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Immunomodulatory Potential of *Simarouba glauca* DC., Leaf and Stem Bark extract, *in vivo*

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ABSTRACT: Natural products are celebrated to primarily modulate the immune system in nonspecific ways. A number of plant-based principles have been acknowledged and isolated with potential immunomodulation activity which validate their use in traditional folklore medicine and can form the base of further specified research. Here, we investigated the immunomodulatory activity of *Simarouba glauca* DC., leaves and stem bark extract at the doses of 100, 200 and 300 mg/kg body weight in mice followed by the immunostimulatory activity as cyclophosphamide suppressed immune system, phagocytic activity using carbon clear test, T-cell population test and anti-inflammatory activity were carried out. In the carbon clearance test, SGL and SGB extracts exhibited a significantly high phagocytic index indicating the stimulation of reticuloendothelial system. A significant decrease in mean difference, in the foot paw thickness in carrageenan-induced paw edema activity, indicates its anti-inflammatory activity. Both SGL and SGB aqueous extract treated groups at 200 and 300 mg/kg doses showed a significant increase in T-cell population due to raise in T-cell rosette formation. These results confirm the immunomodulatory activity of SGL and SGB aqueous extract, which is a known immunomodulator in indigenous medicine.

Keywords: Immunomodulators, *Simarouba glauca* DC., T-cell rosette assay, anti-inflammatory.

INTRODUCTION

The emergence of antimicrobial resistance (AMR), is one of the major global public health threat. It can lead to longer hospital stays and increased mortality rates. It can also limit the effectiveness of treatments for a wide range of infections, from minor illnesses to life-threatening conditions like pneumonia, sepsis and tuberculosis (Rajagopala *et al.*, 2011). As pharmaceuticals are highly priced, traditional plant-acquired remedies are available. The supposed "Green Wave "caused by Increased bionomical awareness has led to increased participation all over the globe in herbal formulations. Currently, immunomodulation has been a key component of effective disease management (Aslam and Ahmad 2016).

The human body has an astonishingly classy immune system with an arm of innate and adaptive immunity. The innate immunity is the most hastily acting immunity that provides first protection against pathogens which generally depends on neutrophils, macrophages, dendritic cells, and monocytes. On the other hand, T and B cells are involved in adaptive immunity to enhance protection by maintaining immune system homeostasis. (Beutler, 2004; Saroj *et al.*, 2012). In response to the pathogen, leukocytes perform many phagocytic activities, including chemotaxis, adhesion of leukocytes to vascular endothelial cells, and pathogen engulfment. The phagocytes migrate towards the chemoattractants such as complement (C3a and C3b) and utilize a similar signal transduction system, namely G protein–coupled receptor that is, complement C5a receptor (C5aR), to promote the interaction of chemotactic factor and its receptor to stimulate cytoskeletal reorganization, calcium mobilization, and degranulation in heterologous cell types (Luster, 2001). The phagocytosis triggers the generation of superoxide radical (O_2^-) and release of reactive oxygen species (ROS), for instance hydroxyl radical hypochlorous acid (HOCL), myeloperoxidase (MPO) and induced nitric oxide (iNOS). Macrophages, in addition, modulate adaptive immunity by presenting antigens to $CD4^+$ T cells through major histocompatibility complex (MHC) class II antigens. $CD4^+$ T cells act upon four subpopulations, which embrace Th-1, Th-2, Th-17, and $CD4^+$ T regulatory cells (Chapel *et al.*, 2006). The $CD4^+$ T cells help B cells to develop into plasma cells to produce antibodies and as well activate T cells to become activated cytotoxic T cells (Beutler, 2004; Luckheeram *et al.*, 2012). The proinflammatory cytokines (TNF-α, IL-1, IL-11) and anti-inflammatory cytokines (IL-4, IL-10, and IL-13) also play essential

roles in immune response as intercellular messenger molecules amid stimulating phagocyte migration and coordinating early responses of monocytes, macrophages, dendritic cells, and lymphocytes (Shaikh, 2011).

Several clinical disorders are associated with imbalanced immune system. In many such conditions, the immune system is a target of numerous drug like molecules called as immunomodulators which modulates the immune system to respond against a malady or illness (Geetha *et al*., 2005). These immunomodulators exert their activity either by immunostimulation (as in the case of cancer and AIDS treatment) or by immunosuppression (as in the case of treatment of autoimmune diseases). Currently, immunomodulation has been a key component of effective disease management. Indian medicinal plants manifest miraculous effects in curing a range of human diseases and disorders which are superiorly called "elixirs of life". *Simarouba glauca* DC., a plant well known as the paradise tree, belongs to the *Simaroubaceae* family which has been found to have therapeutic potential due to the presence of components called quassinoids (Fausto *et al*., 2005; Almeida *et al*., 2007).

Most of the studies carried out so far have focused mainly on infectious diseases and hardly any studies have been conducted to understand their potential immunomodulating properties. Keeping this in view, the current work was therefore designed to study the immunomodulatory potency of *Simarouba glauca* DC., leaf and stem bark aqueous extract using cyclophosphamide induced immunosuppression model by evaluating the various hematological parameters and other parameters such as carbon clearance test, anti-inflammatory test, the T-cell population in presence of sheep red blood cells (SRBCs) as antigen in Swiss albino mice.

MATERIALS AND METHODS

Sample collection and preparation of extract samples. The *Simarouba glauca* DC., plant leaf and stem bark samples were collected from 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 days until it is free from 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 min. 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, Ham *et al.*, 1954).

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).

Immunomodulatory activity of *S. glauca* DC., leaves and stem bark extracts. The SGL and SGB extracts were evaluated for immunostimulatory effect in the cyclophosphamide-induced immuno suppressed mice model. The different parameters analyzed were carbon clearance test, T-cell population evaluation in presence of SRBCs as an antigen, and anti-inflammatory potential.

Preparation of Sheep RBCs Antigen. To obtain only red blood cells, fresh SRBCs were collected in sterile Alsever's solution (1:1) and washed two to three times with phosphate-buffered saline (pH 7.2) by centrifugation (3000 × g for 10 min). Then it was kept aside in PBS at 4ºC for use at the time of immunization.

Cyclophosphamide induced immune suppression. Cyclophosphamide (CP) induced immune suppression was performed by following the standard protocols (Hemalatha *et al*., 2011; Jayathirtha and Mishra 2004). Briefly, 36 mice were fasted for 18 h and divided in to nine groups of four mice each. The Group I received PBS orally (1 mL/100g b.w), group II received the immunosuppressant (cyclophosphamide), group III received positive control (levamisole), groups IV, V, VI, VII, VIII and IX were administered only with cyclophosphamide (30 mg/kg, i.p./day) for the initial three days. Further, the group II was kept for 16 days without any treatment. The immunosuppressed group III was treated orally with levamisole and considered as a positive control (50 mg/kg/day). The groups IV, V, and VI were treated orally with SGL (100, 200, and 300 mg/kg/day) and groups VII, VIII and IX orally with SGB (100, 200 and 300mg/kg/day), respectively, for 15 days. The blood samples collected in anticoagulant-coated tubes on the 16th day were used for the determination of total WBC, differential WBC, RBCs, platelets, hemoglobin concentration, and the number of splenocytes.

In-vivo Carbon clearance test. The carbon clearance test was performed according to the standard method with slight modifications (George *et al*., 2014). This parameter was studied for seven days in all the mice groups. Group I received PBS (1mL/100g, p.o/day) and group II was treated with levamisole (50mg/kg, p.o/day). The SGL extract was administered at doses of 100, 200 and 300mg/kg, p.o/day in groups III, IV, and V, respectively. The groups VI, VII, and VIII, received SGB extract at doses of 100, 200 and 300mg/kg, p.o./day, respectively, for seven days. The blood was withdrawn from mice through retro-orbital plexuses or tail cut method on the 9th day. Afterwards, India ink [India ink (3mL): saline (4mL): 3% gelatin (4mL)] was injected through an intravenous route (tail vein), and the blood was drawn at 0 and after 15 min of injection. This was followed by treatment with sodium carbonate

(0.1%) solution (2 to 4 mL) with the blood samples (25-50µL) to lyse the erythrocytes. Further, absorbance was measured at 660nm and the carbon clearance was calculated using the equation;

$K = (\text{Loge } OD_1 - \text{Loge } OD_2)/15$

OD_1 and OD_2 represent the optical density of the blood sample at 0 and 15 min respectively.

Phagocytic Index (α) = $(\sqrt[3]{K})$ x Weight of Animal/Weight of liver + Weight of Spleen

T- cell population by erythrocyte rosette assay. This assay was performed as per the standard method which was followed for 10 days (Dhasarathan *et al*., 2010; Smriti *et al*., 2012). Group I received PBS (1 mL/100g, p.o./day), and Group II was treated with levamisole (50mg/kg, p.o/day). Group III, IV and V was administered with SGL extract at doses of 100, 200 and 300mg/kg, p.o/day respectively. In addition, groups VII, VIII, and IX were treated with SGB extract for ten days at doses of 100, 200 and 300mg/kg, p.o./day, respectively. On the 0^{th} day, all mice were sensitized with 0.1 mL of SRBC (1×10^8 cells, i.p.), except the control group. On the 11^{th} day, blood was drawn from both control and treated groups via retro-orbital plexus in anticoagulant-coated vials. Vials were placed in a 45-degree slant position at 37°C for 1 h and the supernatant containing lymphocytes was collected. An equal volume (0.25 mL) of suspension and 0.5% SRBCs were mixed in a vial and incubated for 5 min at 37°C. After centrifugation of mixture at 1000 rpm for 5 min, the obtained supernatant was stored for 2 h at 4°C, and subsequently, lymphocytes were counted using the graduated slide. Those, T – cells attached with three or more SRBCs were considered as a rosette and counted under microscope.

Anti-inflammatory activity by Carrageenan-induced paw edema in mice. Carrageenan-induced paw edema in mice is a well-established model of acute inflammation for screening anti-inflammatory agents. The anti-inflammatory activity of SGB and SGL samples of varied concentrations (100mg, 200mg, and 300mg) was studied using carrageenan-induced paw edema in mice. Briefly, inflammation was induced in the right hind paw of mice by subcutaneous injection of 0.1 ml 1% λ carrageenan. The mice were pretreated with intra-peritoneal injection of standard drug, diclofenac sodium (10 mg/kg), and the test samples (SGB and SGL) for 1 h before administration of carrageenan. Edema in the paw was measured at hourly intervals from 0 h up to 24 h using Plethysmometer and compared with the control. The inhibitory effect was determined by using the following formula:

$$\% \text{ Inhibition} = \frac{(C_t - C_o)\text{ control} - (C_t - C_o)\text{ treated}}{(C_t - C_o)\text{ control}} \times 100$$

Where $(C_t - C_0)$ control is the difference in the size of paw at 24 h in control mice, and $(C_t - C_0)$ treated is the difference in the size of paw at 24 h in mice treated either with the standard drug or SGB/SGL (Huang *et al*., 2012; Vikas and Vijay 2011; Akindele and Adeyemi 2007).

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 with a difference in p values (* = $p<0.05$, ** = $p<0.01$, or *** = $p<0.001$).

RESULTS

Effect of SGL and SGB against drug-induced immunosuppression. The immunostimulatory effect of SGL and SGB aqueous extracts was assessed in cyclophosphamide (CP) induced immunosuppressed mice. Administration of CP (30 mg/kg, i.p) produced a significant decrease in the total RBCs from 7.47 ± 0.23 to 5.92 ± 0.20, WBC counts from 4.50 ± 0.26 to 3.13 ± 0.14, and % hemoglobin from 9.93 ± 0.08 to 6.40 ± 0.32, neutrophils from 34.57 ± 0.47 to 26.00 ± 0.31, lymphocytes from 10.10 ± 0.26 to 7.36 ± 0.23, platelets from 6.97 ± 0.23 to 3.13 ± 0.27 and splenocytes from 1.72 ± 0.20 to 0.89 ± 0.09 when compared to control. Besides, levamisole, SGL, and SGB extracts treatment for 15 days stimulated the immune cells as compared to CP induced group and control. The SGL and SGB extracts at varied concentrations (100, 200, and 300 mg/kg) stimulated the immune cells in a dose-dependent manner. The values were found to be highly significant when compared to the control and CP-induced group (Tables 1 and 2).

Table 1: Immunostimulatory effect of *Simarouba glauca* DC., leaf (SGL) aqueous extract.

Parameters	Control	CP (30mg/kg)	LM (50mg/kg)	SGL (100mg/kg)	SGL (200mg/kg)	SGL (300mg/kg)
RBC (10^6/mm^3)	7.47±0.23	5.92±0.20*a	8.96±0.12	8.10±0.55	8.50±0.30	9.36±0.26*ab
Hemoglobin (g/dL)	9.93±0.08	6.40±0.32**a	11.23±0.33***ab	10.50±0.45*b	10.83±0.48*b	12.23±0.44**ab
WBC (10^3/mm^3)	4.50±0.26	3.13±0.14*a	10.66±0.33***ab	7.6s3±0.02*b	9.14±0.3**ab	11.28±0.13***ab
Neutrophils (%)	34.57±0.47	26.00±0.31*a	69.26±0.93***ab	55.66±0.33*ab	62.89±0.93**ab	66.27±0.89***ab
Lymphocytes (%)	10.10±0.26	7.36±0.23**a	14.33±2.64***ab	13.20±2.2*b	16.5±0.5**ab	21.22±0.20***ab
Monocytes (%)	0.02±0.0	0.6±0.01	0.23±0.04	0.32±0.1	0.30±0.0	0.21±0.05
Eosinophils (%)	0.20±0.01	0.55±0.0	0.26±0.06	0.32±0.1	0.29±0.01	0.22±0.01
Basophils (%)	0.01±0.0	0.11±0.02	0.04±0.01	0.02±0.0	0.12±0.01	0.05±0.0
Platelets (10^9/L)	6.97±0.23	3.13±0.27***a	8.7±0.23**ab	7.65±0.55*b	7.88±0.8**b	8.81±0.55**b
Splenocytes (10^8cells/mL)	1.72±0.2	0.89±0.09**a	2.73±0.33**ab	1.86±0.5*b	3.15±1.3**b	4.12±0.6**b

Data were expressed as Mean ± SEM (n=5). Statistical significance * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) compared with control and CP-treated groups. The alphabet **a**: Significance between control and CP induced group, **b**: Significance between SGL extracts and Levamisole groups with control.

Table 2: Immunostimulatory effect of *Simarouba glauca* DC., stem bark (SGB) aqueous extract.

Parameters	Control	CP (30mg/kg)	LM (50mg/kg)	SGB (100mg/kg)	SGB (200mg/kg)	SGB (300mg/kg)
RBC (10^6/mm^3)	7.47±0.23	5.92±0.20*a	8.96±0.12	7.93±0.3	8.26±0.32	9.65±0.5*ab
Hemoglobin (g/dL)	9.93±0.08	6.40±0.32**a	11.23±0.33***ab	10.79±0.4*b	11.03±0.48*b	12.12±0.2**ab
WBC (10^3/mm^3)	4.50±0.26	3.13±0.14*a	10.66±0.33***ab	7.84±0.10*b	9.07±0.1**ab	12.08±0.3***ab
Neutrophils (%)	34.57±0.47	26.00±0.31*a	69.26±0.93***ab	58.56±0.20*ab	63.27±0.89**ab	68.65±0.63***ab
Lymphocytes (%)	10.10±0.26	7.36±0.23**a	14.33±2.64***ab	13.42±0.2*b	15.9±0.5**ab	21.72±0.2***ab
Monocytes (%)	0.02±0.0	0.6±0.01	0.23±0.04	0.34±0.03	0.31±0.03	0.20±0.05
Eosinophils (%)	0.20±0.01	0.55±00	0.26±0.06	0.33±0.01	0.29±0.02	0.21±0.4
Basophils (%)	0.01±0.0	0.11±0.02	0.04±0.01	0.02±0.0	0.11±0.04	0.05±0.1
Platelets (10^9/L)	6.97±0.23	3.13±0.27***a	8.7±0.23**ab	7.72±0.34*b	8.01±0.2**b	9.83±0.33***b
Splenocytes (10^8cells/mL)	1.72±0.2	0.89±0.9**a	2.73±0.33**ab	1.98±0.8*b	3.63±0.3**b	4.26±0.2**b

Data were expressed as Mean ± SEM (n=5). Statistical significance * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) compared with control and CP-treated groups. The alphabet **a**: Significance between control and CP induced group, **b**: Significance between SGB extracts and Levamisole groups with control.

Effect of SGL and SGB on Carbon clearance. The oral administration of SGL and SGB aqueous extract in mice was found to increase the clearance of carbon particles from blood as indicated by a highly significant phagocytic index in a dose-dependent manner (Fig 1a and 1b,). The SGL extract showed remarkable phagocytic index value (α) at 100mg/kg (3.44±0.02; p<0.05), 200mg/kg (4.27±0.12; p<0.01), and 300mg/kg (5.71±0.18; p<0.001) and these values for SGB were 3.78±0.66 (p<0.05), 4.92±0.20 (p<0.01) and 6.30±0.33 (p<0.001) at 100, 200 and 300 mg/kg, respectively.

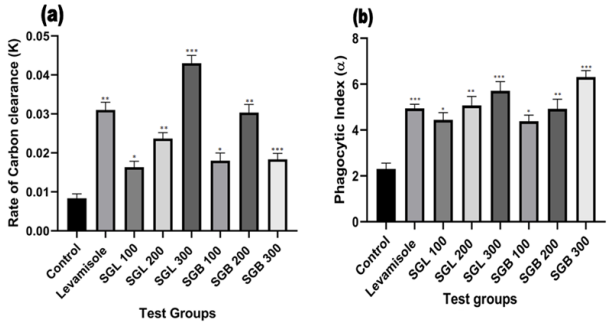

Fig. 1. (a) The dose-dependent effect of SGL and SGB administration on carbon clearance. (b) The dose-dependent effect of SGL and SGB administration on phagocytic index. Data were expressed as mean ± SEM (n=5) and the statistical significance of these were expressed by comparing with control as * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Effect of SGL and SGB on T-cell erythrocyte rosette formation. After treatment with SGL and SGB extracts for 10 days, the T-cell population was recorded in a dose-dependent manner at 100, 200, and 300mg/kg as compared to control (Fig. 2). The number of T-cell rosette formation (flower-like arrangement of T-cell and SRBC) against SRBCs was found to be moderately increased at 100 and 200mg/kg (p<0.01) for both SGL and SGB samples; the values were found to be significant as compared to control. Besides, a significant number of T-cell rosettes were mounted at 300mg/kg (p<0.001), and the levamisole-treated group also showed high T-cell rosettes (p<0.001) as compared to control (Fig. 2).

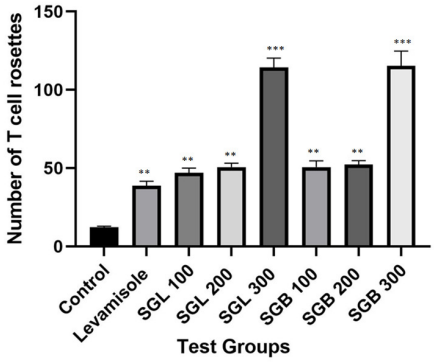

Fig. 2. Dose-dependent effect of SGL and SGB on T-cell rosettes formation. Data were expressed as mean ± SEM (n=5) and the statistical significance of these were expressed by comparing with control as * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Effect of SGL and SGB on anti-inflammatory activity. The anti-inflammatory activity of SGL and SGB extract against acute pedal edema was performed which showed significant reduction in paw thickness when compared to control (Table 3). It was observed that the SGL (300mg/kg) extract exhibited maximum and SGB (300mg/kg) extract showed moderate anti-inflammatory activity against Carrageenan-induced hind paw edema. The inhibition obtained with SGL and SGB extracts was comparable with the standard diclofenac sodium drug (Table 3).

Table 3: Anti-inflammatory assay by Carrageenan induced paw edema in mice.

Test groups	Paw thickness of mice (cm)						%Decrease in paw thickness at 24 h
	0h	1h	2h	3h	4h	24h	
Control	0.026±0.0008	0.03±0.001	0.035±0.019	0.042±0.009**	0.048±0.014***	0.049±0.005***	-
Diclofenac sodium (10mg/kg)	0.03±0.006	0.027±0.007	0.029±0.008**	0.029±0.007**	0.029±0.007**	0.021±0.004***	33%
SGL (100mg/kg)	0.046±0.002	0.048±0.004***	0.046±0.004	0.046±0.002	0.046±0.002	0.032±0.005**	27.77%
SGL (200mg/kg)	0.04±0.004	0.048±0.004***	0.048±0.004	0.044±0.002	0.044±0.002	0.028±0.002***	30%
SGL (300mg/kg)	0.036±0.004	0.05±0.002***	0.046±0.002	0.042±0.002	0.042±0.002	0.024±0.002***	33.33%
SGB (100mg/kg)	0.04±0.004	0.052±0.004***	0.046±0.004	0.048±0.004	0.048±0.005	0.03±0.004**	25.5%
SGB (200mg/kg)	0.032±0.002	0.052±0.004***	0.046±0.002	0.048±0.004	0.046±0.004	0.032±0.005*	20%
SGB (300mg/kg)	0.042±0.003	0.05±0.003***	0.036±0.002	0.04±0.003	0.042±0.004	0.031±0.003**	28.57%

DISCUSSION

Immunosuppression is a state of impermanent or permanent immunity dysfunction and can make the host more sensitive to foreign bodies due to the smash-up of the immune system. In recent years, immunotherapy has been developed as a promising therapy in form of complementary treatment for the diseases caused by viral infections (Varade *et al*., 2021; Pizzolla *et al*., 2016). Natural products are known to primarily stimulate the nonspecific immune responses (innate immunity), in which the mediators of immune system including cytokines, macrophages, neutrophils, acute-phase proteins and monocytes provide instant defense against pathogens (Federico *et al*., 2020).

The hematopoietic system is highly susceptible to the effect of nitrogen mustards including CP (Gulati *et al*., 2002). In addition to its ability to damage cellular DNA, CP has significant immunomodulatory activity, affecting several classes of immune cells. The current experimental evidence suggests that CP caused significant depression in biochemical markers such as erythrocytes, WBC, neutrophils, and splenocytes due to the alkylation of these precursors of granulopoiesis in the bone marrow. Apart from this, CP is also well known for causing thrombocytopenia (Jones, 1996). Most of these chemotherapeutic agents available today are immunosuppressant, cytotoxic, and exert huge side effects that are highly evident in cancer chemotherapy. Plant based immunomodulators are often employed as supportive or adjuvant therapeutics to overcome the undesired effects of cytotoxic chemotherapeutic agents and to restore normal health (Ben Sghaier *et al*., 2011). Some of the earlier reports of immunomodulatory effects of plant extracts have shown to increase the level of these biochemical parameters (Fan *et al*., 2013). In this study, the treatment with SGL and SGB extracts for 15 days stimulated the immune cells significantly as compared to CP induced and control group in a dose-dependent manner. This is probably due to the presence of various phytocomponents such as triterpenoids, quassinoids, cardiac glycosides, tannins, flavonoids and diterpenoids for stimulating hematopoiesis. Likewise, the standard drug, levamisole, also found to escalate the immune stimulation up to an extent which is lower as compared to higher dose of both SGL and SGB extracts. This was found to be consistent with earlier studies of the effect of ethanolic extract of *Shirishadi* on CP-induced immunosuppression indicating a good protection by increasing all the hematological parameters. The WBC count, RBC count, and % hemoglobin values observed were better than the control group (Divya *et al*., 2012). The hydroalcoholic extract of *Quisqualis indica* Linn. flower extract is a potent immunostimulant, the dose 150mg/kg of this extract showed significant ($p<0.05$) immunomodulatory activity by stimulating specific and non-specific immune mechanism (Yadav and Mohanty 2011).

The carbon clearance test was carried out to evaluate the effect of SGL and SGB extracts on the reticuloendothelial system (RES). The phagocytic cells such as macrophage is the major differentiated cell in the mononuclear phagocyte system (MPS) (Goldsby *et al*., 2003). Cells of the RES and MPS are known to be important in the clearance of particles from the bloodstream. When colloidal ink containing carbon particles was injected directly into the systemic circulation, the rate of clearance of carbon from the blood by the macrophage goes through a respiratory burst where more oxygen is consumed to supply the energy required for producing reactive oxygen species

(ROS). For an antigen mopping, reactive oxygen species require vitamin A, E, C, D, and the elements, Zn^{2+} and Cu^{2+} along with flavonoids and terpenoids which helps macrophages to remain active in antigen processing via cytokines (IL-1, IL-6, and TNF-α) (Deng *et al*., 2014). Additionally, a genuine alarm by macrophages and dendritic cells towards tissues invading infections is potential enough to switch from innate immunity to adaptive immunity. Comparably, pre-treatment with SGL and SGB extracts for up to a week enhanced the macrophage activity by two- to five-fold in the clearance of Indian ink in the circulating system with 200 and 300mg/kg doses, respectively, when compared to control as showed by significantly enhanced phagocytic index. A study found that the polyherbal formulation of *Boerhavia diffusa* whole plant and the ethanolic root extract of *Asparagus racemosus* (Shatavari) in different ratio (1:1, 1:2 and 2:1) at a dose of 100mg/kg increase rate of carbon clearance from blood and also a significant alteration in blood parameter in cyclophosphamide group when compared to standard (Amrita *et al*., 2021).

Withal, both SGL and SGB aqueous extracts displayed a positive response in our T-cell population test. An increase in the T cell rosette formation indicates the effect of extracts on cell-mediated immunity. The extracts showed a dose-dependent activity which may stimulate $CD4^+$ and $CD8^+$ cells resulting in an increase in T-cell immune response significantly. As such, the measurement of rosettes provides the most sensitive information on the T-cell population. The 10-days treatment with SGL and SGB aqueous extract raised the T-cell erythrocyte rosettes formation to nine-fold at a higher dose as compared to the control. These reliable findings in our experiment are aligned with the previous data (Kori *et al*., 2009). Eventually, the study showed that the *Moringa oliefera* stimulates both the cell-mediated and the humoral-mediated immune system at lower doses (Adouko *et al*., 2020; Sudha *et al.,* 2010) and *Trigonella foenum graecum* whole plant has shown significant increase in lymphocytes and T-cell rosettes formation at the dose 500mg/kg when compared to standard (Levamisole) (Sneha *et al*., 2014).

Carrageenan-induced paw edema is a generally used model for evaluating the anti-inflammatory activities of test compounds. This type of inflammation is biphasic, the initial phase is due to the release of histamine, 5-hydroxytryptamine, leukotrienes, kinins, and cyclooxygenases in the first hour of the administration of carrageenan, and the delayed phase has been linked to the production of prostaglandins, bradykinin, neutrophil infiltration, etc. (Setiawati *et al*., 2017; Huang *et al*., 2011; Brooks *et al*., 1991). In the present investigation, SGL and SGB extracts significantly ameliorated carrageenan induced paw edema after 24 h. In our study, we observed that SGL and SGB extracts could significantly reduce the exudation of plasma proteins in the paw of mice. This result suggests that the anti-inflammatory effect of SGL and SGB extracts may be due to the inhibition of cyclooxygenase synthesis and this effect is similar to that produced by nonsteroidal anti-inflammatory drugs such as diclofenac sodium (Huang *et al*., 2011). This was found to be consistent with the results of *in vivo* activity of hydroalcoholic extract of *Erythrinaindica* bark has showed significant activity in dose dependent manner, when compared to standard drug Aspirin and the ethanolic leaves extract 600mg/kg of *Ficus carica* Linn. exhibited maximum anti-inflammatory effect which is 75.90% in acute inflammation and in chronic study showed 71.66% reduction in granuloma weight (Fahmy *et al*., 2018; Bhagyasri *et al*., 2017; Vikas and Vijay 2011).

CONCLUSIONS

Overall, the present study affirms that the aqueous extract of *Simarouba glauca* DC., leaves and stem bark is an effective immunomodulatory agent. The effectiveness of extract-treated animals in overcoming the side effects of CP-induced immunosuppression provides evidence for the balancing and adaptogenic effectiveness by exhibiting a similar mechanism as that of levamisole in restoring immunosuppression. These results also support the fact that water-soluble components derived from these extracts have an important effect on the immune system and can effectively modulate immunological interactions. Thus, based on this preliminary study, it can be concluded that the SGL and SGB aqueous extracts hold the promise for its use as a novel immunostimulating agent. However, further detailed studies are needed to validate these findings.

FUTURE SCOPE

Simarouba is a genus of trees and shrubs in the family Simaroubaceae, native to the neotropics. As other genus of *Simarouba glauca* DC., includes *Simarouba amara* and *Simarouba versicolor* are well known for its pharmacological use worldwide, *Simarouba glauca* bark and leaf extracts are well known for its different types of pharmacological properties such as haemostatic, antihelmenthic, antiparasitic, antidysentric, antipyretic and anticancerous. The active content such as glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, simaroubidin, simarolide, simarubin, sistosterol is mainly evolved in pharmacological activities of this plant. Though, medicinal plants are one of the best sources for immunomodulators to control infectious diseases, by the use of immunomodulators rather antibiotic, host immune system can be boosted to defend against the pathogens. The immunomodulators can be the best alternatives for antibiotics which co-operates with the host immune system rather than acting against pathogen and thus reducing the chance of resistance against the immunomodulators. These pondering shall hopefully elicit the attention of researchers and egg on advance studies on these plant-based immunomodulation products as latent therapy for the management of infectious diseases, counting viral ones such as COVID-19.

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Conflict of Interest. None.

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