RESEARCH PAPER

Assessment of acute and sub-acute oral toxicity of aqueous leaf and stem bark extract of *Simarouba glauca* DC.

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ABSTRACT

The purpose of this research study is to examine the toxicological effects of *Simarouba glauca* DC., leaves (SGL) and stem bark (SGB) aqueous extract in mice as per the conventional acute oral toxicity test. In this study, the mice were administered a single dose of a leaf or stem bark aqueous extract at varying concentrations (1000 to 5000mg/kg b.w.). The mice were observed for body weight, general behavioral changes, adverse effects and mortality up to 14 days, post-treatment. In sub-acute toxicity studies, the leaf or stem bark aqueous extract was given orally to mice at varying doses of 100, 250, and 500mg/kg b.w for 28 days. The body weight, food and water intake were monitored throughout the study period and the relevant hematological, biochemical and histopathological evaluation was carried out at the end of the experiment. By acute toxicity study, the LD_{50} was found to be >5000mg/kg for both SGL and SGB groups. Likewise, in sub-acute toxicity studies of SGL and SGB-treated mice, the mean body weight was found to be significant ($p<0.001$). The hematological and biochemical evaluation of both extracts affirmed that the dose-dependent variations are not statistically significant. The histopathological findings suggest that SGL and SGB extracts are nontoxic to the liver, kidney and spleen. In conclusion, all these data suggest that the *Simarouba glauca* extracts are non-toxic and could be used as a safe herbal medicine against various infectious diseases and for the treatment of obesity.

Keywords: *Simarouba glauca* DC., Acute and sub-acute oral toxicity, Serum enzymes, Liver, Kidney, Spleen.

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INTRODUCTION

Traditional medicines are being used extensively in health promotion, prevention, and treatment of both physical and mental illnesses over the years. In circumstances where conventional medicine is ineffective for the treatment of advanced infectious diseases, ancient traditional knowledge is now being used in a holistic approach to modern health care (Homeber et al., 2012; Singh et al., 2001). Even after the availability of modern medicines, ethnobotanicals have retained their utility for ancient and cultural reasons. During the past two decades, public interest in natural therapy has greatly increased to thoroughly screen medicinal plants for their therapeutic value and has forced researchers to check the safety profile. It is very essential to scientifically validate the safety and efficacy of traditional medicines before using them as drugs (Yuan et al., 2016). In this regard, traditional systems of medicine have drawn greater attention to *in vivo* research studies to determine the toxicity of medicinal plants and their products. All these efforts should clarify, if any, the health risks posed by the environmental and oral exposures of these plant extracts to support effective risk management decisions. Among

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numerous medicinal plants, *Simarouba glauca* DC., has a long history of its use as an herbal medicine in many parts of the world. According to prevalent knowledge, stem bark and leaf extract of this plant have immense importance as they display a wide spectrum of ethnopharmacological activities such as hemostatic, anti-parasitic, anti-dysenteric, antipyretic, anti-cancerous and anti-viral properties. These extracts have been found to possess active components such as glaucarubin, quassinoids, benzoquinone, simarubin, simaroubidin, melianone and sistosterol that are implicated as the key metabolites responsible for the pharmacological activities (Manasi and Gaikwad 2011; Almeida et al., 2007; Saraiva et al., 2006).

Recently, it was also reported that *Simarouba glauca* DC., extract could down-regulate bacterial quorum sensing (QS) which elevates the medicinal value of the plant. QS is a bacterial cell-cell communication process that involves the production, detection, and response to extracellular signaling molecules (Vadakkan et al., 2018; Waters et al., 2005). The QS is a key regulatory system in dozens of clinically-relevant bacteria to regulate the communal production of virulence factors to colonize the hosts successfully (Novick and Geisinger, 2008; Ruby, 1996). By suppressing bacterial QS, it is possible to inhibit the virulent mechanism of the pathogen either by enzymatic or non-enzymatic inhibition which leads to its silence in the host system by assisting the host defense mechanism for successful clearance of the pathogen (Vadakkan et al., 2018; Waters and Bassler, 2005). Keeping in view the importance of bacterial QS inhibition, to date very limited ethnobotanical agents are recognized as QS inhibiting agents that have been successfully taken to the clinical level. This is mainly due to the paucity of biocompatibility data and scanty *in vivo* toxicity information. Due to its high biocompatibility, the huge exploitation of medicinal plants has raised numerous questions about anonymous toxicity. Therefore, it is imperative to fully comprehend the toxicological effects of ethnobotanical agents for their clinical usage. In the case of *Simarouba glauca* DC., leaves and stem bark extract, the apparent safety and toxicity data are not available. In this study, we have analyzed the acute and sub-chronic toxicity of *Simarouba glauca* DC., leaves and stem bark extract in Swiss albino female mice and thereby purge the anarchy over its side effects.

Methods

Sample collection and preparation of extract samples

The *Simarouba glauca* DC., plant leaf and stem bark samples were collected from the botanical garden (Smritivana) of Kuvempu University, Shankaraghatta, Karnataka, India. The collected samples were thoroughly rinsed with tap water and dried at room temperature for 7 to 8 days until it is free of moisture. Subsequently, 10g of dried sample (leaf and bark) was soaked in 100 ml of water (w/v,1:10) and subjected to boiling for 15 to 20 minutes under high pressure. After cooling, the extracts were filtered through Whatman No. 1 filter paper. The extracts, SGL (*Simarouba glauca* leaf) and SGB (*Simarouba glauca* bark) were further subjected to lyophilization and the obtained powder was dissolved in distilled water to get the desired concentrations for toxicity evaluation [Adeyi et al., 2013; Helida et al., 2014].

Experimental animals and their maintenance

A total of 72 female Swiss albino mice in the weight range of 23-25g were used for the study. The test animals were housed under standard laboratory conditions of 22 ± 3°C room temperature, 30–70% relative humidity, with 12 h light and 12 h dark cycle. The mice were housed in polypropylene cages and provided with a commercial pelleted diet and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days before the commencement of studies. The study was conducted following the internationally accepted guidelines for the use of experimental animals (NGSMIPS/IAEC/MARCH-2018/102) (National Research Council, 2011)

Acute toxicity studies

Acute toxicity evaluation was conducted *in vivo* according to OECD guidelines423 (OECD 2002) to determine LD_{50}. A total of forty-four female Swiss albino mice were used after one week of acclimatization. The acute toxicity of *Simarouba glauca* DC., leaf (SGL), and stem bark (SGB) plant extracts was carried out using the groups of four mice ($n = 4$) by administering varied doses (1000, 2000, 3000, 4000, and 5000mg/kg b.wt), while the control group received only the vehicle to establish a comparative negative control (Shetty et al., 2007; Diener and Schlede 1999). The animals were observed periodically during the first 24 h after oral administration of the extracts and then once in a day for further 14 days. The mice were observed for mortality and behavioural changes during

$$LD_{50} = \sqrt{\frac{D_0 + D_{100}}{2}}$$

this period. The lethal dose (LD_{50}) of SGL and SGB was calculated as shown below:

D_0 = Highest dose that resulted in no death; D_{100} = Lowest dose that resulted in death.

Assessment of sub-acute toxicity

The oral sub-acute toxicity study was carried out *in vivo* according to OECD guideline 407(OECD 2002). Adult healthy female Swiss albino mice (22–25g) were divided into 7 groups (n=4) and were kept under standard conditions. Group I was the control and the other test groups received the plant extracts (SGL and SGB) at different doses of 100, 250 and 500 mg/kg body weight for 28 consecutive days (Allaoui et al.., 2011). All the mice were observed at least twice a day to record any symptoms of ill-health or behavioural changes such as changes in the skin and fur, the activity of eyes, respiration rate, circulatory, and somato motor activity. The food and water intake were recorded during the study period of 28 days. At the end of the study period, all the surviving animals were fasted overnight and anesthetized with ether. The heparinized blood samples were collected for determining the hematological parameters and the serum from non-heparinized blood was carefully collected to evaluate clinical blood parameters. Animals were sacrificed after the blood collection and the internal organs such as liver, kidney, and spleen were weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examinations (Hor et al., 2012). The relative organ weight (ROW) of each animal was then calculated as follows;

$$\text{ROW (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Hematological analysis

On the 28th day of treatment, the blood samples were collected from the orbital sinus of mice from each group, under light ether anaesthesia after overnight fasting. The blood samples were collected in tubes containing EDTA as an anticoagulant. Later, the hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (HB), percentage and total count of lymphocytes, and percentage of monocytes were determined (Yuet Ping et al., 2013).

Serum biochemical analysis

The collected non-heparinized blood was allowed to remain in tubes for 45 min after which it was centrifuged at 3500 rpm for 10 min to separate the serum. The separated serum was used to evaluate biochemical parameters such as ALT, ALP, AST, total protein, BUN, and Cholesterol (Das et al., 2015; Yasmin et al., 2012).

Histopathological analysis

Histological analysis was done to evaluate the effect of SGL and SGB extracts on different tissue/organs of mice. Organs were collected and washed with 0.9% saline solution and then fixed in a formaldehyde-calcium solution. Sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (HE). The general structure of tissue, degenerative changes, necrosis and inflammation were analyzed using 40X photomicrography (Bigoniya et al., 2015; Cunha et al., 2009).

Statistical analysis

The statistical variations were determined using the software Graph pad prism 8.4.3 version with one-way ANOVA with Dunnett's post-test and two-way ANOVA with Tukey's post-test. All the data were considered significant when *$p<0.05$, **$p<0.01$, or ***$p<0.001$.

RESULTS

Acute toxicity study

The acute toxicity studies were conducted to determine the short-term adverse effects of extracts when administered during a period of 24h. The assessment of acute toxicity data showed that SGL and SGB extracts when administered orally at different concentrations were relatively safe; no death of mice was observed neither during the first 4 h of continuous observation nor after 24 h of recorded observation during the 14 days of the study period. The morphological characteristics such as the color of fur and skin appeared normal.

Further, the gain in body weight during the study period among the treated animals was comparable to their respective controls; a significant increase in the body weight of mice was evident. The final body weight on the 14^{th} day for the SGL control group (26.91±0.23g) and the SGB control group (27.23±0.12g) were

comparable to that of respective varied treatment groups.

The food and water intake in SGL and SGB treated groups was determined in a dose-dependent manner and compared to their respective control groups. During the study period, at varied concentrations of the extract, a significantly less amount of food and water intake was observed in the SGL 5000mg/kg treated group (155.67±16.62 g/day and 140.92±16.47 ml/day, $p<0.001$) and SGB 5000mg/kg treated group (169.12±3.39g/day and 144.57±15.05ml/day, $p<0.001$) as compared to the control group (222.97±4.37 g/day and 212.21±5.37ml/day) after 14days of treatment, respectively (Table 1 and 2).

There was no salivation, diarrhoea, lethargy, unusual behaviour, or altered respiration during this period. This suggests that the LD_{50} of both SGL and SGB extracts exceeded 5000 mg/ kg.

Table 1: Body weight gain, food and water consumption of mice treated orally with aqueous extract of *Simarouba glauca* DC., leaf (SGL) after 14 days of treatment.

Test Animals	Acute Toxicity (SGL)					
	Control	**1000mg/kg**	**2000mg/kg**	**3000mg/kg**	**4000mg/kg**	**5000mg/kg**
Final body weight (g)	26.91±0.23	24.98±0.33^{ns}	25.46±1.03^{ns}	25.85±0.51^{ns}	26.00±0.65^{ns}	27.66±0.74^{ns}
Food intake(g/day)	222.97±4.37	200.01±13.54^{ns}	195.62±12.93^{ns}	190.03±13.27*	182.85±13.62**	155.67±16.62***
Water intake (ml/day)	212.21±5.37	196.07±9.01^{ns}	200.35±8.58^{ns}	184.35±4.92*	171.57±15.43**	140.92±16.47***

Data were expressed as mean ±SEM (n=4). The statistical significance was expressed as *($p<0.05$), **($p<0.01$), ***($p<0.001$) and *ns* (not significant) when compared to the control.

Table 2: Body weight gain, water, and food consumption of mice treated orally with aqueous extract of *Simarouba glauca* DC., stem bark (SGB) after 14 days of treatment.

Test Animals	Acute Toxicity (SGB)					
	Control	**1000mg/kg**	**2000mg/kg**	**3000mg/kg**	**4000mg/kg**	**5000mg/kg**
Final body weight (g)	27.23±0.12	25.90±0.56^{ns}	26.01±1.55^{ns}	26.56±1.02^{ns}	26.81±0.49^{ns}	27.87±0.50^{ns}
Food intake (g/day)	222.97±4.37	219.45±7.39^{ns}	202.21±10.77^{ns}	190.21±0.77*	188.42±11.75**	169.12±3.39***
Water intake (ml/day)	212.12±5.37	209.57±1.05^{ns}	199.57±5.56^{ns}	191.28±13.78*	171.28±13.72**	144.57.±15.05***

Data were expressed as mean ±SEM (n=4). The statistical significance was expressed as *($p<0.05$), **($p<0.01$), ***($p<0.001$) and *ns*(not significant) when compared to the control.

Sub-acute toxicity study

Sub-acute toxicity analysis suggested that oral administration of SGL and SGB extracts did not cause any noticeable toxicity or changes in regular behavioural aspects of mice. After 28 days of treatment, there was no mortality which suggested that SGL and SGB extracts are safe in all the tested concentrations. In the current study, although there was a significant decrease in the body weight throughout the study period when compared to its control it was not significant when compared day-1 control. The percent body weight gain, however, was significantly higher in the control group as compared to the treated groups (Fig. 1 and 2). Bodyweight gain studies indicated that there is no significant relationship between concentrations of SGL and SGB administrated versus variations in body weight. There was significant difference in the group treated with SGL 500mg/kg b.w. and in all the SGB 100, 250 and 500mg/kg b.w. treated groups. However, significant difference was noted with respect to relative organ weight of liver but, no significant difference was noted in the organs kidney and spleen weight whereas, of the study mice when compared to the control (Fig.7).

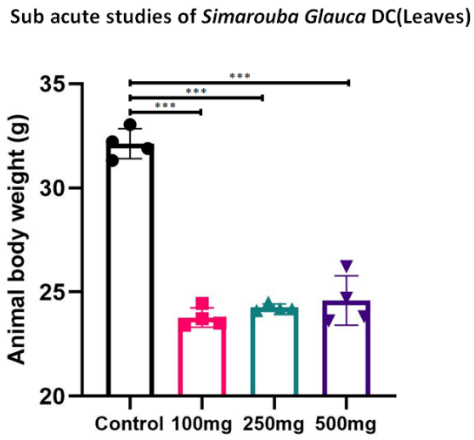

Figure 1: Effect of *Simarouba glauca* DC., leaf extract (SGL) on body weight of female mice. Values are expressed as mean ±SEM (n=4). The data was compared with control and expressed in terms of statistical significance (***, $p < 0.001$).

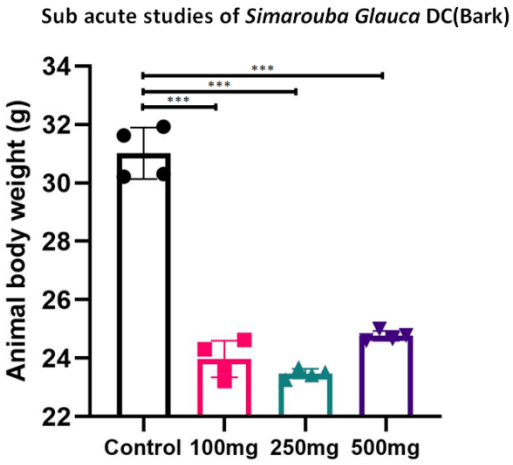

Figure 2: Effect of *Simarouba glauca* DC., stem bark extract (SGB) on body weight of female mice. Values are expressed as mean ±SEM (n=4). The data was compared with control and expressed in terms of statistical significance (***, $p < 0.001$).

Assessment of hematological parameters

According to the data, a dose-dependent variation was observed but there was no significant change in hematological parameters of treated animals as compared to the control (Tables 3 and 4) which indicates that there was no lysis of blood cells or inhibition in blood cells synthesis by the active constituents of *Simarouba glauca* DC. However, there was a slight increase in the neutrophils count in the SGL (83.66±3.17, 83.00±3.92 and 83.00±4.04) as well as SGB (79.00±6.08, 83.33±4.37 and 88.66±1.85) treated groups at 100, 250 and 500mg/kg b.wt, respectively, when compared tocontrol group (71.66±3.48) after 28 days of treatment as shown in Tables 3 and 4. The above-observed variations are normal and within the standard range and statistically non-significant ($p > 0.05$).

Table 3: Effect of daily administration of *Simarouba glauca* DC., leaf extract (SGL) extract for 28 days on hematology profiles of the control and treated Swiss albino female mice in the sub-chronic toxicity study subacute toxicity study.

Parameters	Sub-chronic toxicity of SGL				
	Control	**100mg/kg**	**250mg/kg**	**500mg/kg**	**Normal Std Range**
RBC (10^6/mm^3)	11.00±0.11	10.66±0.08^{ns}	10.19±0.25^{ns}	9.86±0.25^{ns}	8–15
WBC (10^3/mm^3)	12.63±0.23	11.00±0.25^{ns}	12.13±0.23^{ns}	10.88±0.05^{ns}	7-56
Neutrophils (%)	71.66±3.48	83.66±3.17^{ns}	83.00±3.92^{ns}	83.00±4.04^{ns}	40-75

Lymphocyte (%)	34.00±1.15	16.00±3.05^{ns}	17.00±3.21^{ns}	22.00±1.52^{ns}	25-40
Eosinophils(%)	0.00±0.00	0.00±0.00^{ns}	0.66±0.33^{ns}	0.66±0.66^{ns}	01-04
Monocyte (%)	0.33±0.33	0.33±0.33^{ns}	0.33±0.33^{ns}	0.00±0.00^{ns}	01-08
Hb (g/dL)	14.16±0.14	12.06±0.23^{ns}	12.63±0.29^{ns}	12.43±0.43^{ns}	12-15

Data were expressed as mean ±SEM (n=4). *ns*(not significant) when compared to the control.

Table 4: Effect of daily administration of *Simarouba glauca* DC., bark extract (SGB) extract for 28 days on hematology profiles of the control and treated Swiss albino female mice in the sub-chronic toxicity study.

Parameters	**Sub-chronic toxicity of SGB**				
	Control	**100mg/kg**	**250mg/kg**	**500mg/kg**	**Normal Std Range**
RBC(10^6/mm^3)	11.00±0.11	9.69±0.23^{ns}	9.78±0.21^{ns}	10.17±0.14^{ns}	8–15
WBC(10^3/mm^3)	12.63±0.23	11.20±0.26^{ns}	11.30±0.23^{ns}	11.96±0.14^{ns}	7-56
Neutrophils(%)	71.66±3.48	79.00±6.08^{ns}	83.33±4.37**	88.66±1.85***	40-75
Lymphocyte(%)	34.00±1.15	15.00±1.73^{ns}	15.33±2.40^{ns}	31.00±2.64^{ns}	25-40
Eosinophils(%)	0.00±0.00	0.33±0.33^{ns}	0.33±0.33^{ns}	0.00±0.00^{ns}	01-04
Monocyte(%)	0.33±0.33	0.33±0.33^{ns}	0.00±0.00^{ns}	0.00±0.00^{ns}	01-08
Hb(g/dL)	14.16±0.14	12.60±0.32^{ns}	12.66±0.38^{ns}	13.00±0.17^{ns}	12-15

Data were expressed as mean ±SEM (n=4). *ns* (not significant). When compared to the control.

Assessment of biochemical parameters

According to serum biochemical analysis, the data from the mice administered with the respective doses of SGL and SGB showed highly significant differences in serum ALT, ALP and AST activities. In the case of SGL, the increase in ALT was significant at 100mg/kg ($p<0.01$), and highly significant at 250 and 500mg/kg ($p<0.001$), whereas AST and ALP levels were significantly increased ($p<0.001$) in all three treatment doses as compared to control (Table 5). Likewise, in the SGB treated groups data, the increase in ALT levels was significant at 100mg/kg ($p<0.01$); 250 and 500mg/kg ($p<0.001$), whereas AST and ALP levels were significantly increased ($p<0.001$) in all three treatment doses as compared to control (Table 6). In addition, the cholesterol levels were also slightly elevated ($p<0.05$) in both SGL and SGB treated (500mg/kg) groups when compared to control. Meanwhile, BUN and the total protein did not alter significantly in both SGL and SGB-treated groups compared as compared to the control group after 28 days of treatment. Overall, because of the inherent property of the extracts, although there was a slight increase in the activities in sozme of the groups, all the values are within the normal standard range as compared to the control.

Table 5: Effect of daily administration of *Simarouba glauca* DC., leaf extract (SGL) extract for 28 days on biochemical profiles of the control and treated Swiss albino female mice in the sub-chronic toxicity study.

Parameters	**Sub-chronic toxicity of SGL extract**				
	Control	**100mg/kg**	**250mg/kg**	**500mg/kg**	**Normal Std Range**
Total Protein	4.033±0.08	4.43±0.08^{ns}	4.86±0.12^{ns}	4.17±0.06^{ns}	3.5–7.2
ALT(U/L)	40.43±1.10	54.06±1.75**	60.66±4.13***	69.7±3.41***	26-77
AST(U/L)	92.43±1.18	122.26±5.26***	133.40±4.86***	166.13±4.67***	54-269
ALP(U/L)	60.90±1.64	104.23±3.40***	110.9±5.05***	149.83±1.47***	45-222
BUN(mg/dL)	17.33±0.26	17.70±0.23^{ns}	17.96±0.20^{ns}	19.53±0.45^{ns}	12-28
Cholesterol(mg/dL)	37.33±1.45	42.83±0.97^{ns}	42.09±1.30^{ns}	50.0±1.15*	26-82

Data were expressed as mean ±SEM (n=4). The statistical significance of these was expressed as *NS* (not significant), *p<0.05, **p<0.01 and ***p<0.001 when compared to the control.

Note: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and blood urea nitrogen (BUN).

Table 6: Effect of daily administration of *Simarouba glauca* DC., bark extract (SGB) extract for 28 days on biochemical profiles of the control and treated Swiss albino female mice in the sub-chronic toxicity study.

Parameters	Sub-chronic toxicity SGB				
	Control	100mg/kg	250mg/kg	500mg/kg	Normal Std Range
Total Protein	4.033±0.08	4.46±0.12^{ns}	4.86±0.20^{ns}	4.26±0.14^{ns}	3.2–7.2
ALT(U/L)	40.43±1.10	56.16±0.49**	58.06±0.31***	60.36±0.32***	26-77
AST(U/L)	92.43±1.18	143.16±6.47***	183.76±5.89***	187.4±6.51***	54-269
ALP(U/L)	60.90±1.64	116.63±2.80***	118.16±2.54***	137.36±1.56***	45-222
BUN(mg/dL)	17.33±0.26	18.33±0.29^{ns}	19.03±0.14^{ns}	21.03±0.78^{ns}	12-28
Cholesterol(mg/dL)	37.33±1.45	43.76±1.38^{ns}	44.66±0.98^{ns}	50.13±0.94*	26-82

Data were expressed as mean ±SEM (n=4). The statistical significance of these was expressed as *ns*(not significant), *p<0.05, **p<0.01 and ***p<0.001 when compared to the control.

Note: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and blood urea nitrogen (BUN).

Histopathological investigation

Histopathology is the microscopic examination of biological tissues from the body under a microscope to spot the signs and characteristics of the disease. Our study on tissuehistology of organs like liver, kidney, and spleen revealed the compatibility of SGL and SGB extracts in test animals (Figure 3 and 4). Liver histology of animals treated with both SGL and SGB extract at the doses of 100 and 250mg/kg body weight revealed predominantly normal hepatic architecture with normal central vein, hepatic sinusoids, and mild distortion of portal triad. There were no significant degenerative hepatocytes in the treated groups. Only the animals treated with SGL and SGB extract at 500mg/kg body weight showed mild feathery degeneration in the hepatocytes, however, normal lobular architecture was displayed (Figure 3 and 4). In the case of kidney of animals treated with SGL and SGB extract at 100 and 250mg/kg body weight, there was normal renal architecture with the normal orientation of glomeruli and tubules, with mild inflammatory changes and no degenerative features were seen. In the case of animals treated with SGL and SGB extract at 500mg/kg body weight, there was normal cortex and medulla with normal glomerular foci. Mild tubular hyalinization with congestion was observed, but there was no evidence of toxic changes. In the case of spleen, animals treated with SGL and SGB extract at 100 and 250mg/kg body weight, foci of mild sinusoidal dilatation seen with normal white pulp and red pulp architecture. The animal treated with SGL 500mg/kg body weight spleen, there was occasional hemorrhagic and congested areas was noted. Moreover, in animal treated with SGB 500mg/kg body weight spleen, mildly distorted spleenic architecture and sinusoidal space dilatation was observed, but there was no evidence of toxic changes.

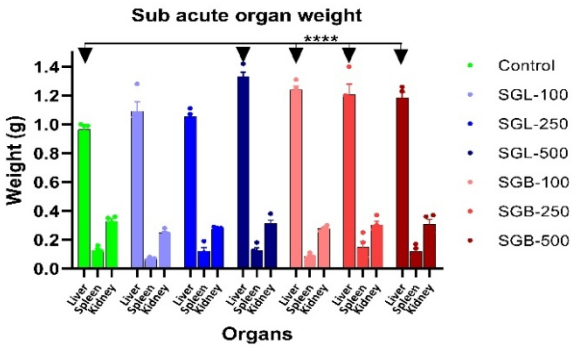

Figure 3: Relative organ weight of Swiss albino female mice (liver, spleen and kidney) after 28-days of treatment with the *Simarouba glauca* DC., leaf extract (SGL), and stem bark extract (SGB). Values are expressed as mean ±SEM (n=4). The data was compared with control and expressed in terms of statistical significance (***, $p < 0.001$).

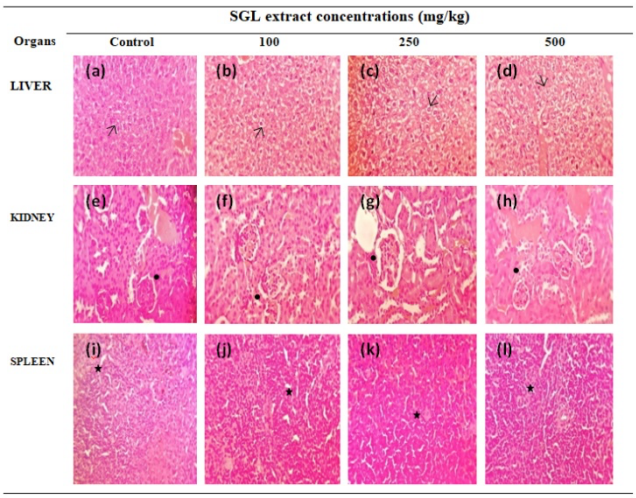

Figure 4: Histopathological analysis of sub-acute toxicity studies of *Simarouba glauca* DC., leaf extract (SGL) on Swiss albino mice. Photomicrograph analysis of organs treated with *Simarouba glauca* DC. SGL extract (H & E, 400×). a) Control liver: Normal hepatic architecture (arrow headed); (b &C) SGL 100 and 250mg/kg body weight treated liver: showing mild distortion of portal triad (arrow headed); d) SGL 500mg/kg body weight treated liver: mild feathery degeneration in the hepatocytes (arrow headed); e) Control Kidney: normal renal architecture(·); (f & g) SGL 100 and 250mg/kg body weight treated kidney: normal orientation of glomeruli and tubules with mild inflammatory changes(·); h) SGL 500mg/kg body weight treated kidney: Mild tubular hyalinization with congestion (·); i) Control spleen: Normal splenic histology with normal white pulp and red pulp architecture; (j & k) SGL100 and 250mg/kg body weight treated spleen: foci of mild sinusoidal dilation (*); l) SGL 500mg/kg body weight treated spleen: Occasional hemorrhagic and congested areas(*).

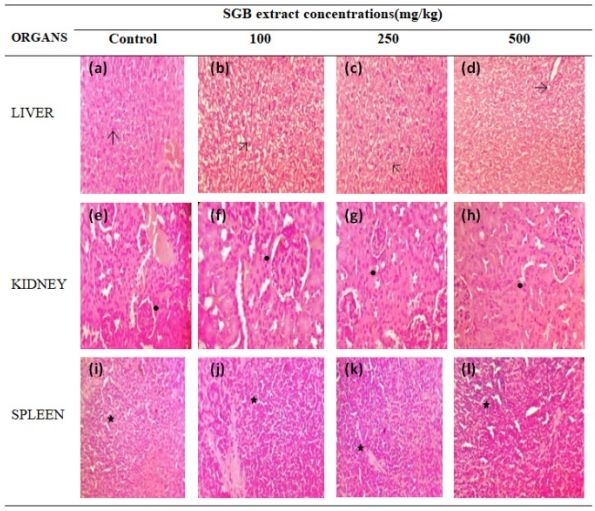

Figure 5: Histopathological analysis of sub-acute toxicity studies of *Simarouba glauca* DC. stem bark extract (SGB) on Swiss albino mice. Photomicrograph analysis of organs treated with *Simarouba glauca* DC., SGB extract (H & E, 400×). a) Control liver: Normal hepatic architecture (arrow headed); (b &C) SGB 100 and 250mg/kg body weight treated liver: showing mild distortion of portal triad (arrow headed); d) SGB 500mg/kg body weight treated liver: mild feathery degeneration in the hepatocytes (arrow headed); e) Control Kidney: normal renal architecture(·); (f & g) SGB 100 and 250mg/kg body weight treated kidney: normal orientation of glomeruli and tubules with mild inflammatory changes(·); h) SGB 500mg/kg body weight treated kidney: Mild tubular hyalinization with congestion (·); i) Control spleen: Normal splenic histology with normal white pulp and red pulp architecture; (j & k) SGB100 and 250mg/kg body weight treated spleen: foci of mild sinusoidal dilation (*); l) SGB 500mg/kg body weight treated spleen: mild sinusoidal space dilatation (*).

Discussion

The plant, *Simarouba glauca* DC., has long been implicated to have potent medicinal applications. Despite its utility against various ailments, there is scanty information regarding its toxicity analysis. As per the available literature, there are no oral acute toxicity studies regarding no-observed-adverse-effect-level (NOAEL) for the period of 14 days at a single dose of 1000, 2000, 3000, 4000, and 5000mg/kg b.wt. The data obtained from our studies indicates that the extract has no toxicity even at a concentration of 5000 mg/kg b.w, and the LD_{50} is considered to be >5000 mg/kg b.wt. As per the literature, several plant extracts exhibited null effects on animal mortality such as the leaf extract of Moringaoleifera Lam. (Moringaceae) (Awodele et al., 2012) and aqueous extract of the leaves of Myrianthusarboreus P. Beauv. (Cecropiaceae) as per the toxicity studies in Wistar rats (Awounfack et al., 2016).

However, for applications in treating chronic disorders like cancer, diabetes, etc., the safety of the extracts needs to be confirmed from its subacute toxicity study. Thus, we performed sub-acute toxicity study at the concentrations of 100, 250, and 500mg/kg b.w of the extract as per OECD guidelines (Muhammad et al., 2015). Our data has shown that there is a gradual decrease in the body weight over a study period of 28 days upon treatment with SGL and SGB. The researchers have shown that an increases or decreases in the body weight is accompanied by the accumulation of fats and physiological alterations in responses to the

plant extracts rather than to the toxic effects of chemicals or drugs that escort to decrease appetite and, hence lower caloric intake by the animal (Teo et al., 2002; Nirogi et al., 2014).

Alhough in our findings both control and treated group mice appeared to be well during the entire study period, there was an increase in the body weight of mice in the control group but there was a significant decrease in the treated mice at all the concentrations used. The weight loss could be due to the gastrointestinal irritation which reduces food consumption in contrast to metabolic derangement (Wren et al., 2002; Lee et al., 2012). Prominently, these extracts appear to exhibit anti-obesity drug properties. Natural anti-obesity agents such as flavonoids, polyphenols and terpenoids help in managing the body weight through several potential mechanisms, the most common mechanism is lowering the plasma lipid levels and reduction in levels of circulating fat and stored fat which are the key factors in obesity management (Sun et al., 2016). Consequently, the reduction in oxidative stress, inhibition of pancreatic lipase activity and suppression of adiponectin, a component released from adipocytokine may helpful for counteracting the risk of obesity-associated carcinogenesis (Wang et al., 2016). Proper dopamine regulation, helps in secreting the respective hormones which maintain satiety and appetite suppression, inhibiting digestive enzymes of fat or sugar from food. According to the literature, there are around 54 families of plants with 11 different parts that have shown anti-obesity potential (Mohamed et al., 2014; Zhang et al., 2014). It is also evident that there is no significant relationship between the concentration of SGL and SGB extracts on the organ weight. It has been shown that the stem-bark ethanol extract of *S. Versicolor* (which belongs to the same family *Simaroubaceae*), administered to *Wister* rats for 30 days did not result in any observable signs of toxicity or mortality (Oliveira et al., 2016).

Although some parameters observed in our study (erythrocyte count, WBC, Hb, total count, and lymphocytes) showed variations in groups treated with high dose of SGL and SGB extracts when compared to the control group, the values were within the normal range. However, SGL and SGB extracts positively enhanced the production of neutrophils by three-fold as compared to control due to the migratory imbalance of lymphocytes. This pragmatic dose-dependent variation in hematology is due to the inherent property of the test extracts but not due to the toxicity (Kelley et al., 2007; Nowru et al., 2013). Nevertheless, the observed values for all the hematological values were within the standard and normal range reported for mice and the variations were statistically non-significant (Abba et al., 2018).

Biochemical and histological analyses were done to understand the effect of SGL and SGB extracts on the function of vital organs such as liver, kidney and spleen which suggested that the extract did not amend the appropriate functioning of organs. Detoxification is a key function of the liver. The damage is usually assessed by the determination of serum transaminases (ALT and AST). ALT is found primarily in the liver and is the most sensitive marker for liver cell damage. In our present study, the ALT, AST and ALP were elevated in all the SGL and SGB-treated groups. This could probably due to exposure to higher dose treatment for a prolonged period and the extracts could have caused mild injury to liver, lungs, and kidney as the values were within the normal range. However, AST is a non-specific enzyme whose activity in serum could be due to injury to various other vital organs in the body (Akanmu et al., 2020; Mujahid et al., 2017).

The histopathological findings confirmed that this elevation could have been probably due to very mild damage that was observed in the liver and kidney tissues. The kidney is primary target of toxins as it receives one-fourth of total cardiac blood flow (Okumu et al., 2017; Debelo et al., 2016). Our study has shown that the difference in values for blood urea nitrogen and total protein were negligible as compared to control. Further, these values were within the reference range for mice indicating that the plant extract has no adverse effects in the functioning of kidney.

Further, the photomicrographs of tissue histological analysis suggested no significant alterations in the tissue level organization of liver, kidney and spleen which confirm the safety of SGL and SGB aqueous extracts in the tested concentration range. These observations from our study matches with similar toxicity assessment analysis of ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice (Muhammad et al., 2015) and hydroethanolic extract of Dolichandra unguis-cati L. leaves in rats (Juliana et al., 2017).

Conclusion

The short-term use of leaves and stem bark extracts of *Simarouba glauca* DC. at doses up to 5000 mg/kg b.w has not resulted in toxic manifestations in mice. Hence, SGL and SGB extract tested in this study falls in Category 5 under the GHS safety category according to OECD guidelines 423. However, long-term administration of the extracts (beyond 14 days) has associated with mild dose-dependent histopathological

changes in the liver, kidney, and spleen of mice, as well as minor alterations in hematological and biochemical parameters. However, the observed variations are within the normal range and statistically not significant. Considering the ethnomedicinal claims of using SGL and SGB samples for various ailments, our study has shown its utility as a good natural anti-obesity agent. In conclusion, our current data could be useful as a valuable information on the safety aspect of leaves and stem bark of *Simarouba glauca* DC. Thus, the data obtained here could therefore will be a stepping stone for any further detailed work on the evaluation of genotoxicity, carcinogenicity, and mutagenicity which could be indispensible in leading to complete safety profile analysis of this paradise tree.

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