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Subacute Effect of *Euphorbia neriifolia* Linn. On Hematological, Biochemical and Antioxidant Enzyme Parameters of Rat

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Abstract: *E. Neriifolia* is an herb extensively used in the Indian system of medicine, is a small deciduous tree of the family Euphorbiaceae. As traditional medicine the plant is useful in abdominal troubles, bronchitis, tumors, leucoderma, piles, inflammation, enlargement of spleen, anemia, ulcers, fever and in chronic respiratory troubles. *E. neriifolia* predominantly contains sugar, tannins, flavonoids, alkaloids and triterpenoidal saponin. The plant reported to have mild CNS depressant, wound healing and immunomodulatory activity of leaf hydro-alcoholic extract. Saponin separated from *E. neriifolia* leaf posses good hemolytic and *in-vitro* antioxidant activity. The present study was undertaken to investigate the effect of sub-acute administration of *E. neriifolia* leaf extract on some haematological, biochemical, histological and antioxidant enzyme status of rat liver and kidney following 21 and 45 days treatment. The animals were observed for gross physiological and behavioral responses, food and water intake and body weight changes. Free radical scavenging activity and histopathology was done on liver and kidney samples. *E. neriifolia* extract treatment extreme significantly ($p < 0.001$) reduced serum lipid profile along with glucose establishing its catabolic property. *E. neriifolia* showed an extremely significant ($p < 0.001$) rise in liver and kidney SOD along with liver catalase and decrease in liver lipid peroxidation. These features indicate that *E. neriifolia* upto 400 mg/kg daily dose is safe and has potential to be consumed for long time in management of various diseases.

Brief Synopsis: *E. neriifolia* is an herb extensively used in the Indian system of medicine. Plant reported to have mild CNS depressant, wound healing and immunomodulatory activity. The study investigates effect of sub-acute administration on haematological, biochemical and antioxidant enzyme status. The results indicate *E. neriifolia* upto 400 mg/kg daily dose is safe and has potential for long time consumption.

Key words: *Euphorbia neriifolia* · Leaf · Sub-acute · Biochemical parameters · Serum marker

INTRODUCTION

From prehistoric times, various communities and civilizations throughout the world are using herbal medicines. For the past several decades, people are increasingly consuming herbal medicines without prescription. They are traditionally considered as harmless since they belong to natural sources. Herbal formulations have reached widespread acceptability as therapeutic agents like anti-diabetics, anti-arthritics, aphrodisiacs, hepatoprotective, cough remedies, memory enhancers and adaptogens. Phytomedicines consisting of many chemical constituents have complex pharmacological effects on the body. Apart from determining efficacy, the safety of herbal products should also be assessed, as not all herbal medicines are harmless. In this context the incidence of 1991 and 1992 at Brussels, Belgium, in which 30 women treated with a Chinese herbal sliming preparation died from renal failure caused by the presence of aristocholic acid can be taken in to account [1].

The associated safety risks for some herbal medicines are believed to be low but the collated knowledge on the relative safety of herbal medicines remains poor.

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The accurate scientific assessment of herbal medicine is a prerequisite for global harmonization of herbal health claims. In this regard the World Health Organization (WHO) has set specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines. Most over-the-counter herbal products like ginseng have drawn great public attention but there are several case reports of adverse reactions of herbal drugs mentioned in the literature which are generally considered safe. An example is the occurrence of muscular weakness due to hypokalemia in long term users of herbal anthranoid laxatives [2]. Although preliminary assessment of efficacy can be obtained through the results of *in vitro* testing and experiments on animals but the effect of long term consumption should also be explored. It is very much needed to review continually and assess the safety of botanicals, with an emphasis on surveillance of the use of these products to identify unknown hazards or risk associated with their continuous use.

However, all the adverse reactions do not occur immediately after starting the therapy. In normal practice herbal medicines are prescribed for relatively longer durations (2 weeks to 3 months) and during that period there is possibility of patient's self-medication and some other medicines are occasionally co-administered. It is therefore necessary and also highly desirable to clarify what kind of effect the herbals have after long term uses on body weight, relative organ weight, haematological parameters, serum enzyme parameters, antioxidant enzyme status and histopathology of liver and kidney.

There are over 1500 species of Euphorbias in the world ranging from annual weeds to trees. *Euphorbia neriifolia* Linn. (Euphorbiaceae) grows luxuriously around the dry, rocky, hilly areas of North, Central and South India. *E. neriifolia* is a herb full of spine, popularly known as 'sehund' or 'thohar'. Leaves are thick succulent, 6-12 inch long, ovular in shape. In traditional system leaves are used as aphrodisiac, diuretic, cough and cold, bleeding piles and in ano-rectal fistula [3]. The tribal population of Chattishgarh region uses the milky latex as an ingredient of aphrodisiac mixture. Latex is used to de-root skin warts, earach and in arthritis [4]. Plant is bitter, laxative, carminative, improves appetite, useful in abdominal troubles, bronchitis, tumors, leucoderma, piles, inflammation, enlargement of spleen, anemia, ulcers, fever and in chronic respiratory troubles [5]. Natives of Chhattisgarh use externally boiled 'thohar' milk in castor oil with salt to cure the deep cracks in soles of legs. The milk of 'thohar' is also used commonly like aloe gel in case of burns. 'thohar' milk can be used successfully for healing of wounds. Application of lukewarm 'thohar' leaves reduces itching pain and swelling in piles [6].

E. neriifolia hydroalcoholic extract was found to contain sugar, tannins, flavonoids, alkaloids, triterpenoidal saponin on preliminary phytochemical analysis. Several triterpenoids like Glut-5-en-3β-ol, Glut-5(10)-en-1-one, taraxerol and β-amyrin has been isolated from powdered plant, stem and leaves of *E. neriifolia* [7, 8]. Antiquorin have been isolated from ethanol extract of fresh root of *E. neriifolia* [9]. Neriifolione, a triterpene and a new tetracyclic triterpene named as nerifoliene along with euphol were isolated from the latex of *E. neriifolia* [10, 11].

E. neriifolia is easily available in large quantity in the dry hilly areas of North and Central India. This plant can be used as a cheap source of active therapeutics as propagation of these plants is easy and cheap which can be grown in large number with very less expenses. *E. neriifolia* latex showed wound healing activity in guinea pig by increasing epithelization, angiogenesis, tensile strength and DNA content in wounds [12]. We have already reported mild CNS depressant, wound healing and immunomodulatory activity of leaf hydro-alcoholic extract [13-16]. Saponin separated from *E. neriifolia* leaf posses good hemolytic and *in-vitro* antioxidant activity but it is devoid of antibacterial activity upto 10 mg/ml concentration [17]. This study was performed to assess effect of long term administration of *E. neriifolia* leaf alcoholic extract once a day continuously for 21 and 45 days on growth, hematology and biochemistry of experimental animals.

MATERIALS AND METHODS

Collection and Extraction of Plant Material: *E. neriifolia* leaves were collected from cultivation field hedge plants of suburban areas of Bhopal (latitude 23.21°, longitude 77.84°, BHOP), Madhya Pradesh, India, in September 2005. The plant was identified with the help of available literature and authenticated by Dr. AP Shrivastava, taxonomist and Principal, P.K.S Govt. Ayurveda College, Bhopal, India. A voucher specimen was deposited in the herbarium of department (No. 1085).

The leaves were air dried under shade and milled into coarse powder, extracted in Soxhlet extractor successively with different organic solvents such as petroleum ether (60-80°C), chloroform, acetone and 95% ethanol in increasing order of polarity [7]. The marc was dried in hot air oven below 50°C before extracting with next solvent. The extracts obtained with each solvent was distilled to

remove 1/4th of solvent then the extracts were dried using a vacuum oven below 30°C and percentage weight calculated in terms of w/w. 95% ethanolic extract was dark brown in colour and extractive value was 4.85 % (w/w) of the dry weight of starting material. Presence of triterpenoidal steroids was confirmed by the Salkowski test and Noller's test [18]. Presence of saponin was confirmed by Froth test and Hemolysis test [19]. Presence of flavonoids was confirmed by Shinoda test and Alkaline reagent test [20].

Test Animals: Laboratory bred Wistar albino rats of both sexes (150-200 g) maintained under standard laboratory conditions at 22 ± 2°C, relative humidity 50±15% and photoperiod (12-h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water were provided *ad libitum*. In order to avoid diurnal variation all the experiments were carried out at same time of the day i.e. between 10 a.m. to 5 p.m. Approval was obtained from Institutional Animal Ethical Committee (approved body of Committee for the Purpose of Control and Supervision of Experiments on Animals, Chenni, India) of Radharaman College of Pharmacy, Bhopal, before carrying out the experiments and care provided to the animal was as per the WHO 'guidelines for the care and use of animals in scientific research.

Determination of LD_{50}: Ld_{50} was determined according to the guidelines of Organization for Economic Co-operation and Development (OECD) following the Up and Down method (OECD guideline No. 425) and Fixed dose method (OECD guideline No. 420). Based on these agreements a *Limit test* was performed to categorize the toxicity class of the compound and then *Main test* was performed to estimate the exact LD_{50} [21]. The limit test was started from 2000 mg/kg dose. LD_{50} was found greater then the test dose so the test substance could be classified in the hazard classification as class 5, 2000 mg/kg<LD_{50} <5000 mg/kg in the Globally Harmonized System (GSH). LD_{50} of *E. neriifolia* leaf extract was found to 2779.71 mg/kg from main test [22]. A dose range of 100, 200 and 400 mg/kg was selected for *E. neriifolia* leaf extract. The doses selected for the study starts from 1/7 of LD_{50}, a dose range of 100, 200 and 400 mg/kg was selected for *E. neriifolia* leaf extract.

Study Protocol: Animals were divided into four groups of 16 rats in each, group 1 served as control received 2% CMS (0.5 ml/100gm) only, group II, III and IV were treated with *E. neriifolia* extract 100, 200 and 400 mg/kg, p.o dose respectively on daily basis for 45 days. Eight rats from each group were sacrificed on 21st day and remaining eight rats on 45th day two hours after last dose administration to assess all the parameters.

The animals were observed for physiological and behavioral responses, mortality, food and water intake and body weight changes. Body weight of the animals was noted before and after extract treatment for 21 and 45 days and percent increase in body weight was calculated. The animals were sacrificed by cervical dislocation and blood collected by cardiac puncture in clean dry heparinised tubes, used for estimation of hematological parameters. Another aliquot of blood was allowed to coagulate for 30 min in room temperature and centrifuged at 3000 rpm for 5 minutes. The supernatant serum was separated and used for estimation of marker enzyme. The animals were quickly dissected to remove liver, kidney, spleen and heart, washed with cold saline solution, pressed between filter paper pads and weighed. Relative organ weight (weight of organ/100 gm of body weight) was calculated and recorded. A part of liver and kidney was preserved in cold saline for estimation of free radical scavenging activity and reminder in Aqua Bouine's fluid for histopathology.

Haematological Parameters: Estimation of hemoglobin content (Sahli's Hemoglobinometer), total WBC and RBC count (Neubauer hemocytometer; Feinoptik, Germany) was done using standard technique and differential WBC count (neutrophil, eosinophil, basophil, lymphocyte and monocyte) was done by Leishman's staining method [23].

Estimation of Blood Marker Enzymes: Serum alkaline phosphatase [24], total protein content [25], total cholesterol [26], serum triglycerides [27] and high density lipoprotein [28] were determined spectrometrically (Shimadzu UV-1700; pharmaspec) using the commercially available standard kit (Span Diagnostics Ltd., India). Serum low density lipoprotein [29] content was calculated from known concentration of total cholesterol, triglyceride and HDL cholesterol. Glucose content of serum was estimated following the method of Trinder [30] using a standard kit of Transasia Bio-medicals Ltd., Daman. Serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase [31] was estimated using kit obtained from Stangen Immuno Diagnostics, Hydrabad.

Estimation of Free Radical Scavenging Ability of Liver and Kidney: Lipid peroxides was estimated as thiobarbituric acid reactive substance malondialdehyde

(MDA) at 532 nm following the method of Ohkawa *et al*. [32]. The level of lipid peroxidases was expressed as nM of MDA/ mg of liver protein. Peroxidase is a class of important enzymes such as NAD-peroxidase, NADP-peroxidase and glutathione peroxidase involved in scavenging of hydrogen peroxide. Liver homogenate 0.5 ml (10% in 0.1 M KCl) was added to 1 ml of potassium iodide and sodium acetate solution each then the absorbance was noted at 533 nm. To the above solution 20 µl of hydrogen peroxide solution was added and the change in absorbance was again noted after 5 min. One unit of peroxidase activity is defined as change in absorbance per min and expressed in terms of units/min/mg of protein.

Catalase was determined as per the method of Aebi [33]. Decomposition of H_2O_2 by catalase is directly proportional to decrease in absorbance at 340 nm. The results were expressed as unit of H_2O_2 decomposed/min/mg of protein, using 71 as molar extinction coefficient of H_2O_2. Superoxide dismutase was estimated as per the method of Misra and Fridovich [34]. One enzymatic unit of SOD is the amount of protein in the form of enzyme present in 100 µl of sample required to inhibit the reduction of 24 micromolar nitro-blue tetrazolium (NBT) by 50% and is expressed as unit/mg of protein. All these estimations were done on Shimadzu (UV-1700 pharmaspec) spectrophotometer.

Histological Study of Liver and Kidney: Permanent tissue slides of liver and kidney sections were prepared and stained (Haemotoxylene and Eosin) based on method of Nanji *et al*. [35].

Statistical Analysis: All data are presented as means ± SEM. Experimental data was analysed using one-way ANOVA followed by Student's *t*-test to compare the difference between the control and treated values. P value < 0.05 were considered significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS

Body Weight and Relative Organ Weight: Administration of *E. neriifolia* extract (400 mg/kg) for 21 and 45 days resulted in significant increase in relative weight of kidney ($p < 0.05$) and spleen ($p < 0.05$ and $p < 0.001$). As shown in Table 1. *E. neriifolia* extract after 21 and 45 days treatment resulted in a dose dependent decrease in body weight gain, at 400 mg/kg dose it showed only 6.25 and 9.37% increase in body weight respectively compared to 21.88 and 40.61% of control.

Heamatological Parameters: *E. neriifolia* extract treatment showed non-significant increase in total RBC, WBC and Hb content at all the tested doses. *E. neriifolia* extract treatment at 200 and 400 mg/kg dose showed extremely significant ($p < 0.001$) rise in percentage of neutrophil and lymphocyte as shown in Table 2.

Serum Biochemical Parameters: *E. neriifolia* extract treatment caused extremely significant ($p < 0.001$) decrease in serum SGOT at 21 days as well as 45 days treatment in all the tested doses. Extract treatment showed no significant alteration of serum SGPT at all the tested doses on 21 days and 45 days compared to control. Extract caused extremely significant ($p < 0.001$) rise in serum ALP at 400 mg/kg dose on 21 days treatment. On 45 days treatment the increase in serum ALP concentration was extremely significant ($p < 0.001$) at all the tested doses. Serum cholesterol level decreased significantly ($p < 0.01$ and 0.001) at 200 and 400 mg/kg dose after 45 days treatment. Decrease in serum triglyceride concentrations was extremely significant ($p < 0.001$) at 400 mg/kg dose after 45 days treatment. *E. neriifolia* extract treatment did not have any significant effect on serum protein content. Extract administration extreme significantly ($p < 0.001$) decreased serum LDL at 200 and 400 mg/kg doses on 45 days treatment. *E. neriifolia* significantly ($p < 0.05$) increased serum HDL only at 400 mg/kg dose after 45 days treatment. Extract initially at 21 days treatment had non-significant effect on serum glucose, on the other hand after 45 days treatment it decreased serum glucose at 200 and 400 mg/kg dose which is extremely significant ($p < 0.001$). All the results are tabulated in Table 3.

Free Radical Scavenging Ability of Liver and Kidney: Effect of *E. neriifolia* extract treatment on antioxidant parameters of liver and kidney was tabulated in Table 4. Liver lipid peroxidase activity was decreased both at 21 and 45 days treatment which was extremely significant ($p < 0.001$) and it has non-significant activity on kidney lipid peroxidase level. Extract treatment initially at 21 days treatment showed extremely significant ($p < 0.001$) rise in peroxidase levels of both liver and kidney. Afterwards on 45 days treatment the changes were extremely significant ($p < 0.001$) only at 400 mg/kg dose. Liver catalase activity showed extremely significant ($p < 0.01$ and 0.001) rise in all tested doses after 45 days treatment. On the other hand it had non-significant effect on kidney catalase activity. *E. neriifolia* showed extremely significant ($p < 0.001$) rise in liver and kidney SOD at all the tested doses on 45 days treatment.

Table 1: Effect of daily oral administration of *E. Neriifolia* extract upto 6 weeks on relative body and organ weight of rats

		Relative organ weight (gm/100 gm of body weight) M±SEM				
Day	Treatment (mg/kg, p.o)	Liver	Kidney	Heart	Spleen	% increase in body weight
21	Vehicle control	3.034±0.156	0.803±0.047	0.379±0.026	0.234±0.044	21.88
	E. n extract (100)	3.243±0.462ns	0.805±0.022ns	0.385±0.033ns	0.257±0.011ns	20.13
	E. n extract (200)	3.661±0.223ns	0.877±0.085ns	0.412±0.052ns	0.279±0.041ns	15.50
	E. n extract (400)	3.060±0.375ns	1.049±0.073*	0.456±0.066ns	0.388±0.015*	6.25
45	Vehicle control	3.305±0.678	0.926±0.034	0.384±0.059	0.286±0.055	40.61
	E. n extract (100)	3.823±0.137ns	0.997±0.081ns	0.412±0.065ns	0.425±0.093ns	32.20
	E. n extract (200)	4.070±0.721ns	1.050±0.096ns	0.445±0.049ns	0.581±0.014*	21.33
	E. n extract (400)	4.226±0.850ns	1.256±0.040*	0.520±0.082ns	0.836±0.058***	9.37

Data expressed as Mean±SEM. n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 and ns = not significant when compared to respective control group

Table 2: Effect of daily oral administration of *E. Neriifolia* extract upto 6 weeks on peripheral blood parameters of rats

		Hb	RBC	WBC	Differential leukocyte count in % (M±SEM)				
	Treatment	(gm/dl)	(10^6/mm^3)	(10^3/mm^3)					
Day	(mg/kg, p.o)	M±SEM	M±SEM	M±SEM	N	L	M	E	B
21	Vehicle control	11.70±0.50	4.80±0.13	6.02±0.82	52.45±1.27	45.28±1.94	1.30±0.12	0.77±0.17	0.20±0.11
	E. n extract (100)	12.18±0.63^{ns}	5.93±0.92^{ns}	6.35±0.48^{ns}	53.92±1.47^{ns}	45.80±1.22^{ns}	0.03±0.01**	0.22±0.12^{ns}	0.03±0.01^{ns}
	E. n extract (200)	13.05±0.59^{ns}	6.05±0.46^{ns}	6.27±0.31^{ns}	41.64±1.49***	57.54±1.35***	0.40±0.13*	0.40±0.14^{ns}	0.02±0.01^{ns}
	E. n extract (400)	14.00±0.58*	7.20±0.38^{ns}	5.95±0.22^{ns}	40.52±1.28***	57.93±1.31***	0.52±0.24^{ns}	0.90±0.21^{ns}	0.13±0.11^{ns}
45	Vehicle control	12.20±0.97	5.31±0.25	7.20±0.53	53.14±1.51	44.17±1.59	1.02±0.17	1.20±0.15	0.47±0.14
	E. n extract (100)	13.91±0.80^{ns}	6.13±0.55^{ns}	6.46±0.83^{ns}	48.52±1.39^{ns}	49.08±1.40^{ns}	1.04±0.33^{ns}	0.79±0.42^{ns}	0.56±0.23^{ns}
	E. n extract (200)	14.36±0.68^{ns}	7.04±0.62^{ns}	6.86±0.50^{ns}	39.37±1.44***	58.54±1.30***	1.10±0.42^{ns}	0.82±0.24^{ns}	0.17±0.12^{ns}
	E. n extract (400)	14.50±0.74^{ns}	7.28±0.81^{ns}	7.05±0.42^{ns}	40.02±1.72***	58.86±1.50***	0.94±0.36^{ns}	0.12±0.11^{ns}	0.06±0.01^{ns}

Data expressed as Mean±SEM. n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 and ns = not significant when compared to respective control group. N = Neutrophil, L = Lymphocyte, M = Monocyte. E = Eosinophil and B = Basophil

Table 3: Effect of daily oral administration of *E. Neriifolia* extract upto 6 weeks on serum biochemical parameters of rat

	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (U/l)	Cholesterol	Tri-glyceride	Protein	HDL	LDL	Glucose
Day	(mg/kg, p.o)	M±SEM	M±SEM	M±SEM	(mg/dl) M±SEM	(mg/dl) M±SEM	(gm/dl) M±SEM	(mg/dl) M±SEM	(mg/dl) M±SEM	(gm/dl) M±SEM
21	Vehicle control	44.72±2.66	47.21±3.15	65.74±4.34	91.70±3.22	63.50±3.70	7.60±0.11	42.67±2.86	36.33±2.11	109.72±5.30
	E. n extract (100)	27.46±2.04***	43.37±3.22^{ns}	72.19±4.56^{ns}	143.95±5.31***	64.87±3.40^{ns}	8.62±0.37^{ns}	45.17±3.10^{ns}	66.35±3.94***	111.64±4.73^{ns}
	E. n extract (200)	11.92±1.30***	39.22±2.94^{ns}	90.03±4.90*	103.75±4.95^{ns}	63.46±3.52^{ns}	9.49±0.73^{ns}	45.39 3.22^{ns}	26.63±2.55^{ns}	103.95±4.56^{ns}
	E. n extract (400)	10.65±1.05***	39.01±2.78^{ns}	163.71±6.14***	97.16±4.33^{ns}	61.29±3.90^{ns}	10.07±0.84^{ns}	49.25±3.04^{ns}	17.27±1.79***	97.16±4.80^{ns}
45	Vehicle control	45.55±3.71	46.19±3.22	68.24±3.84	104.72±5.17	65.17±3.07	7.82±0.40	45.40±3.10	46.29±2.12	112.63±4.68
	E. n extract (100)	15.33±1.66***	43.92±2.99^{ns}	103.17±5.03***	105.33±5.17^{ns}	61.25±3.14^{ns}	7.65±0.59^{ns}	48.32±3.16^{ns}	44.76±2.33^{ns}	96.54±4.84^{ns}
	E. n extract (200)	9.87±0.94***	44.27±3.42^{ns}	129.53±5.09***	82.48±4.30**	58.65±2.53^{ns}	7.04±0.38^{ns}	56.80±3.56^{ns}	13.95±1.70***	82.48±4.60***
	E. n extract (400)	8.31±0.85***	43.18±2.55^{ns}	138.97±5.61***	73.23±3.16***	42.21±2.76***	6.68±0.51^{ns}	60.36±3.67*	4.43±0.16***	43.23±3.58***

Data expressed as Mean±SEM. n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 and ns = not significant when compared to respective control group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum pyruvate oxaloacetate transaminase, ALP = Alkaline phasphatase, HDL = High density lipoprotein, LDL = Low density lipoprotein.

Table 4: Effect of daily oral administration of *E. Neriifolia* extract upto 6 weeks on liver and kidney antioxidant enzyme parameters of rat

		Lipid peroxidase (nmol/mg of protein) M±SEM		Peroxidase (unit/min/mg of protein) M±SEM		Catalase (unit/min/mg of protein) M±SEM		SOD (unit/mg of protein) M±SEM	
	Treatment								
Day	(mg/kg, p.o)	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
21	Vehicle control	28.65±1.28	6.78±0.50	2.68±0.26	1.17±0.13	3.31±0.37	5.36±0.76	1.86±0.12	1.71±0.23
	E. n extract (100)	22.13±2.46^{ns}	6.82±0.22^{ns}	5.13±0.57*	1.83±0.44^{ns}	4.68±0.57^{ns}	5.14±0.48^{ns}	3.19±0.47^{ns}	2.73±0.77^{ns}
	E. n extract (200)	15.62±1.41***	6.74±0.36^{ns}	8.91±0.37***	3.59±0.23***	6.47±0.83*	4.55±0.52^{ns}	4.75±0.38***	4.60±0.25**
	E. n extract (400)	18.54±1.35**	5.99± 0.42^{ns}	7.65±0.81***	4.56±0.14***	7.14±0.90**	3.88±0.16^{ns}	4.71±0.62***	4.54±0.42**
45	Vehicle control	29.94±1.22	7.44±0.34	2.71±0.45	1.30±0.25	3.16±0.47	4.17±0.48	1.76±0.23	4.31±0.69
	E. n extract (100)	10.26±0.87***	8.90±0.33^{ns}	2.74±0.23^{ns}	1.70±0.13^{ns}	6.15±0.57**	3.82±0.33^{ns}	13.40±0.95***	26.46±2.32***
	E. n extract (200)	3.96±0.52***	8.67±0.45^{ns}	4.58±0.87^{ns}	2.33±0.22^{ns}	7.32±0.60***	3.60±0.31^{ns}	26.06±1.12***	47.58±2.24***
	E. n extract (400)	7.78±0.69***	8.48±0.68^{ns}	4.47±0.41^{ns}	5.47±0.42***	6.70±0.45***	2.79±0.15^{ns}	25.46±1.43***	47.15±2.14***

Data expressed as Mean±SEM. n = 6, *P<0.05, **P<0.01, ***P<0.001 and ns = not significant when compared to respective control group. SOD = Super oxide dismutase

Histological Study of Liver and Kidney: Histopathological studies of the liver and kidney samples of the treated rats showed normal architecture with structural and functional integrity.

DISCUSSION

E. neriifolia did not show mortality on 45 days continuous treatment, all animals were found to be normal during and at the end of observation period. Leaf extract initially produced marked depression of central nervous system, reduction in motor activity and depression of auditory and visual reflexes. Unsteady gait and eyelid closure was also observed in the animals but these effects were normalized after few days. The extract treated groups showed decreased body weight gain compared to control. Organ body weight ratios are normally investigated to determine whether the drug treatment has any effect on size and weight of the vital organs comparative to total body weight. However the significant increase in both, spleen and kidney weight for 45 days extract treatment may be attributed to it beneficial effect to the body and also its immunostimulant properties [15]. Since kidney is concerned with excretion of foreign substances including plant extract and incase of tissue necrosis the organ might have been reduced. The extract did not have significant effect on RBC, WBC or hemoglobin content but had increased percentage of neutrophils and lymphocytes which in turn signifies its immunopotentiating effect.

The measurement of enzyme activities in tissue or body fluid plays a significant and well defined role in investigation and diagnosis of diseases [36]. Tissue enzyme assay can indicate cellular damage long before structural damage of tissues can be picked up by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by sub-acute or chronic use of plant extract. Alkaline phosphatase is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum functionality [37]. However extract treatment decreased serum SGOT with no effect on SGPT. Increment in the enzyme activity following 21 and 45 days extract administration may be attributed to enhancement of membrane component including alkaline phosphatase into the extra cellular fluids or stimulation of the enzyme molecule by the phosphate group for the phosphorylation of ethanolamine and choline. This is needed for the synthesis of two major membrane phospholipids, phosphatidylethanolamine and phosphatidylcholine, respectively with the attendant consequence of affecting membrane fluidity and increasing the permeability of the epithelial cells [38]. Enhancement in alkaline phosphatase activities may lead to increased availability of plant extract or activation of the enzyme molecule in situ [39]. It is often employed to assess the integrity of plasma membrane [40] and endoplasmic reticulum.

E. neriifolia treatment reduced serum lipid profile (cholesterol, triglyceride and LDL) along with glucose which is extremely significant, establishing its catabolic property. It showed an extremely significant rise in liver and kidney SOD along with liver catalase. The extract also showed extreme significant decrease in liver lipid peroxidation, which signifies the reported and well defined antioxidant activity on liver and kidney of the treated animals [17]. Some plants contain heterogeneous alkaloids and tannins so some toxic effects are expected on long term uses, however components like reducing sugars, flavonoids, amino acids and other compounds present might have helped in reducing the toxicity [41].

CONCLUSION

E. neriifolia leaf extract had potent analgesic, anti-inflammatory, mild CNS depressant, wound healing activity along with humoral and cell mediated immunostimulating activity. *E. neriifolia* reduced serum lipid profile and glucose signifying catabolic property with added *in vivo* and *in vitro* antioxidant activity. Phytochemical study revealed presence of triterpenoidal saponin, reducing sugar, tannins, flavonoids and alkaloids. The catabolic and antioxidant effect of the extract may be due to presence of saponin and flavonoids. This plant has immense potential and have broad spectrum of activity on several ailments. *E. neriifolia* is easily available in large quantity in the dry hilly areas of North and Central India. The global changing scenario is showing a tendency towards use of nontoxic plant products having good traditional medicinal background. This plant can be used safely for longer duration as a cheap source of active therapeutics for alleviation of commonly occurring ailments by the poor and under privileged people of India.

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