RESEARCH ARTICLE

Immunomodulating potential of *Calotropis procera* (ait.) root bark ethanolic extract on experimental animal

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ABSTRACT

The aim of the study was to explore the immunomodulatory activity of ethanolic extract (CPE) of *Calotropis procera* Ait. root bark in experimental mice. Ethanolic extract of the root bark of C. procera was evaluated for immunomodulatory activity using immunological tests in mice, viz. the humoral mediated antibody titre (AT), delayed-type hypersensitivity (DTH), peritoneal macrophage count, vascular permeability, haematological profile i.e. total red blood cell count (RBC), total leucocyte count (TLC), % neutrophils and % lymphocytes) and cyclophosphamide-induced myelosuppression (CIM) at three dose levels (50, 100 and 200 mg kg^{-1}). Oral administration of CPE at the doses of 50, 100 and 200 mg/kg in mice dose dependently enhanced the serum primary and secondary antibody level in mice and promoted the delayed-type hypersensitivity (DTH) against SRBC as compared with control group by increasing the mean foot pad thickness at 24, 48 and 72 h. Peritoneal macrophages in mice were significantly augmented ($P<0.05$). In hematological profile the TLC and % lymphocyte count increased significantly but the neutrophil count was decreased compared with the control. The percentage reduction in vascular permeability was increased in CPE (50, 100 and 200 mg/kg; p.o.) treated groups (32, 45 and 62 % resp.) compared to the control group. CPE prevented myelosuppression in mice treated with cyclophosphamide. The above findings suggest that the ethanolic extract of root bark of C. procera stimulate defense system by modulating several immunological parameter and can be complementary medicine for the management of immunodeficiency disorders.

Keywords: *Calotropis procera* ethanolic extract (CPE), Immunomodulation, Humoral response, Delayed type hypersensitivity, SRBCs

1. INTRODUCTION

The immune system is involved in etiology as well as pathophysiologic mechanisms of various diseases [1]. The modulation of the immune response with the aid of various bioactives inorder to alleviate certain diseases is an active area of interest [2]. Apart from being facilitatory stimulation or suppressive, certain agents modulate pathophysiological processes and immune responses, hence called immunomodulatory agents [3]. An excess of materials like proteins, lectins, polysaccharides, etc. which are plant-derived have been shown to stimulate the immune system [4]. Some of the plants with established immunomodulatory activity like *Viscum album*, *Panax ginseng*, *Asparagus racemosus*, *Azadirachta indica*, *Tinospora cordifolia*, *Polygala senega*, and *Ocimum sanctum* [5]. As an alternative, natural products reported to have influence on the immune system have also been investigated, such as sugarcane extracts [6], plant flavonoids[7], phenols [8], synthetic peptides derived from the beetle *Allomyrina dichotoma* defensin [9] and glutamine [10].

Calotropis procera Ait., a laticiferous plant of family Asclepiadaceae is well known for its multifarious medicinal properties. Different parts of this plant have been used in the traditional medicinal system for the treatment as an antineoplastic [11,12], anti microbial [13], anti-inflammatory [14,15], Anti-arthritic [16], antioxidant [17] agent and allergenic and immunological responses due to latex proteins [18]. The root of this plant is used as a carminative in the treatment of dyspepsia [19]. The ethanolic extract of the plant and chloroform fraction of its root have been shown to inhibit ulcer formation induced by various agents [20,21,22]. Besides, the chloroform extract of the roots has been shown to possess anti-inflammatory and analgesic properties [23].

The effects of the latex of *C. procera* on immune responses have been particularly investigated previously. The latex-methanol extract reduced the inflammatory stimulus induced by Freund's adjuvant

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on the experimental arthritis model [16] also dialysis and centrifugation of the latex provide a set of proteins that shows different effects on cell-mediated immunity [24].

The present study was therefore undertaken to explore *in vivo* immunomodulatory activities of *C. procera* root bark ethanolic extract on cellular (T cell) and humoral (B cell) mediated immune responses using animal models to the antigenic challenge by sheep RBCs, haemagglutination antibody (HA) titre and delayed type hypersensitivity (DTH) and by peritoneal macrophage count, vascular permeability, hematological profile and cyclophosphamide-induced myelosuppression.

2. EXPERIMENTAL

2.1. Collection of plant material

The fresh roots of the *C. procera* plant were collected at the flowering stage in the hot season from the city Indore, (M.P.) India. The plant was authenticated from Botanical Survey of India, Pune, India. Voucher specimen was kept at the centre with number (BSI/WRC/Tech./2010/GAPCAP1).

2.2. Chemicals, drugs and blood cells used

All the chemicals of AR grade were used and were obtained from Kasliwal Brothers, Indore, India. Cyclophosphamide was obtained as gift sample from Biochem Pharmaceutical Industries, Mumbai, India.

The sheep red blood cells (SRBCs) were collected in Alsever's solution and washed three times with pyrogen free saline and adjusted to concentration of 0.1 ml containing 1x 10^8 cells for immunization [25].

2.3. Preparation of ethanolic extract

The root bark of *C. procera* was shade dried and pulverized to coarse powder. The powdered root bark (500 g) was defatted with petroleum ether and then cold macerated with 90% ethanol. The extract was filtered and concentrated in rotary evaporator. The dried extract was stored in airtight container and refrigerated.

2.4. Preliminary phytochemical tests

The ethanolic extract of *C. procera* were subjected to preliminary phytochemical screening [26, 27], following standard procedure for the qualitative detection of various plant constituents.

2.5. Animals

Swiss albino mice of either sex (procured from MHOW Veterinary College, Indore, India.), weighing between 20 to 25 g, were used for the experiment the animals were housed under standard condition of temperature (25 ± 2º C) and 12 h / 12 h light / dark cycles. They were fed with standard pellet diet and water ad libitum. The animals were allowed to acclimatized one week before the experimentation. All experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) under supervision of CPCSEA (465/01/ab/CPCSEA).

2.6. Acute toxicity studies

The method described by the OECD guideline no. 425 in a stepwise procedure using the Acute Oral Toxicity –Up-and-Down Procedure was employed in the determination of the LD50. Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for the study. Animals were divided into four groups of three animals each and fasted overnight. 5, 50, 300, and 2000 mg/kg b.w. doses were administered to the Group I, II, III, and IV, respectively. After administration of extracts various parameters like body temperature, CNS activity, micturition, defecation etc. were observed for 24 hours [28].

2.7. Haemagglutinating antibody (HA) titre and Delayed type hypersensitivity (DTH)

Mice were divided into five groups each containing six mice. Group I was control and given sodium carboxy methyl cellulose (0.5%, p.o.). Group II was given standard drug (25 mg/kg, p.o.) for five days i.e, one day prior to sensitization (day 1), on day of sensitization (day 0) and (day 1, 2 and 3) after

sensitization with sheep red blood cells (day -1, 0, 1, 2 and 3). Group III to IV were given alcoholic extract of *C. procera* (three dose levels 50, 100 and 200 mg/kg p.o.) for eight days (from day 0 to day 7) after sensitization with sheep red blood cells.

The swiss albino mice were immunized by injecting 0.1 ml of sheep red blood cells suspension containing 1x 10^8 cells i.p. on day 0. Blood samples were collected from individual mouse by retro-orbital puncture on day +7 for primary antibody titre and on day +14 for secondary antibody titre. The blood samples were centrifuged and serum was collected. Antibody levels were determined by the haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two serial dilutions of pooled serum samples were made in 25ml of 1% suspension of sheep red blood cells in saline. After mixing, the plates were incubated at 37ºC for 1h, and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as antibody titre. On the day +7, the thickness of the right hind foot pad was measured using vernier caliper (Mitutoyo Digimatic). The mice were then injected 1x 10^8 sheep red blood cells in right hind footpad. The foot thickness was again measured at 24 h. The difference in paw thickness was taken as the measure of DTH response [29,30].

2.8 Peritoneal Macrophage count

Animals were treated with ethanolic extract of *C. procera* (three dose level 50,100 and 200 mg/kg, p.o.) for 20 days. On day 21 peritoneal fluids was collected in 5 ml of cold phosphate buffer saline (PBS, pH 7.2), transferred to a glass petridish and kept at 37°C for 1h. The supernatant was then discarded and cold 2% EDTA solution was added to it and kept at 4ºC for 30 minutes. It was then centrifuged and the pellet was suspended in 1 ml PBS. The macrophage count was determined after staining with 1% neutral red solution in PBS, using haemocytometer [31].

2.9. Vascular Permeability in mice

Mice were divided into six groups, each six mice. Group I was control (Na CMC, 0.5%), Group II was standard (Dexamethasone, 10 mg/kg). Group III was Standard (Ibuprofen, 100 mg/kg). Group IV-VI, were given ethanolic extract of *C. procera* (50,100 and 200 mg/kg, p.o.). Mice were then injected with 0.2% solution of Evans blue dye (0.25% w/v in normal saline) intravenously after 30 min of oral administration of the drug. Fifteen minutes later the mice were injected intraperitoneally (1 ml/100 g of body weight) with freshly prepared 0.6% of acetic acid (v/v). After 30 min of acetic acid injection the peritoneal cavities were washed with 5ml of heparinised sterile normal saline and centrifuged (3000 rpm) for 10 min. Absorbance of the supernatant was measured at 610 nm using a spectrophotometer (Shimadzu, UV-1601) [32].

2.10. Haematological profile

Ethanolic extract of *C. procera* (at three dose level 50, 100 and 200 mg/kg, p.o.), were administered orally for 20 days. Blood samples were collected at day 0 and then on day 21 after drug treatment total WBC, total RBC, percent neutrophil and lymphocyte and haemoglobin were determined using haemocytometer and haemometer [33].

2.11 Cyclophosphamide-induced immune suppression

Mice were divided into five groups, each containing six mice. Group I was served as control (Na CMC, 0.5%), Group II was standard (Dexamethasone, 1 mg/kg). Groups III, IV and V received test extract CPE at doses of 50, 100 and 200 mg/kg, p.o., respectively, for 15 days prior to administration of cyclophosphamide. Group II to V received cyclophosphamide at a single dose of 200 mg/kg, s.c., this day was labeled as day zero. On day 16, blood was collected from retro-orbital plexuses of individual animals and white blood cell count was determined using haemocytometer. Total WBC and absolute

neutrophil counts were performed prior to (on day 0) and on day 3 after injection of cyclophosphamide and were compared with the values of the control group [34].

2.12. Statistical analysis

Data are expressed as mean ± S.E.M. Statistical evaluation was carried out using one-way (ANOVA followed by Tukey's-Kramer multiple comparison test) using "Graphpad" version 5.00 for Windows 2007, Graphpad software, San Diego California USA. The values of $p < 0.05$ were considered to be statistically significant.

3. RESULTS

3.1. Phytochemical tests

The overall average yield of *C. procera* root bark extract was calculated to be 12.3%. Preliminary phytochemical screening revealed that petroleum ether extract contain lipids where as ethanolic extract showed the presence of tannins, flavonoids, alkaloids, phenols, steroids, terpenoids, carbohydrates, reducing sugars, glycosides and proteins respectively.

3.2. Acute toxicity studies

The LD_{50} was estimated to be 1000 mg/kg (p.o.) in mice. The toxicity data were represented by 'Irwin Table for Acute Toxicity'. During observation the animals exhibited decreased mobility, respiratory distress (gasping) with eventual immobility but no convulsions or loss of righting reflex prior to death.

3.3. Haemagglutinating antibody (HA) titre

The HA titre was used to assess humoral immune response. A dose-related increase in both primary and secondary antibody titre was observed in mice treated with ethanolic extract of CPE.

The augmentation of the humoral immune response to sheep red blood cells (SRBCs) by ethanolic extract is evidenced by increase in the antibody titre in the blood of mice (Table 1).

3.4. Delayed-type hypersensitivity (DTH) reactions

The cell-mediated immune response was assessed by DTH reaction, i.e. foot pad reaction. The ethanolic extract produced a significant, dose-related increase in DTH reactivity in mice. Increase in DTH reaction in mice up to 72 hours in response to T cell dependent antigen revealed the stimulatory effect of ethanolic extract on T cells (Table 2).

3.5 Peritoneal Macrophage count

Effect of morphometric changes of mouse peritoneal macrophages was evaluated. CPE showed significant rise in macrophage count in the dose dependent manner (Table 3).

3.6. Vascular Permeability in Mice.

Ethanolic extract of *C. procera* caused dose related reduction in vascular permeability. Treatment with ethanolic extract at higher dose (i.e., at 200 mg/kg; 61.5%) has shown similar result that of standard drug Ibuprofen (100 mg/kg; 67.4%). Dexamethasone, a standard drug at 10 mg/kg showed 23.7 % inhibition which was markedly less than the effect showed by all the doses i.e., 50, 100 and 200 mg/kg oral dose (Table 4).

3.7. Peripheral blood count

A significant, dose-related increase in the WBC count was observed, but percent neutrophils count was found to be significantly reduced at higher dose (200 mg/kg), percent lymphocyte count were found to be increased in mice treated with *C. procera* (50-200 mg/kg, p.o.) for 15 days (Table 5).

3.8. Cyclophosphamide-induced immuno suppression

Injection of a single dose of 200 mg/kg cyclophosphamide subcutaneously produced an increase of total leucocyte count but fall in absolute neutrophil count on the third day. The animals treated

for 15 days with CPE (50, 100 and 200 mg/kg/day) showed a significant leucocytosis with predominant neutrophilia. The leucopenia and neutrophilia, which occurred in control animals following cyclophosphamide, was less marked in the treated animals. No mortality was found in the groups with given treatments. In the positive control group i.e, standard (Dexamethasone 1 mg/kg) where drug was co-administered with cyclophosphamide and treatment discontinued after cyclophosphamide, no leucocytosis or protection against neutropenia occurred. This is indication that *C. procera* extract is immune boosting and immune restorative **(**Table 6**)**.

4. DISCUSSION

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells. To evaluate the effect of *C. procera* on humoral response, its influence was tested on sheep erythrocyte specific haemagglutination antibody titre in mice. Cyclophosphamide at a dose of 200 mg/kg, p.o., showed significant inhibition in antibody titre response, while *C. procera* was found to significantly enhance the production of circulating antibody titre. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis [35].

DTH is a part of the process of graft rejection, tumor immunity, and, most important, immunity to many intracellular infectious microorganisms, especially those causing chronic diseases such as tuberculosis [36]. DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilation, macrophage accumulation [37] and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing [38]. In the present study, SRBC, a sensitizer which acquires antigenicity [39], were used to elicit contact hypersensitivity reaction in mice. It was found that *C. procera* dose-dependently potentiated the DTH reaction induced by SRBCs. Increase in DTH reaction in mice in response to thymus-dependent antigen revealed the stimulatory effect of *C. procera* on T-lymphocytes and accessory cell types required for the expression of reaction [40].

Phagocytosis and killing of invading microorganisms by macrophages constitute body's primary line of defense. Macrophages are an integral part of the immune system, acting as phagocytic, microbicidal and tumoricidal effector cells [41]. Through interaction with lymphocytes, macrophages play an important role in the initiation and regulation of immune response. Macrophages also present antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. When activated, macrophages increase the phagocytic activity and release various materials such as cytokines and reactive intermediates and then carry out nonspecific immune responses [42]. In view of the pivotal role played by the macrophages, *C. procera* was found to significantly increase the peritoneal macrophages. The results indicate that *C. procera* might have enhanced the capacity of the monocyte macrophage system [38].

Vascular permeability in mice was used to evaluate the inhibitory activity against increased vascular permeability which is induced by a phlogistic substance [43]. Mediators of inflammation, such as histamine, prostaglandins and leucotrienes are released following stimulation e.g. of mast cells. This leads to a dilation of arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated and edemas are formed. The increase of permeability was recognized by the infiltration of the injected vital dye Evan's blue.

Since *C. procera* augmented the circulating antibody titre, it was thought worthwhile to evaluate its effect on peripheral blood count and cyclophosphamide-induced immunosuppression. Chronic administration of *C. procera* significantly ameliorated the total white blood cell count and also restored the myelosuppressive [44] effects induced by cyclophosphamide.

5. CONCLUSION

The investigation revealed that *C. procera* stimulates both the cellular and the humoral immune responses suggesting its therapeutic usefulness. However further investigations to establish exact role of *C. procera* that contributes to immunostimulatory effect are under process.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

The author declares no conflicts of interest.

Table 1: Effects of ethanolic extract of *Calotropis procera root bark* on 1º and 2º antibody titre to antigenically challenged mice

Group	Treatment	Dose (mg/kg)	Mean haemagglutinating antibody (HA) titre	
			1º HA titre	2º HA titre
I	Control	--	91.42 ± 12.93	102.92 ± 11.92
II	Cyclo	25	45.71 ± 23.42	50.21 ± 9.82
III	CPE	50	192.00 ± 28.62^{b}	200.23 ± 20.02
IV	CPE	100	213.33 ± 26.98^{c}	310.33 ± 25.62
V	CPE	200	320.00 ± 64.00^{a}	380.55 ± 32.05

All values are mean ± S.E.M, Control 0.2% Sodium carboxy methyl cellulose; n=6 per group. Comparison of I with II, III, IV, and V; Comparison of II with III, IV, and V. $^{a}P < 0.001$, very significant; Vs Control, $^{b}P < 0.05$, significant; $^{c}P < 0.001$, very significant Vs Cyclophosphamide by Tukey's-Kramer Multiple Comparison test (n = 6); 1º HA, primary haemagglutinating; 2º HA, secondary haemagglutinating. Cyclo: Cyclophosphamide 25 mg/kg, CPE: *Calotropis procera* extract

Table 2: Effects of *Calotropis procera* root bark on SRBCs (1×10^{8} cells, i.p.) induced DTH

Groups	Treatment	Dose mg/kg	0 min	1h	3h	12h	24h	72 h
I	Control	--	1.63 ± 0.06	3.6 ± 0.08	3.43 ± 0.05	2.78 ± 0.08	2.02 ± 0.02	2.37 ± 0.07
II	Cyclo	25	1.48 ± 0.04	2.97 ± 0.10	3.20 ± 0.08	3.14 ± 0.15	2.70 ± 0.12	2.81 ± 0.12
III	CPE	50	1.47 ± 0.04	2.97 ± 0.14	3.45 ± 0.08	2.63 ± 0.03$^{\#\#}$	2.13 ± 0.06^{c}	2.57 ± 0.05^{ns}
IV	CPE	100	1.42 ± 0.05	2.60 ± 0.16	3.55 ± 0.07$^{\#\#}$	2.71 ± 0.07$^{\#}$	2.17 ± 0.06^{c}	2.82 ± 0.10^{a}
V	CPE	200	1.52 ± 0.05	2.86 ± 0.04	3.51 ± 0.05$^{\#}$	2.78 ± 0.06$^{\#}$	2.27 ± 0.03^{c}	2.87 ± 0.06^{a}

All values are mean ± S.E.M, Control- 0.2% Sodium carboxy methyl cellulose; n=6 per group, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, Vs Control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, Vs Cyclophosphamide. CPE: *Calotropis procera* extract, Cyclo: Cyclophosphamide 25 mg/kg.

Table 3: Effect of *Calotropis procera* on *in vivo* peritoneal macrophage in mice

Group	Treatment	Dose (mg/kg)	Peritoneal macrophage in thousand/mm^{3})
I	Control	---	3.08 ± 0.56
II	CPE	50	6.07 ± 0.38^{a}
III	CPE	100	7.90 ± 0.14^{a}
IV	CPE	200	9.27 ± 0.91^{a}

All values are mean ± S.E.M, Control 0.2% Sodium carboxy methyl cellulose; n=6 per group. Comparison of I with II, III, IV. $^{a}P < 0.05$ Vs control; CPE: *Calotropis procera* extract.

Table 4: Effect of *Calotropis procera* on *in vivo* acetic acid induced vascular permeability in mice.

Groups	Treatment	Dose mg/kg)	Concentration of dye (µg / mouse)	Inhibition (%)
I	Control	-	5.65 ± 0.03	---
II	Dexa	10	4.31 ± 0.02	23.7
III	Ibu	100	1.84 ± 0.27	67.4
IV	CPE	50	1.83 ± 0.06^{ns}	32.4
V	CPE	100	3.08 ± 0.09^{b}	45.4
VI	CPE	200	2.17 ± 0.26^{a}	61.5

Data represent mean ± SEM (n=6) $^{a}P < 0.01$ compared with control group; $^{b}P < 0.001$ compared with Standard; $^{ns}P > 0.05$, non-significant. The statistical difference was evaluated by One-way ANOVA followed by Tukey's-Kramer Multiple Comparison test. Data shows increase in concentration of dye due to excessive vascular permeability in mice, reduction of dye concentration by drug treatment. Dexa: Dexamethasone, Ibu: Ibuprofen, CPE: *Calotropis procera* extract.

Table 5: Effect of chronic administration of *Calotropis procera* on peripheral blood count.

Groups	Treatment	Dose mg/kg	Haemoglobin concentration in g	RBC (x 10^6 cells/µl)	WBC (x 10^3 cells/µl)	Neutrophils (%)	Lymphocytes (%)
I	Control	--	14.20 ± 0.18	12.07 ± 0.34	11.15 ± 1.22	32.33 ± 1.45	58.33 ± 2.94
II	Dexa	1	13.01 ± 0.21	11.16 ± 0.57	9.15 ± 1.49	11.50 ± 5.50	78.0 ± 3.52
III	CPE	50	14.50 ± 0.20	11.22 ± 0.40	13.67 ± 1.25^{c}	30. 45 ± 2.55 ^{ns}	65.36 ± 6.41^{a}
IV	CPE	100	14.76 ± 0.24	11.24 ± 0.42	16.55 ± 1.35^{c}	27.50 ± 2.50^{ns}	69.33 ± 6.11^{b}
V	CPE	200	14.89 ± 0.17	11.27 ± 0.35	26.95 ± 1.23^{c}	15.33 ± 3.18^{a}	75.20 ± 4.70 ^{b}

Values are expressed as mean ± S.E.M. of six observations. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ as compared to control. ^{ns}P states as nonsignificant. The statistical difference was evaluated by One-way ANOVA followed by Tukey's-Kramer Multiple Comparison test. Dexa: Dexamethasone, CPE: *Calotropis procera* extract.

Table 6: Effect of chronic administration of *Calotropis procera* on cyclophosphamide (200mg/kg, s.c.) induced myelosupression

Groups	Treatments	Dose mg/kg	WBC (x 10^3 cells/µl)		Neutrophils (%)	
			DAY 0	DAY 3	DAY 0	DAY 3
I	Control	--	11.1 ± 1.2	3.5 ± 0.8	32.3 ± 1.4	5.5 ± 1.5
II	Dexa	1	9.1 ± 1.4^{ns}	2.2 ± 0.5	11.5 ± 5.5^{b}	1.5 ± 0.5
III	CPE	50	13.5 ± 1.1^{b}	4.2 ± 1.7	30.6 ± 2.5^{ns}	5.8 ± 1.6
IV	CPE	100	16.5 ± 1.3^{c}	7.2 ± 1.2	27.5 ± 2.5^{ns}	7.2 ± 1.7
V	CPE	200	26.9 ± 1.2^{c}	8.9 ± 2.7	15.3 ± 3.1^{a}	8.0 ± 1.0

Values are expressed as mean ± S.E.M. of six observations. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ as compared to control. $^{ns}P > 0.05$ states as non significant. The statistical difference was evaluated by One-way ANOVA followed by Tukey's-Kramer Multiple Comparison test; Dexa: Dexamethasone, CPE: *Calotropis procera* extract

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