each fasting rat was determined. The amount of the CO extract was administered in the form of single dose by means of a gavage utilizing stomach tube, after each dosing withholding of food was ensured for a period of 3-4 hours. Moreover, following the calculation of exact lethal dose, initially only one animal was tested and observed for survival then four additional rats was consequently tested; they were monitored for mortality up to 14 days, if three or more rats survived than LD_{50} is recognized as > 2000 mg/kg while if three or more animal will die than LD_{50} is considered as <2000 mg/kg.

2.5 Oral Toxicity Study with Single dose

In order to estimate the acute toxicity of CO extract, selection of the method was based on "OCED Test guideline on acute oral toxicity" [11]. This was initiated by taking three groups of rats consisting of 6 rats in each group, they were administered three different doses of CO extract in a graded dose manner 250, 500 and 1000mgby oral route, one more group received the vehicle (0.9% saline solution) in equivalent volume orally. Before the dose administration of CO extract, rats were fasted overnight with no restriction of water, the calculated dose was administered by means of a gavage utilizing stomach tube as a single dose and subsequently food was withheld 3-4 hours after administration of CO extract.The observation period consists of total 14 days, on the first day every six hours followed by once daily. Conversely, the entire period of study rats were keenly evaluated to perceive the signs of central nervous system like tremors and convulsion as well as change in level of activity and their reactivity to the handling.

2.6 Oral Toxicity in Rats by Repeated dose Administration

The sub-acute oral dose toxicity study of CO extract was performed inaccordance with the method described by the former researchers to evaluate the sub-acute toxicity [12,13]. On daily basis manner for 14 days, three different doses (250, 500 and 1000 mg/kg/day) of the CO extract

were given orally to three groups of randomly selected male rats (6 each). One additional group received the vehicle (0.9% saline solution) in equivalent volume orally and food and water for the rats freely available all the time. Selection of the above mentioned graded doses were done based on the finding of lethal dose in this study as >2000mg. During the entire study time, the rats were evaluated for the following parameters:

2.7 Cage Side Observation and Mortality

Weight of each animal was determined before the administration of the extract on each day for the entire duration of 14 days.

On the day 14th, rats were given the last dose and then fasted overnight. Subsequently, they were anesthetized, and three samples of the blood were collected using retro-orbital puncture for following analyses.

2.8 Hematological and Biochemical Analysis

Analysis of the whole blood was performedto determine complete blood counting parameters like, Red blood cells count (RBC), Hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells count (WBC) and platelets count (PLT). This was performed by utilization ofhematology analyzer (ADVIA® 2120i, Siemens AG, Germany). The samples of the blood were obtained in plastic tubes with pre-added anti-clotting EDTA. (BD Vacutainer®) and submitted for hematology analysis.

While for routine coagulation studies together with prothrombin time (PT) and activated partial thromboplastin time (aPTT) was performed with the coagulation analyzer (Sysmex® CS-2100, Siemens AG, Germany) and for this, the blood samples were collected into plastic test tubes (BD Vacutainer® Citrate Tubes with 3.2% buffered sodium citrate solution). Finally, for the serum biochemical analysis the blood samples were collected into serumseparator tubes (without anticoagulant) and allowed to stand on ice for the purpose of complete clotting. The serum for biochemical analysis, blood samples were obtained in serum separator tubes with no addition of anticoagulant and kept in a stand on ice with the intention to achieve complete clotting of the blood. Consequently, blood samples in the clotted state were subjected to centrifuge at the speed of 3000rpm for duration of 15 min and serum was separated and stored at - 800C. Serum sample was analyzed using (ADVIA 1800 Chemistry System, Siemens AG, Germany) for: the determination of biochemical parameters: urea, creatinine (CREA), glucose (GLU), total cholesterol (CHOL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP).

2.9 Statistical Analysis

The statistical analysis of the results of this study was done by using statistical analyzer social science software (SPSS) version 16 (IBM®, USA). Multiple evaluation were performed by the one-way analysis of variance ANOVA), repeated one way ANOVA and Tukey's *post adhoc* test. Moreover, the computation of statistically significant difference among the mean value was measured at p value of less than 0.05 ($p<0.05$) and 0.001($p<0.001$). The values revealed in the text and tables were characterized as ± SEM. The graphs were prepared by the software of GraphPad Prism, version 5.

3. RESULTS

3.1 Body Weight

Statistically insignificant variation (Table 1) in average post-treatment rats' body weights was observed in all CO-treated groups after daily oral treatment with CO (250, 500 and 1000 mg/kg/day) for a period of 14 days in comparison with control group ($p>0.05$).

3.2 Oral Lethal dose in Rats

The result of orally administered CO extract in the dose of 2000 mg/kg to five rats revealed no mortality for 14 days period. Hence, the extract was found to be safe at that dose and the oral lethal dose was estimated to be higher than the tested dose (2000 mg/kg).

3.3 Toxic Effect of Single Oral dose in Rats

In this study, no deaths were observed during the 14 days period up to 1000 mg/kg dose level. At tested doses, rats revealed no symptoms associated with toxicity, such as convulsions, ataxia or diarrhea. A remarkable observation in animals throughout this study has revealed sedation with weak response to stimuli like

Table 1. Rats body weight at baseline and after 14 days of daily oral administration with CO extract at dose of 250, 500 and 1000 mg/kg/day.

Group	Rats' body weight (g)	
n = 6	Pre-treatment	Post-treatment
Control (0.9% saline)	186.36±9.20	209.43±10.50
CO (250 mg/kg/day)	175.73±7.36	199.53±10.40
CO (500 mg/kg/day)	179.33±8.50	204.66±7.23
CO (1000 mg/kg/day)	177.16±9.62	203.33±6.50

*Data is expressed as means ±SEM; n: Number of animals/groups; CO: Commiphora opobalsamum; *p<0.05 in comparison to the related control group value*

sounds or thrust. Otherwise, all treated rats during the entire study were quite normal as compared with the control.

3.4 Repeat dose Oral Toxicity in Rats

3.4.1 Mortality and cage side observation

After daily oral dose of rats with CO extract at graded dose of 250, 500 and 1000 mg/kg/day for 14 days, no mortality and no obvious perceptible signs of toxicity were observed, and they survived being active and healthy up to 14 days. All the treated rats throughout the study were quite normal as compared with the control.

3.5 Hematological Analysis

Outcome of hematological analysis revealed that (Fig. 1.) after oral 14 days treatment with CO extract in graded doses of 250 and 500 mg/kg/day, no significant attenuation of RBC count was scrutinized in comparison with the control group (p>0.05), conversely remarkable reduction in RBC was perceived in CO group (1000 mg/kg/day) compared with control group (p<0.001).

However, in contrast similar graded dose and same duration of CO extract produced noteworthy attenuation of HGB in contrast with the control group p<0.05, p<0.05 and p<0.001, respectively). The results also showed non-significant decrease in the HCT values in comparison with the control group(p>0.05) when treated with CO extract in the dose of 250 and 500 mg/kg/day for a period of 14 days, however CO extract in the dose of 1 gm/kg/day revealed a similar pattern of significant attenuation of HCT when compared with its effect on HGB in similar dose as mentioned above.

Furthermore, the effect of CO extract in the identical graded dose and duration was also evaluated on other hematological parameters like MCV (fL), MCH (pg) and MCHC (g/dL) in rats (Table 2) illustrated identical prototype of outcome in accordance with results of RBC count HGB and HCT.

When parallel effects of CO extract was done in analogous doses and duration of therapy in rats (Fig. 2A and Fig. 2B) to perceive its effect on WBC and platelets, remarkably, non-significantly decreased the WBC count corresponding to the control group (p>0.05) was observed with CO (250 mg/kg/day),nevertheless CO extract in higher doses(500 and 1000 mg/kg/day)for 14 days demonstrated significant difference of WBC count in comparison with the control group (p>0.05 and 0.001 respectively) was observed.

However, non-significant difference was observed in PLT of all CO-treated groups after daily oral treatment with CO (Fig. 2A and Fig. 2B) in graded doses 250, 500 and 1000 mg/kg/day for a period of 14 days in comparison with (Fig. 2A and Fig. 2B) with the control group (p>0.05). In addition, coagulation parameters were studied by observing the effect of oral treatment with CO in the dose of 250, 500 and 1000 mg/kg/day) for a duration of 14 days in contrast with control group on PT and aPTT (Table 3.) andnomomentous dissimilarity was demonstrated (p>0.05) among the two groups.

3.6 Biochemical Parameters

In this study, the analysis of Urea, CREA and GLU (Table 4) demonstrated inconsequential differences after 14 days of oral treatment with CO extract in graded doses contrast with control group (>0.05).

Regarding lipid profile (Table 5) oral treatment with extract of CO in graded doses for 14 days similar insignificant decreased the CHOL, LDL, HDL and TG was demonstrated in comparison with the control group (>0.05).

Furthermore, concerning the parameters of liver function tests (Fig. 3) the results illustrated insignificant increase in AST and ALT after 14 days of oral treatment with CO (250, 500 and 1000 mg/kg/day) contrast with control group (>0.05). Similarly, unremarkable decrease in ALP was observed with analogous doses and duration of CO extract after 14 days of oral treatment with CO (250, 500 and 1000 mg/kg/day) in contrast with the control group (>0.05).

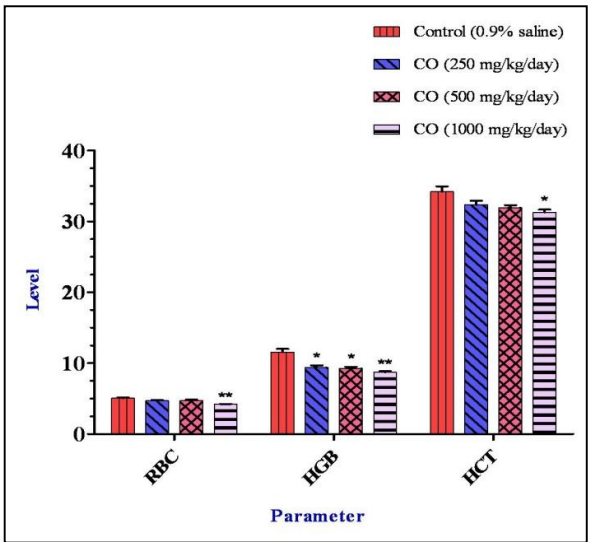

Fig. 1. The effect of daily oral treatment with CO extract in graded doses for 2 weeks on RBC HGB and HCT in rats

*Data is revealed as means ± SEM; n= 6 rats/group; CO: Commiphora opobalsamum; RBC: Erythrocyte; HGB: Hemoglobin; HCT: Hematocrit *p<0.05, **p<0.001 in contrast with thecontrol group values*

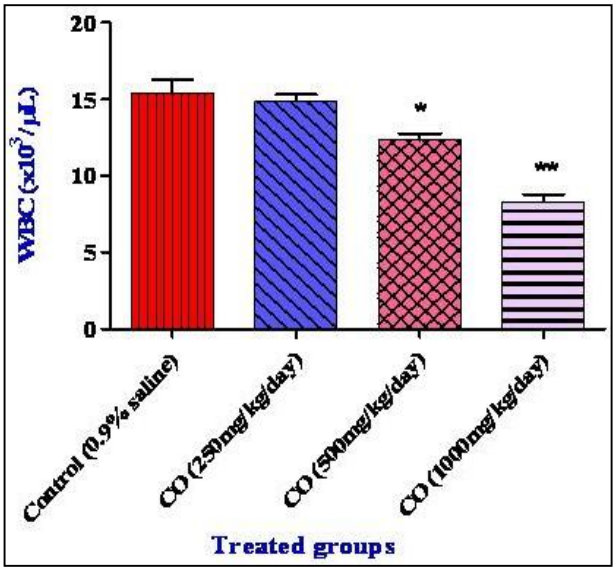

Fig. 2A. The outcome of daily oral treatment with CO extract in graded doses for 2 weeks on WBC in rats

*Data is revealed as means ±SEM; n= 6 rats/group; CO: Commiphora opobalsamum; WBC: White blood cell. *p<0.05, **p<0.001 in contrast with the concerned control group values; accomplished by using One-way ANOVA and Tukey HSD post hoc test.*

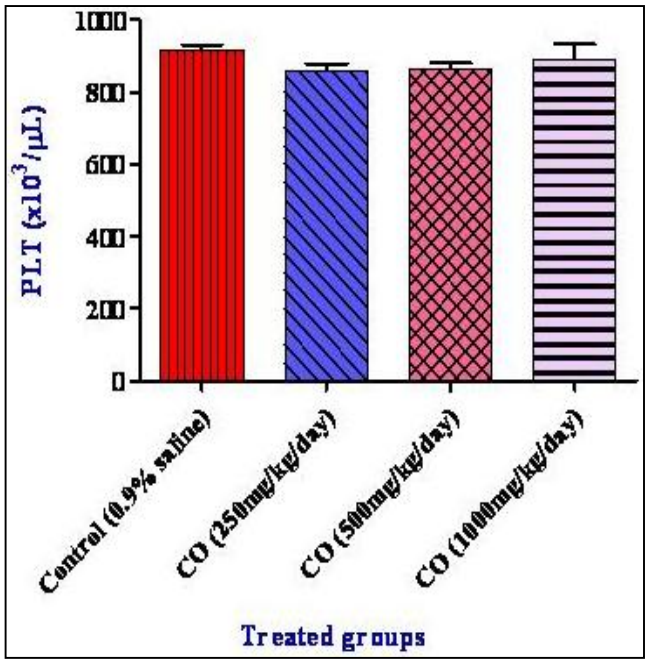

Fig. 2B. The effect of daily oral therapy of CO extract in graded doses for 2 weeks on PLT in rats

*Data is illustrated as means ±SEM; n= 6 rats/group; CO: Commiphora opobalsamum; PLT: Platelet. *p<0.05 in contrast with the concerned control group values; accomplished by using One-way ANOVA and Tukey HSD post hoc test*

Table 2. The daily oral treatment of the rats with CO extract in graded doses for 2 weeks and its effect on MCV, MCH and MCHC

Groupn = 6	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control (0.9% saline)	62.4±2.2	22.6±0.9	36.5±0.5
CO (250 mg/kg)	60.8±1.5	19.9±0.4	32.7±0.3
CO (500 mg/kg)	59.3±2.6	19.6±1.0	33.0±0.3
CO (1000 mg/kg)	63.8±1.6	20.6±0.3	32.3±0.8

*Data is expressed as means ±SEM; n=Number of animals/group; CO:Commiphora opobalsamum;MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; *p<0.05, **p<0.001 in contrast with the control group values*

Table 3. The effect of daily oral treatment with CO extract in graded doses for 2 weeks on PT and aPTT in rats

Group	Parameters	
n = 6	PT (sec)	aPTT (sec)
Control (0.9% saline)	11.43±0.51	27.66±2.08
CO (250 mg/kg /day)	12.20±0.91	29.33±1.52
CO (500 mg/kg /day)	11.66±1.20	27.00±3.00
CO (1000 mg/kg /day)	11.16±0.28	28.33±4.16

*Data is expressed as means ±SEM; n: Number of animals/group; CO: Commiphora opobalsamum; PT: Prothrombin time; aPTT: Activated partial thromboplastin time; *p<0.05 in contrast with the control group values*

3.7 Thyroid Function Markers

Finally, insignificant difference was demonstrated in thyroid function markers (TSH and FT4) (Table 6) after 14 days of oral treatment with CO in graded doses in contrast with the control group (>0.05).

Table 4. The effect of daily oral therapy of CO extract (250, 500 and 1000 mg/kg/day) for 14 days on Urea (mmol/L), CREA (µmol/L) and GLU (mmol/L) in rats

Group	Parameters		
n = 6	Urea (mmol/L)	CREA (µmol/L)	GLU (mmol/L)
Control (0.9% saline)	7.90±1.95	30.00±1.00	6.86±1.41
CO (250 mg/kg /day)	6.20±0.43	34.33±8.08	7.03±1.25
CO (500 mg/kg /day)	9.43±2.28	38.00±10.53	8.50±0.95
CO (1000 mg/kg /day)	7.30±1.69	26.50±2.12	7.80±1.13

*Data is illustrated as means ±SEM; n= 6 rats/group; CO: Commiphora opobalsamum; CREA: Creatinine; GLU: Glucose. *p<0.05 in contrast with thecontrol group values*

Table 5. The effect of daily oral treatment with CO extract in graded doses for 2 weeks on CHOL, HDL and LDL in rats

Group	Parameters			
n = 6	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TG (mmol/L)
Control (0.9% saline)	1.89±0.28	1.43±0.19	0.27±0.08	0.39±0.11
CO (250 mg/kg /day)	1.56±0.12	1.19±0.17	0.20±0.03	0.34±0.11
CO (500 mg/kg /day)	1.73±0.48	1.31±0.41	0.23±0.10	0.39±0.05
CO (1000 mg/kg /day)	1.39±0.07	1.04±0.13	0.17±0.10	0.36±0.01

*Data is expressed as means±SEM; n:Number of animals/group; CO: Commiphora opobalsamum; CHOL: Cholestrol; HDL: High density lipoprotein; LDL: Low density lipoprotein; TG: Triglyceride; *p<0.05 in contrast with the control group values*

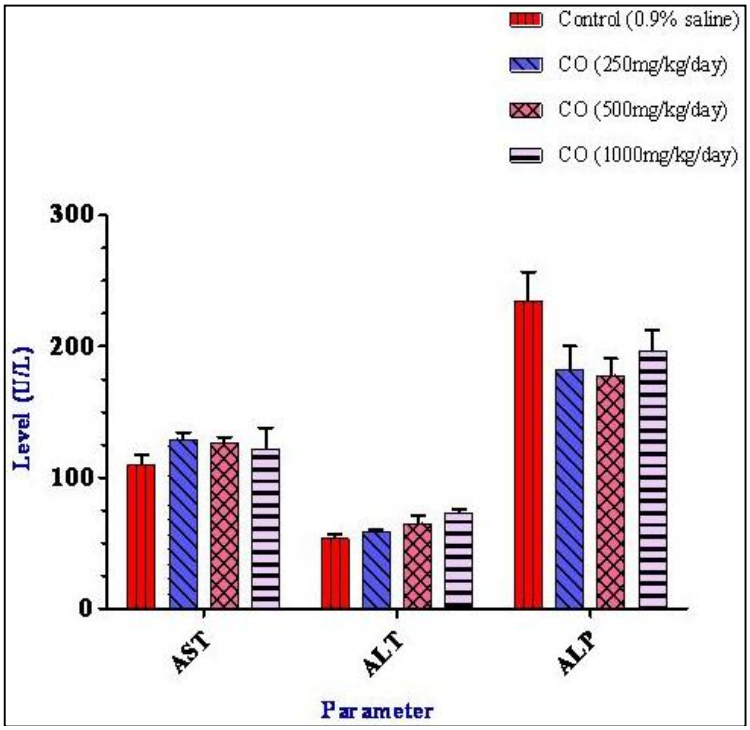

Fig. 3. The effect of daily oral treatment with CO extract graded doses for 2 weeks on AST, ALT and ALP in rats

*Data is revealed as means ±SEM; n= 6 rats/group; CO: Commiphora opobalsamum; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP : Alkaline phosphate.*p<0.05 in contrast with the control group values*

Table 6. The effect of daily oral administration with CO extractin graded doses for 2 weeks on thyroid function markers in rats

Group	Parameters	
n = 6	TSH (µIU/mL)	FT_4 (pmol/L)
Control (0.9% saline)	0.037±0.003	21.63±0.60
CO (250 mg/kg /day)	0.035±0.002	21.23±5.72
CO (500 mg/kg /day)	0.038±0.004	22.03±6.00
CO (1000 mg/kg /day)	0.034±0.003	20.10±0.56

*Data is expressed as means ±SEM; n =Number of animals/group; CO: Commiphora opobalsamum; TSH: Thyroid-stimulating hormone; FT_4: FreeT_4; * $p<0.05$ in contrast with the concerned control group values*

4. DISCUSSION

The outcome of our toxicological study of CO in these selected models of toxicity remarkably illustrated no perceptible signs of toxicity with nil mortality in rats treated up to 2000 mg/kg limit dose. All the rats survived and being quite healthy equally both in short term study i.e., 24 hours, in addition to its long-term surveillance period i.e., 14 days, this noteworthy finding signify that the oral LD_{50} of the extract CO is quite higher than 2000 mg/kg.

These findings were in concurrence with that mentioned in other studies [14,15] and could be a good initial indicator for safety of the CO extract to the extent of the dose level of 2000 mg/kg/day. However, some attenuation in the intensity of the activity as well as reactivity was noticeable in the dose of 1000 – 2000 mg/kg/day. Similar observations were seen in other study which reported that some decrease in locomotor activity in animals that were treated with *Commiphora molmol*, this could be the reason of elevated content of volatile oil in CO extract; it is quite noticeable that volatile oils are capable of producing depressant action on the central nervous system [10].

As regards the sub-acute toxicity of CO extract, rats were evaluated for different parameters of alteration in body weight, mortality if any, hematological, coagulation and biochemical studies.

Nevertheless, outcome of this experimental work has demonstrated that the rats treated with repeated oral dose of the extract of CO in the graded dose of 250,500 and 1000 mg/kg/day survived with no signs of toxicity for 14 days observation period. The repeat dose oral toxicity assay of CO extract in the graded doses reveals non-significant variation in body weights of rats after 14 days of daily treatment in contrast with control group. By contrast, the results of 28 days treatment with *Commiphora molmol* in graded doses of 250 mg. 500 mg and 1000 mg/kg/day revealed significant decrease in mean body weights and no toxic symptoms or deaths were found and they survived being active and healthy for up to 28 days [16]. However, Rao et al. (2001) reported a significant weight gain in animal groups that were treated with *Commiphoramolmol*100 mg/kg for 3 months as compared to the control animals [14].

It is noteworthy that the daily oral administration of CO for 14 days (Fig. 1) results in a decrease in RBC and HCT more significant in the dose of 1000 mg/kg/day in contrast to the control group ($p>0.001$ and $p>0.05$ respectively).

Moreover, the HGB was significantly decreased at all selected doses of CO 250,500 and 1000 mg/kg/day when compared with the control group ($p>0.001$ and $p>0.05$ respectively) Although, the variations in MCV and PLT were non-significant and comparable to that obtained in the control group ($p>0.05$). Where a significant reduction in MCH and MCHC were recorded in all CO extract treated groups ($p>0.001$ and $p>0.05$ respectively) compared to the control group. A dose-dependent decrease of WBC was showed in CO-treated rats and seems to be significant with the doses of 500 and 1000 mg/kg/day as compared with the control group ($p<0.05$ and $p<0.001$, respectively). These results were contrary to those mentioned in other study which reported that the treatment with *Commiphora molmol* at doses of 250, 500 and 1000 mg/kg/day for 28 days were demonstrated no significant differences of hematological parameters [10,16] reported that the RBC and HGB levels were significantly rise in *Commiphora molmol* treated animals (100 mg/kg/day for 3 months) as compared to the control.

These variations of the normal range of the hematological parameters may be owing to the toxicity of CO. It is pertinent to note that bone marrow is the site of RBC synthesis and paracetamol which is a well-known haem toxicants lead to anemia on chronic use [17], besides several phytochemicals are known to effect the HCT level [18]. Hence, the results of hematological studies may reveal significant and dose-dependent bone marrow inhibition of CO extract on prolonged administration (14 days).

In contrast, the repeat dose oral toxicity assay reveals that the CO extract did not altered the coagulation parameters in rats where a non-significant variation is shown in PT and a PTT in contrast to the control group (p>0.05). The results of former pharmacological studies showed that the *Commiphora mukul* increased fibrinolytic activity and decreased the platelet adhesive index [18].

Furthermore, the biochemistry analyses were conducted in the present work to evaluate the possible alterations in serum glucose, lipid profile, renal and hepatic functions influenced by the CO extract. The daily treatment with CO extract in the graded doses orally for 14 days resulted in a non-significant variation of urea and CREA levels which could be an indicator for the safety of CO extract on kidney functions.

These results were in conformity with those obtained by Rao et al. [14]. In contrast, Abdallah and his colleagues reported significant increases in urea and CREA levels after 28 days duration of therapy with 250, 500 and 1000 mg/kg/day *Commiphora molmol* [16]. It needs to be emphasized that, serum urea and CREA are used as markers to evaluate the renal functions [19]. Seemingly, the first acute marker of renal injury was urea with CREA is the most reliable renal marker whose value is significantly increased when the functioning of the kidneyis markedly reduced [20].

Regarding the fasting serum GLU level, there were non-significant dissimilarity between CO extract treated groups and the control group. CO extract did not show effect on GLU synthesis or metabolism. Similar findings were obtained by Rao et al. [14] in contrast to former results which demonstrated significant reduction in non-fasting serum GLU level after 28 days treatment with *Commiphora molmol* [16].

An additional study illustrated that the water extract of *Commiphora myrrha* was significantly attenuated the serum GLU level in experimentally induce diabetic rats [21].

In contrast, the effect of CO extract on lipids parameters was also explored in this experimental work. The values of cholesterol, LDI, HDL and TG in CO-treated groups showed a non-significant decrease as compared with control group. Despite that the reduction in CHOL and LDL were statistically non-significant in CO-treated rats, the CO extract might have a positive effect on lipid profile with prolonged administration. A former study stated that the treatment with *Commiphora africana* significantly decreased the values of CHOL, LDL and TG as well as significantly increased the HDL value [22].

Among the biochemical parameters measured in the present study were AST, ALT and ALP. Elevated levels of AST and ALT are attributed to the hepatocellular damage (Mukinda and Syce, 2007) [23]. In contrast, the serum ALP concentration is related to the biliary function of the hepatocytes, its high value in the plasma is primarily indicative of increased biliary pressure [24].

In the present study, administration of CO extract for 14 days was produced a statistically insignificant elevation in AST and ALT levels and decrease in ALP level in contrast with the control group. Similar reflection of findings were observed in the past studies of Rao et al. (2001) [14], which illustrate no significant differences in AST and ALT levels in animals that treated with *Commiphora molmol* in contrast to the control group except mild reduction serum AST. While other investigators reported that the AST were significantly increased after 28 days of *Commiphora molmol* administration [25]. Finally, except for RBC, HGB, HCT, MCH, MCHC and WBC, the methanolic extract of CO does not have negative impact on hematological, coagulation, biochemical parameters. It was interesting to note that rats exhibited some extent of sedation or depression effect when treated with CO extract in the present study. In view of its numerous and promising beneficial effects, the current studywas focused to explore the acute and sub-acute toxicological effect of CO extract as well as the estimation of oral LD_{50}. It is noteworthy that this study seems to be the first of its kind in investigating the toxicological effect of

CO as being addressed the LD_{50}, single (acute) and repeat dose (sub-acute) oral toxicity.

5. CONCLUSION

Our current explorative study strongly substantiates that the CO extract have no significant oral toxicity in rats and even the results of 14 days oral treatment indicated that there were no obvious toxic effects at the dosage of 1000 mg/kg/day. The comprehensive sub-acute toxicological effect of CO extracts on hematological and coagulation parameters and biochemical parameters included analysis of urea, creatinine, glucose; lipid profile and thyroid function markers fairly demonstrated no significant harmful outcome even after 14 days treatment with CO.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The present experimental work was officially endorsed by the ethical committee of KingAbdulaziz University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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