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EVALUATION OF ANTIOXIDANT AND HYPOLIPIDEMIC ACTIVITY OF VEDIC GUARD, A POLYHERBAL FORMULATION

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ABSTRACT

The present study was undertaken to evaluate antioxidant and hypolipidemic activity of Vedic guard, a polyherbal formulation comprising of *Terminalia arjuna, Withania sominefera, Bacopa monnierii, Tinosporia cardifolia, Commiphora mukul* and many other plant extracts, all of which are classifieds in Ayurveda as Rasaayanas which are reported to promote antioxidant and hypolipidemic activity. Antioxidant activity has been established in young adult rats against antitubercular drug induced hepatotoxicity but not in geriatric rats. Antioxidant activity was evaluated by hepatoprotectivity against carbon tetrachloride induced liver toxicity for 21 days in geriatric rats using Silymarin as reference standard, and by pyrogallol induced immunosupression for 28 days in young adult rats using levamisole as reference standard. Hypolipidemic activity was evaluated using triton WR1339 induced hyperlipidemic rats as experimental model in acute study. Oxidative damage was evidenced by occurrence of liver cirrhosis, elevation of AST, ALT, and ALP, total bilirubin in blood serum and increased LPO, reduced CAT and GSH in liver homogenate. Vedic guard treatment in geriatric rats with doses of 90mg/kg, 180mg/kg b. w., p. o. and standard silymarin significantly reversed all the changes that were due to CCl_4. Similarly pyrogallol induced immunosupression was evidenced by increased LPO and decreased GSH, SOD, CAT in liver homogenate. Vedic guard treatment in young adult rats at 180mg/kg b. w., p .o. and standard levamisole significantly altered the changes that were due to immunosupression. Hyperlipidemia was evidenced by increased serum total cholesterol, triglycerides, LDL, VLDL and decreased HDL. Vedic guard treatment with lower and higher doses and standard atorvastatin reversed all changes that were due to hyperlipidemia. The Vedic guard showed significant hypolipidemic and antioxidant activity.

INTRODUCTION: Oxidative stress has been postulated to be involved in pathogenesis of variety of diseased states, for e.g., psychiatric disorder like depression and anxiety, immunosupression, endocrine disorders including diabetes mellitus, male impotency, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis. Antioxidants are beneficial compounds that neutralize free radicals before they can attack cells proteins, lipids and carbohydrates and affect cell differentiation and proliferation, block nitrosamine formation, stimulate the immune response, help to maintain integrity of cell membrane and matrixes, and maintenance of normal DNA repair [1].

Hyperlipidemia is associated with risk factors like atherosclerosis, hypertension, type-II diabetes mellitus, obesity, myocardial infarction, congestive cardiac failure, angina pectoris, gall bladder diseases, degenerative joint diseases, sleep apnea, and infertility. Allopathic drugs are available for counteracting liver injury and hyperlipidemia, but the side effects and cost associated with these allopathic drugs necessitates the search for alternatives.

In due regard Vedic Bio labs, Bangalore, has developed a Polyherbal formulation, Vedic guard, each 500mg capsule consisting of dried powder extract of plants such as *Terminalia chebula* (30mg), *Emblica officinalis* (30mg), *Terminalia arjuna* (30mg), *Withania somineferia* (30mg), *Sida cardifolia* (30mg), *Bacopa monnierii* (30mg), *Eclipta alba* (30mg), *Tribulus terrestris* (30mg), *Tinosporia cardifolia* (30mg), *Commiphora mukul* (30mg), *Mesuua ferrea* (30mg), *Pipper longum* (30 mg), *Pureria tuberosum* (30mg), *Glycerhiza glabra* (30mg), *Curcuma longa* (20 mg), *Mica* (15 mg), *Coral* (15 mg),and *Asphaltum* (20mg) [2-4]. Vedic guard is already available in the market as antioxidant capsule to liver toxicity in Ayurvedic system of medicine, but it lacks preclinical evidence. Hepatoprotective activity against antitubercular drugs induced liver toxicity was studied in young rats [5], but effectiveness in geriatric rats was not established as there is shrinkage of liver and reduced liver enzyme activity. Hence the present study was undertaken to evaluate antioxidant activity in geriatric rats. Since many antioxidants like *Tinosporia cardifolia*, *Tribulus terrestris*, *Piper longum*, *Mica*, *Pureria tuberosum* and *Asphaltum* proved to possess hypolipidemic activity; present study is also intended to evaluate hypolipidemic activity of Vedic guard.

MATERIALS AND METHODS:

Drugs and Chemicals: Vedic Guard(procured on 18-08-2009 from Vedic Bio labs Pvt. Ltd, BTM, 2nd stage Bangalore.), Silymarin (Micro labs (P) Ltd., Bangalore.), Levamisole(Ranbaxy laboratories, Mumbai.), AST, and ALT kits (Abhilash Pharma Pvt Ltd Bangalore), DTNB (5-5′ Dithiobis 2 nitrobenzoic acid) (sigma USA), Sodium carboxy methyl cellulose, Phenathizine methosulphate, Nitroblue tetrazolium, NADH (Nictonamide adenine dinucleotide reductase), Pyrogallol and Hydrogen peroxide (SD fine chemicals, Mumbai).

Experimental Animals: Wistar strain of geriatric albino rats weighing (300-350 gm) of age one year and young adult albino rats weighing (200-250 gm) of both sex, were procured from Nimhans animal house Bangalore. They were maintained under standard laboratory conditions (25±2^{0}c, relative humidity 50±15% light and dark cycle of 12 hrs) and fed with standard pellet diet and water ad libitum. The study was carried out at department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore-560070, India from August 2009 to February 2010. All the experimental protocols were approved by the Institutional Animals Ethics

Committee. Test doses of Vedic guard were selected for rats (90, 180mg/kg b. w., p. o.) based on the human dose (2000mg/day) by using dose conversion factor based on body surface area [6].

Rat dose=human dose x0.018…… (For 200gm rat)

The dried powder extracts of polyherbal formulation was suspended in 0.5%w/v sodium carboxy methyl cellulose and standards were prepared in the same manner.

Hepatoprotective activity against carbon tetrachloride induced liver toxicity [7]: Albino wistar rats were divided into 6 groups of 6 animals each. Except Group II (young adult rats) rest all the groups were with geriatric rats. All groups received carbon tetrachloride (30% v/v in olive oil) 1.25ml/kg b. w., p. o. for 7 days to induce hepatotoxicity except Group I which served as vehicle control. Groups IV, V and VI were administered Vedic guard 90 mg/kg b. w., 180 mg/kg b. w., and standard silymarin 25 mg/kg b. w., *p. o.* respectively from 8th day till 14th day. On 15th day, blood was collected by puncturing retro-orbital plexus under light ether anesthesia. Further the liver was excised and used for biochemical estimations.

Biochemical Estimations: The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30°C for 15 min and utilized for the estimation of various bio-chemical parameters namely AST, ALT, ALP and serum total bilirubin. After collection of blood samples the rats were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of glutathione. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of LPO, GSH and CAT.

Estimation of Lipid Peroxidation (LPO): Lipid peroxidation in liver homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, to 0.2 ml of liver homogenate, 0.2ml of 8.1% sodium dodecyl sulphate (SDS), 1.5ml of 20% acetic acid and 1.5ml of 8% TBA were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95°C on a water bath for 60 min. After incubation the tubes were cooled to room temperature and final volume was made to 5ml in each tube. 5.0ml of butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000rpm for 10 min, the upper organic layer was taken and its optical density was read at 532nm against an appropriate blank without the sample. The levels of lipid peroxidation were expressed as nano moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56 \times 105\ M^{-1}cm^{-1}$ [8].

Estimation of Glutathione (GSH): The liver homogenate was added with equal volume of 20% trichloro acetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200µl) was then transferred to a new set of test tubes to which 1.8 ml of the Ellman's reagent (5, 5'-dithio *bis*-2-nitrobenzoic acid) was added. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH [9].

Estimation of CAT: 0.1 ml of liver homogenate was added to cuvette containing 1.9 ml of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as units/mg protein [10].

Estimation of AST: It was estimated by kinetic assay by using enzymatic kits in semiautoanalyzer. To 100µl of serum and working AST reagent 1000µl, was added mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameter, the absorbance was recorded after 60 sec, readings were repeated after every 30 sec i.e., up to 120 sec at 340nm wavelength and determined the mean absorbance change per minute (A/min) [11].

Estimation of ALT: It was estimated by kinetic assay by using enzymatic kits in semiautoanalyzer. To 100µl serum and working ALP reagent 1000µl, was added, mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameter, the absorbance was recorded after 60 sec, readings were repeated after every 30 sec i.e., up to 120 sec at 340 nm wavelength and determined the mean absorbance change per minute (A/min) [11].

Estimation of ALP: It was estimated by kinetic assay by using enzymatic kits in semi auto analyzer. To serum 20µl and working ALP reagent 1000µl, mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameter, the absorbance was recorded after 60sec, readings were repeated after every 30 sec i.e., up to 120 sec at 405 nm wavelength and determined the mean absorbance change per minute (A/min) [12].

Estimation of total bilirubin: The total bilrubin in serum was estimated by Jendrassik and Grof method. To test tubes, serum and reagent -2, 50µl, working reagent 1000µl and reagent-1, 50µl were added, mixed well. Incubated at 37^0 c for 5 min. Absorbance was measured at 546 and 630nm. Total bilirubin was estimated using the formula[13].

Activity against pyrogallol induced immunosupression[14]: Albino rats of Wistar strain, weighing 125-175gm were divided into 5 groups of 6 animals each. Except Group I (Vehicle control) all other groups received pyrogallol 50mg/kg b. w., i. p. daily for 28 days. After one hour of pyrogallol injection groups III, IV, and V rats were administered daily with Vedic guard 90,180 mg/kg b. w., and standard levamisole 50mg/kg b. w., p. o. respectively for 28 days. On day 14^{th} the rats were sensitized with sheep red blood cells ($0.5x10^9$ cells/100gm b. w i .p.), on 21^{st} day, blood was withdrawn from retro orbital plexus, 25 µl of blood mixed with SRBC [$0.025x10^9$ cells $(100\ g)^{-1}$] and the analyzed for presence/absence of humoral immune response [15]. On day 28, blood samples were withdrawn from all rats under light ether anesthesia by retro orbital plexus and the parameters of oxidative stress such as lipid per oxidation, reduced glutathione, catalase and superoxide dismutase were assessed using spectroscopy. On day 29, again sheep red blood cells were injected in the sub plantar region of the hind paw, and an increase in the paw volume was measured after 48 hours to assess cell mediated immune response. On 28^{th} day, the blood was withdrawn from retro orbital plexus and centrifuged at 8000rpm for 20 min. The supernatant was discarded and pellet was dissolved in adequate quantity of 0.1 M phosphate-buffered normal saline to get 5% suspension of RBC.

Biochemical Parameters:

Lipid peroxidation (LPO): To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2 ml of 28% trichloroacetic acid were added and centrifuged. One ml of 1% thiobarbituric acid was added to 4 ml of supernatant, heated in boiling water for 60 min and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) (1.56x 105), and expressed in terms of nanomoles of MDA/g Hb [16].

Superoxide dismutase (SOD): It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 µl of the lysate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of auto oxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD was expressed as units/mg protein[17, 18].

Catalase (CAT): 50 µl of the erythrocyte lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1ml of 30 mM H_2O_2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to one millimoles of H_2O_2 degraded per minute and expressed as units per milligram of protein [10].

Reduced glutathione (GSH): Blood glutathione was measured by addition of 0.2 ml of whole blood to 1.8 ml distilled water followed by 3.0 ml of precipitating mixture (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl to make 100 ml of solution). It was centrifuged at 5000 rpm for 5 min and 1 ml of the filtrate was added to 1.5 ml of the phosphate solution, followed by the addition of 0.5 ml of DTNB reagent. The optical density was measured at 412 nm using spectrophotometer [9].

Lipid lowering activity in triton induced hyperlipidemic rat model of acute study [19]: Albino rats of Wistar strains were divided into 5 groups of 6 rats each. Group I served as Vehicle control, Group II served as triton control, Group III, IV and V were served as treated groups. In acute experiment of 18 hrs, hyperlipidemia was developed by administration of triton WR 1339 at dose 400mg/kg b. w., i. p. to animals of all groups except Group I. After 45 min of triton injection, Group III, IV and V were administered with Vedic guard 90mg/kg b. w., 180mg/kg b. w., and atorvastatin 7.2mg/kg b. w., p. o. After 18 hrs of treatment the animals were anesthetized with light anesthetic ether, 1ml blood was withdrawn from retro orbital sinus .The blood was centrifuged at 2500rpm for 10 min at 4^0 c, serum was separated, used for analysis of total cholesterol, triglycerides, HDL, LDL and VLDL using Semi auto analyzer by enzymatic kits.

Serum Lipid Profile:

Estimation of Serum Total Cholesterol: Serum cholesterol was estimated by the colorimetric, End point CHOD-PAP (Cholesterol oxidase and peroxidase) method using the cholesterol kit. To 2.5µl of the serum sample, 250 µl of the reagent was added mixed well and incubated at room temperature for 10 min. The absorbance of incubated mixture was estimated at 500 nm using microplate reader (Molecular Devices, VERSA).

Estimation of Triglycerides: Serum triglyceride was estimated by the enzymatic, end point,

colorimetric, GPO-POD (Glycerol-3-phosphate oxidase-peroxidase) method using the triglyceride kit. To 2.5µl of the serum sample, 250 µl of the reagent was added mixed well and incubated at room temperature for 10 mins. The absorbance of this incubated mixture was estimated at 500 nm using Microplate reader (Molecular Devices, VERSA).

Estimation of HDL Cholesterol: HDL cholesterol was estimated by HDL-Cholesterol Phosphotungstic precipitation method [20], using the HDL-Cholesterol kit. To 2.5µl of the serum sample, 250 µl of the reagent was added, mixed well and incubated at room temperature for 10 min. The absorbance of this incubated mixture was estimated at 500 nm using microplate reader.

Estimation of VLDL and LDL Cholesterol: VLDL and LDL level were estimated as per the Friedewald formula [21].

VLDL=Triglyceride/5

LDL=Total cholesterol – (HDL + VLDL)

Statistical Analysis: The values were expressed as mean ± standard error of mean (SEM). The results were subjected to statistical analysis by using one- way ANOVA followed by Tukey-Kramer multiple comparison test to calculate the significance difference if any among the groups. P<0.05 was considered as significant.

RESULTS:

TABLE 1: EFFECT OF VEDIC GUARD ON CCL_4 INDUCED LIVER TOXICITY IN RATS

Group and Treatment	Serum of blood				Liver homogenate		
	AST (U/l)	ALT (U/l)	ALP (IU/min)	TOTBIL (mg/dl)	LPO (MMDA/gHb)	GSH (µmol/g Hb)	CAT (Units/mg protein)
I .Vehicle control	196±3.4	232.83±2.725	157.66±9.54	0.40±9.54	74.69±10.573	4.3±0.18	316.73±10.573
II .CCl_4 control (Young adult rats)	216±7.48	237.0±4.88^{++}	202.66±4.66^{+++}	0.785±0.045^{+++}	77.95±3.07	2.093±0.044^{+++}	116.58±3.80^{+++}
III .CCl_4 control	256±7.5^{+++}••	254.66±5.51^{+++}••	216.33±4.76^{+++}••	0.96±0.06^{+++}	94.49±1.8^{+++}•••	2.5181±0.05^{+++}	128.1±2.43^{+++}
IV .Vedic guard (90mg/kg)	217±6.12***	204±3.95***	179.33±3.48**	0.746±0.036	71.2±226***	2.898±0.0093	199±3.67***
V. Vedic guard (180mg/kg)	185±7.92***	184.16±5.21***	166±8.09***	0.64±0.06**	53.7±2.05***	3.179±0.007**	275.58±1049***
VI .Silymarin (25mg/kg)	154.66±8.22***	166±6.339***	137±4.26***	0.366±0.02***	42.65±0.81***	3.74±0.19***	307.48±10.5***

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test. $^{+++}$P<0.001 Vs vehicle control, $^{++}$P<0.01 Vs vehicle control, ***P<0.001 Vs CCl_4 control (geriatric rats), **P<0.01 Vs CCl_4 control (geriatric rats), *P<0.05 Vs CCl_4control (geriatric rats), $^{•••}$P<0.001 Vs CCl_4 control (young adult rats), $^{••}$P<0.01 Vs CCl_4 control (young adult rats)

Carbon tetrachloride (CCl_4) significantly induced hepatotoxicity in Group II and III rats compared to vehicle control rats (Group I) as evidenced by elevated serum biomarkers AST, ALT, ALP and total bilirubin and increased LPO, reduced GSH and CAT in liver homogenate (Table 1). The severity of hepatotoxicity was significantly greater ($P<0.001$) in geriatric rats (Group III) compared to young adult rats (Group II). Vedic guard 90,180 mg/kg b. w., p. o. significantly ($P<0.001$) decreased the activity of serum AST, ALT,ALP, total bilirubin, and reduced the LPO and elevated GSH and CAT in liver homogenate compared to control groups geriatric rats. Silymarin (25mg/kg) also significantly ($P<0.001$) decreased the serum enzymes, total bilirubin and decreased LPO and elevated GSH and CAT in liver homogenate in CCl_4 treated groups compared with the geriatric control group.

Histopathology of light microscopic sections rat liver:

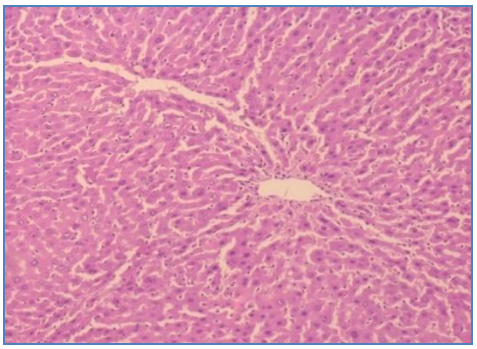

FIG. 1: VEHICLE CONTROL

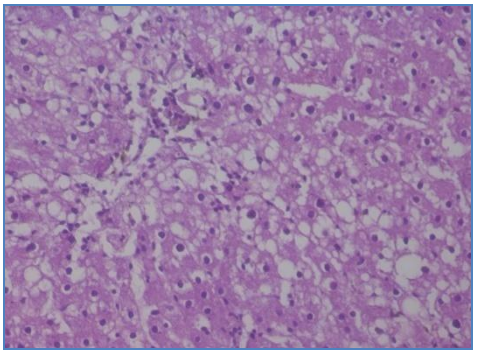

FIG. 2: CCL_4 CONTROL (GERIATRIC RAT)

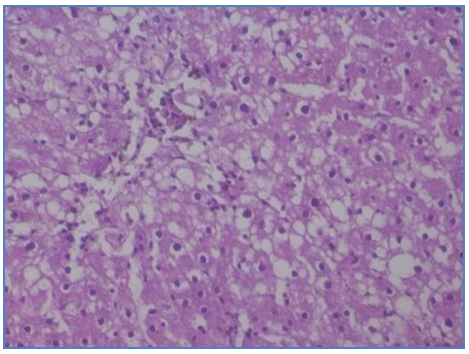

FIG. 3: CCL_4 CONTROL (YOUNG ADULT RAT)

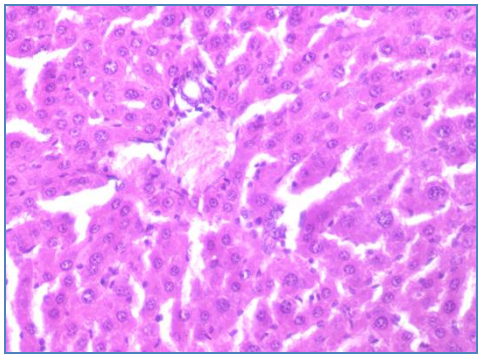

FIG. 4: TREATED WITH VEDIC GUARD (90mg/kg)

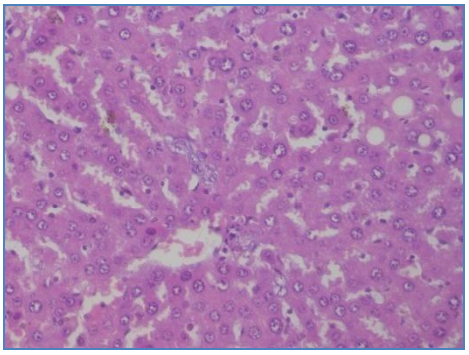

FIG. 5: TREATED WITH VEDIC GUARD (180mg/kg)

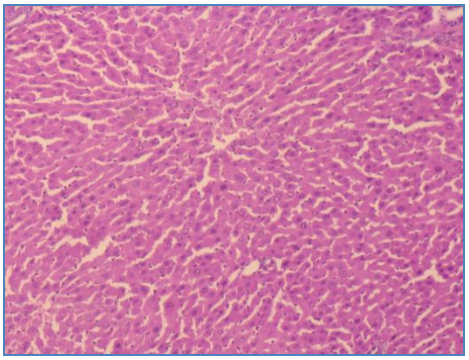

FIG. 6: TREATED WITH SILYMARIN (25mg/kg)

In vehicle control rats, light microscopic sections of liver showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and central vein (**Fig. 1**). Disarrangement of normal hepatocytes with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed on CCl_4 treated animals (**Fig. 2** and **3**). Treatment with Vedic guard at dose 90mg/kg and 180mg/kg b. w., p. o. showed mild fatty change and mild sinusoidal congestion (**Fig. 4** and **5**). Having reversed to a large extent the hepatic lesions produced by the toxin, almost comparable to the vehicle control and the silymarin group (**Fig. 1** and **6**).

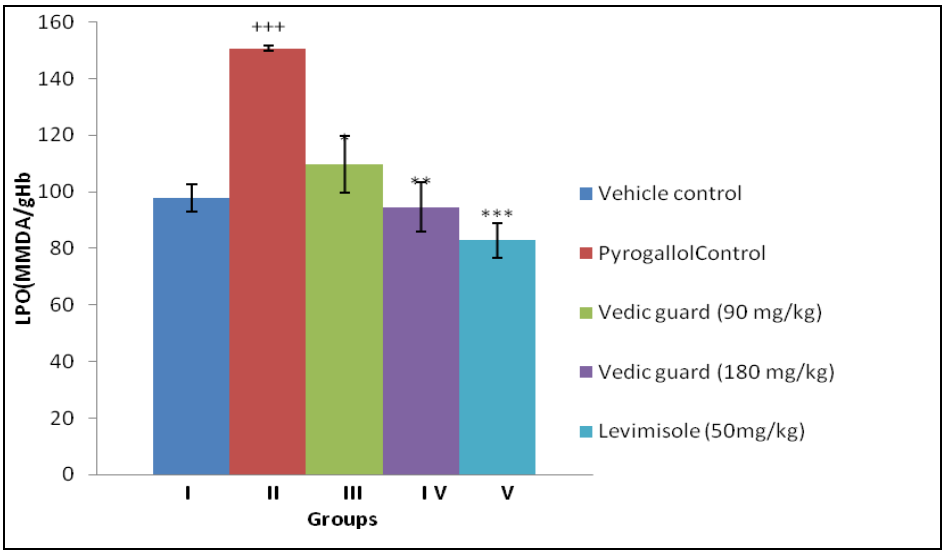

FIG. 7: EFFECT OF VEDIC GUARD ON LIPID PEROXIDATION OF ERYTHROCYTE LYSATE IN PYROGALLOL INDUCED IMMUNOSUPRESSION

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test.
$^{+++}$P<0.001 Vs Vehicle control. ***P<0.001Vs pyrogallol control. **P<0.01 Vs pyrogallol control. *P<0.05 Vs pyrogallol control

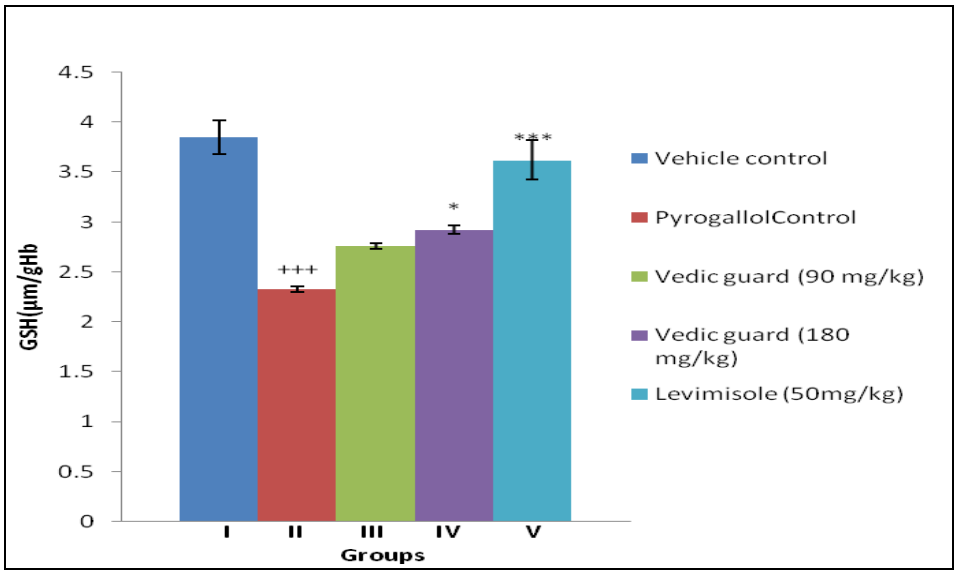

FIG. 8: EFFECT OF VEDIC GUARD ON BLOOD GLUTATHIONE IN PYROGALLOL INDUCED IMMUNOSUPRESSION

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test.
$^{+++}$P<0.001 Vs Vehicle control. ***P<0.001 Vs pyrogallol control. *P<0.05 Vs pyrogallol control

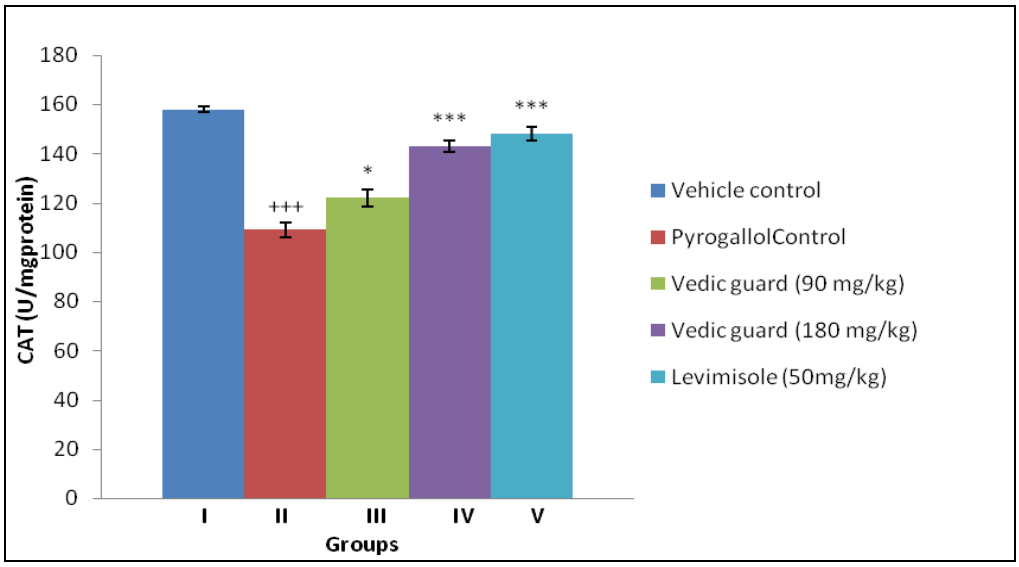

FIG. 9: EFFECT OF VEDIC GUARD ON CATALASE OF ERYTHROCYTE LYSATE IN PYROGALLOL INDUCED IMMUNOSUPRESSION

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test.
$^{+++}$P<0.001 Vs Vehicle control. ***P<0.001 Vs pyrogallol control. *P<0.05 Vs pyrogallol control.

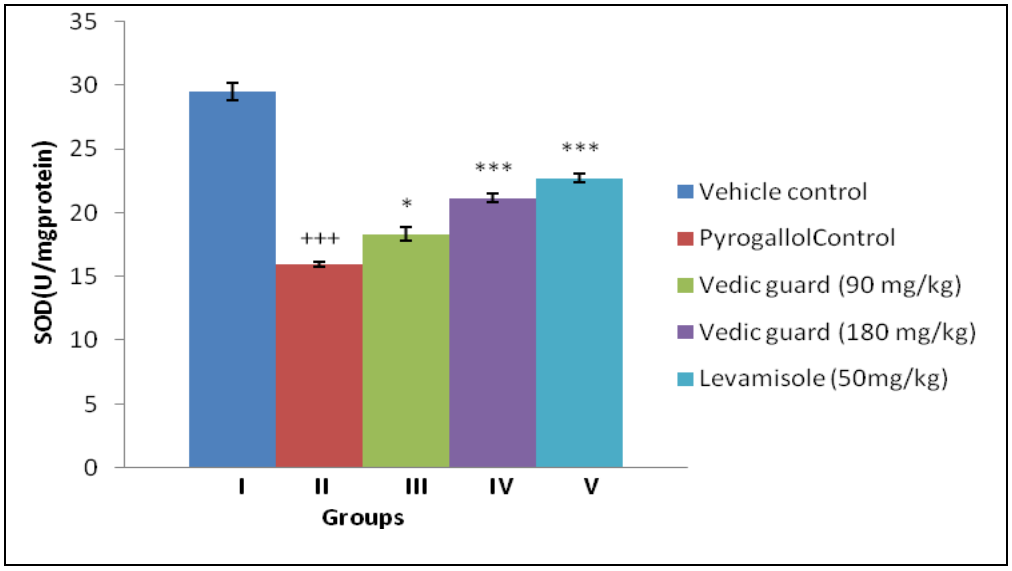

FIG. 10: EFFECT OF VEDIC GUARD ON SUPEROXIDE DISMUTASE OF ERYTHROCYTE LYSATE IN PYROGALLOL INDUCED IMMUNOSUPRESSION

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test.
$^{+++}$P<0.001 Vs Vehicle control. ***P<0.001 Vs pyrogallol control. *P<0.05 Vs pyrogallol control.

Pyrogallol (50mg/kg) b. w., p. o. significantly induced immunosupression, (P<0.001) in group II animals compared to vehicle control (Group I) rats. Since biochemical parameters such GSH, CAT, SOD were reduced, and LPO was elevated (Figures 7-10). In Vedic guard treated animals the LPO was decreased and SOD, CAT and GSH level were increased significantly dose dependently as compared to pyrogallol control. High dose of vedic guard 180mg/kg b. w., p. o. was comparable with reference standard Levmisole.

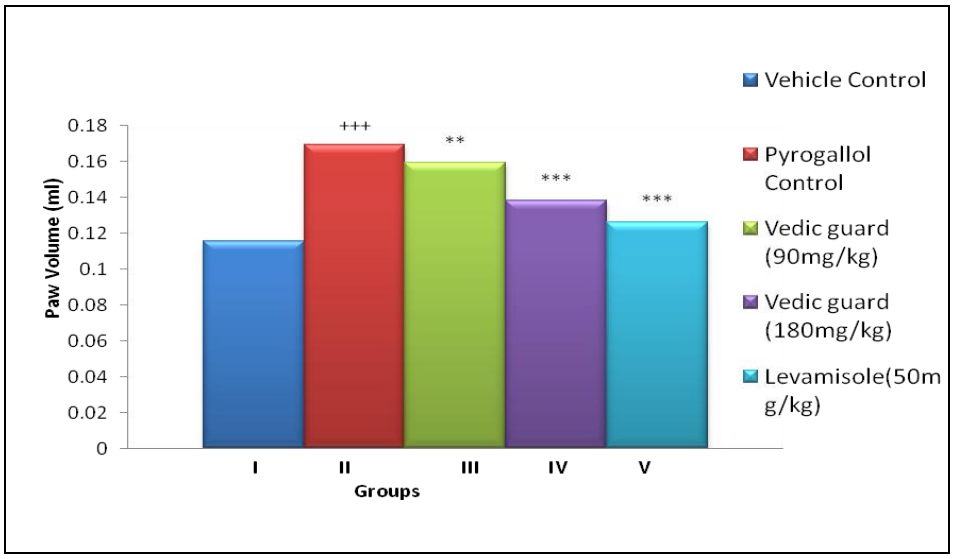

FIG. 11: EFFECT OF VEDIC GUARD ON PAW VOLUME IN PYROGALLOL INDUCED IMMUNOSUPRESSION

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test.
$^{+++}$P<0.001 Vs Vehicle control. ***P<0.001 Vs pyrogallol control. **P<0.01 Vs pyrogallol control.

Upon injecting sheep red blood cells in sub planter region of the hind paw, paw volume increased significantly (P<0.001) in pyrogallol treated control rats compared to vehicle control rats. Treatment with vedic guard 90mg/kg b. w., p. o. and 180mg/kg b. w., significantly reduced paw volume in group III rats (P<0.01) and group IV rats (P<0.001). Effect of Vedic guard 180mg/kg b. w., p. o. was equivalent to standard drug Levamisole in reducing paw volume (**Figure 11**).

TABLE 2: EFFECT OF VEDIC GUARD ON TRITON INDUCED HYPERLIPIDEMIA

Group	Treatment	Biochemical parameters				
		Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)	Serum VLDL (mg/dl)
I	Vehicle control	66.836±0.80	112.435±1.378	32.608±0.608	27.87± 0.52	22.801±0.43
II	Triton Control (400mg/kg)	385.14±5.01^{+++}	513.6±1.584^{+++}	26.618±0.85^{+++}	235.116±0.92^{+++}	108.675±0.81^{+++}
III	Vedic guard (90mg/kg)	282.56±1.30***	427.98±0.87***	37.58±0.85**	169.2±1.04***	87.775±0.69***
IV	Vedic guard (180mg/kg)	256.66±2.22***	376.81±1.128***	38.581±0.788***	138.108±0.70***	76.391±0.56***
V	Atorvastatin (7.2 mg/kg)	171.73±0.97***	224.77±1.95***	39.87±0.33***	68.701±0.33***	65.236±0.51***

n = 6, Values are in mean ±SEM, one-way ANOVA followed Tukey-Kramer multiple Comparison test. $^{+++}$P<0.001Vs control, ***P<0.001 Vs Triton control, **P<0.01 Vs Triton control, *P<0.005 Vs Triton control

Triton WR 1339 at 400mg/kg b. w., i. p. effectively induced hyperlipidemia in Group II control rats, as serum cholesterol, triglycerides, LDL, and VLDL were elevated and serum HDL was reduced significantly (P<0.001) compared to vehicle control Group I rats. Treatment with vedic guard 90mg/kg and 180 mg/kg b. w., p. o. effectively reduced serum biochemical parameters total cholesterol, triglycerides, LDL, and VLDL and significantly enhanced serum HDL compared to control rats (Group II). Hypolipidemic activity of vedic guard 180mg/kg b. w., was equivalent to standard drug atorvastatin.

DISCUSSION: Vedic guard was found to be effective against antitubercular drugs induced hepatotoxicity in young adult rats [5]. As there is physiological and biochemical changes of liver in old age rats due to shrinkage of liver and reduced liver enzymes [22], the present study was intended to evaluate antioxidant activity of Vedic guard in geriatric rats against CCl_4 induced hepatotoxicity. Since immuno-suppressed status enhances oxidative stress, we also wanted to investigate antioxidant activity of Vedic guard is such condition. As many of the plant extracts in the formulation has hypolipidemic activity the purpose of the study was also to investigate hypolipidemic activity of Vedic guard.

In our study intensity of CCl_4 induced hepatotoxicity was greater ($P<0.01$) in geriatric rats compared to young adult rats (Table 1). Even though many compounds including clinically used drugs, can cause hepatocellular damage through metabolic activation to highly reactive compounds such as free radicals, CCl_4 has been selected to induced hepatotoxicity, since it is more extensively studied hepatotoxin both biochemically and pathologically. CCl_4 hepatotoxicity depends on the reductive dehalogenation of CCl_4, catalyzed by cytochrome P-450 in the liver cell endoplasmic reticulum leading to the generation of an unstable complex $CCl^{\bullet}_3$ radical.

This trichloromethyl radical reacts rapidly, which is reported as a highly reactive species. These free radicals attack microsomal lipids leading to its peroxidation and also covalently bind to microsomal lipids and proteins ultimately initiating a site of secondary biochemical processes which is the ultimate cause for the unfolding of the panorama of pathological consequences of CCl_4 metabolism [23]. In view of evaluating the effectiveness of Vedic guard against hepatotoxicity induced by CCl_4, it was examined at lipid peroxide levels and antioxidant enzyme activities in geriatric rats. In the assessment of liver damage by CCl_4 hepatotoxin, the determination of enzyme levels such as AST, ALT and total bilirubin are largely used. The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages.[24]

Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, AST is more specific to the liver, and is thus a better parameter for detecting liver injury [25]. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice [26]. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [27]. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell.

Our results using the model of CCl_4-induced hepatotoxicity in the geriatric rats demonstrated that Vedic guard at the different doses caused significant inhibition of AST, ALT, ALP and total bilirubin levels ($P<0.001$) (Table1) compared to control rats. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the lipid per-oxidation process [28]. High lipid peroxidation values indicate excessive free

radical induced peroxidation and measurement of lipid peroxidation is marker of hepatocellular damage. Treatment with Vedic guard significantly reversed these changes (P<0.001) compared to control rats. Efficacy of Vedic guard was comparable with standard silymarin. Hence it may be possible that the mechanism of hepatoprotection of Vedic Guard is due to its antioxidant effect. GSH is widely distributed in cells. GSH is an intra-cellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis [29].

Treatment with Vedic guard has effectively recovered the reduced glutathione level, (P<0.001) compared to control rats. High dose of Vedic guard was comparable with standard silymarin (Table 1). SOD removes superoxide by converting it to H_2O_2, which can be rapidly converted to water by CAT and glutathione peroxide, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS(Reactive oxygen species) derived from the peroxidative process of xenobiotics in liver tissues [30]. The observed increase of SOD activity suggests that the Vedic guard treatment has an efficient protective mechanism (P<0.001) in response to ROS. And also, these findings indicate that Vedic guard may be associated with decreased oxidative stress and free radical-mediated tissue damage. High dose of Vedic guard 180mg/kg b. w., p. o. was comparable with reference Silymarin 25mg/kg (Table 1).

CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Administration of Vedic guard increases the activities of catalase in CCl_4 induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from CCl_4 intoxication (P<0.001) compared to control rats (Table 1). Histopathological examination of liver sections further substantiated antioxidant activity of Vedic guard. Liver sections of vehicle control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure1).

Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl_4 intoxicated animals (Figure 2 and 3). The liver sections of rats treated with Vedic guard and Silymarin followed by CCl_4 intoxication showed a sign of protection as it was evident by absence of necrosis and vacuoles (Figure 4, 5 and 6). Further antioxidant activity of Vedic guard was evaluated in immuno-suppressed condition induced by pyrogallol. Immune system is vulnerable to the free radical induced oxidative stress. The cellular and humoral components of the immune system are particularly sensitive to increased levels of reactive oxygen species, which may cause premature immunosenscence [4].

Pyrogallol is a strong generator of free radicals, which induces immunosupression. The free radicals on reaction with oxygen generate reactive oxidative free radical intermediates, which repetitively attack polyunsaturated fatty acids in the biomembranes and initiate lipid peroxidation [31], which is a marker of oxidative stress. Thus, immunosupression leads to oxidative stress. In the present study suppression of humoral response to sheep red blood cells was

observed in antigenically challenged, pyrogallol control animals (no clumping due to antigen antibody reaction). The humoral immune response in rats treated with Vedic guard 90,180 mg/kg b. w., p. o. and levamisole 50mg/kg b. w., was positive. As shown in figure 11, the paw volume in pyrogallol control rats was increased significantly (P<0.001) compared to vehicle control rats. Where as in Vedic guard and levamisole treated animals, the paw volume was decreased significantly (P<0.001) compared to pyrogallol control. In pyrogallol control animals, there was increase in LPO and decrease in SOD, CAT, and GSH level compared to normal rats (vehicle control).

In rats treated with Vedic guard, LPO levels were decreased and SOD, GSH, CAT were increased (P<0.001) in dose dependent manner. These results are comparable with that of levamisole (Figures 7 to10). Vedic guard was also evaluated for hypolipidemic activity against triton WR1339 induced hyperlipidemia, since some of the extracts in the formulation were found to have hypolipidemic activity. Triton WR 1339 acts as a surfactant to block the uptake of lipoprotein from the circulation by extra hepatic tissues resulting in an increase in the level of circulatory lipoproteins [32]. In hypolipidemic model, Vedic guard treatment at doses 90mg/kg and 180 mg/kg b. w., p. o. significantly (P<0.001) decreased serum levels of total cholesterol, triglycerides, LDL, VLDL and increased serum HDL dose dependently compared to control rats.

The lipid lowering effect of Vedic guard in rats may be due to inhibition of cholesterol biosynthesis and to increased fecal bile acid excretion. The decrease in serum triglyceride level on administration of Vedic guard could be due to increased catabolism of triglyceride and an inhibition of fatty acetyl-CoA activity and glycerophosphate acetyl transferase. The possible mechanism for increase in serum HDL may be due to inhibition of the action of hepatic TG-lipase on HDL, which may contribute for a rapid catabolism of blood lipids through extra hepatic tissues. The increased HDL facilitates the transport of triglyceride or cholesterol from serum to liver where it is catabolised and excreted out of the body. Hypolipidemic activity of vedic guard 180mg/kg b. w., p. o. is comparable to reference standard atorvastatin 7.2 mg/kg b. w., p. o.

The results of this study demonstrate that Vedic guard has a potent hepatoprotective action against CCl_4 induced hepatic damage in geriatric rats. In animals treated with Vedic guard, the endogenous antioxidants such as SOD, CAT, GSH levels were increased against pyrogallol induced immunosupression leading to oxidative stress. This supports the use of Vedic guard in immunosupressed status. These effects of Vedic guard may be due to its antioxidant and free radical scavenging properties. The study also reveals hypolipidemic activity of Vedic guard upon triton WR 1339 induced hyperlipidemia, as there was reduction in serum cholesterol, triglycerides, LDL, VLDL and increase in serum HDL in treated animals. Further chronic studies are required to confirm hypolipidemic activity of Vedic guard. In all the three models efficacy of Vedic guard 180mg/kg b. w, p. o. was comparable with respective reference standards.

CONCLUSION: Vedic Guard possesses significant antioxidant and hypolipidemic activity, which substantiate its clinical use for all patients groups including geriatrics and in immuno-compromised patients.

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REFERENCES:

1. Azzi A, Davies KJA and Kelly F. Free radical biology terminology and critical thinking, FEBS Lett 2004; 558:3–6.
2. Ramos A, Visozo A, Piloto J, Garcia A, Rodriguez CA and Rivero R. Screening of antimutagenicity via antioxidants activity in Cuban medicinal plants. J Ethanopharmacol 2003; 77(2):31-6.
3. Nadkarni AK and Nadkarni KM. Indian Materia Medica. 2nd ed. Bombay: Popular Book Depot 1982: 28-30.
4. Venkatasubramanian P, Kumar S and Venugopal SN. Use of ksiravidari as a substitute for Vidari as per Ayurvedic descripition. Ind J traditional knowledge 2009; 83:310-8.
5. Razdan R, Ulla I and Dev AMJ. Preventive and curative effects of Vedic Guard against anti-tubercular drugs induced hepatic damage in rats. Phcog Mag 2008; 4(15):182-8.
6. Bharathi KN, Sivaramaiah N, Nagarjuna CG and Gupta AVSSS. Evaluation of Antistress, Anxiolytic and Hypnotic activity of Vedic Calm, A polyherbal formulation. Phcog Mag 2009; 4:124-130.
7. Samudram P, Rajeshwari H, Vasuki, Geetha A and Sathiya M. Hepatoprotectivity Activity of Bi-herbal ethanolic extract on ccl_4 induced hepatic damage in rats. Afr J Biochem 2008; 2(2):61-5.
8. Ohkawa H, Onishi N and Yagi K. Assay for lipid peroxidation in animal tissue by thibarbituric acid reaction. Anal Biochem 1979; 95:351 -8.
9. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82:70-7.
10. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6.
11. Schumann G, Bonora R, Ceriotti F, Ferrard G, Ferrero CA and etal. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 4. Reference procedure for the measurement of catalytic concentration of ALT. Clin Chem Lab Med 2002; 40: 718-24.
12. Tietz NW. Clinical Guide to Laboratory Tests, Philadelphia, W.B. Saunders Company 1983; 54.
13. Shull BC, Less H and Li PK. Mechanism of interference by Hemoglobin in the Determination of total bilirubin. II. Method of Jendrassik Grof. Clin Chem 1980; 26(1), 26.
14. Meera S, Gupta AVSSS and Kumar NS. Immunomodulatory and antioxidant activity of a polyherbal formulation. Inter J Pharmacol 2008; 4(4): 287-91.
15. Joharapurkar AA, Wanjari MM, Dixit PV, Zambad SP, and Umathe SN. Pyrogallol: A novel tool for screening immunomodulators. Ind. J. Pharmacol 2004; 36:355-9.
16. Stocks J and Dormandy TL. Auto oxidation of human red cell lipids induced by Hydrogen peroxide. Br J Hematol 1971; 20:95-111.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265-75.
18. Marklund S and Marklund C. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1984; 7:469-74.
19. Ramesh C and Gitika B. Antidyslipidemic and anti oxidant activities of different Fractions of termanlia Arjuna stem bark. Ind J Clinical Biochem 2004; 19(2):141-8.
20. Warnick GR, Knopp RH , Fitzpatrick V and Branson L. Estimating Low-Density. Lipoprotein Cholesterol by the Friedewald Equation is Adequate for Classifying Patients on the Basis of Nationally Recommended Cut points. Clin Chem 1990; 36(1): 15-19.
21. Johnson R, McNutt P, MacMohan S and Robson R.Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. Clin Chem 1997; 43:2183-4.
22. Castillo C, Salazar V, Ariznavaereta C, Fossati M, Tresguerres JAF and Vara E. Effect of S-adenosyl methonine on age induced hepatocyte damage in old wistar rats. Biogerontology 2005; 6:313-23.
23. Wei YH. Oxidative stress and mitochondrial DNA mutations in human aging. Proc Soc Exp Biol Med 1998; 217:53-63.
24. Sallie R, Tredger JM and Willam R. Drugs and the liver. Part I. testing liver function. Biopharm Drug Disp 1991; 12:251-9.
25. Willianson EM, Okpako DT and Evans FJ. Selection, preparation and pharmacological evaluation of plant material. John Wiley England, 1996; 5:67-9.
26. Drotman RB and Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. Drug Chem Toxicol 1978; 1: 163-71.
27. Moss DW and Butterworth PJ. Enzymology and Medicine. Pitman Medical, London: 1974; 4139.
28. Kurata M, Suzuki M and Agar NS. Antioxidant systems and erythrocyte life span in mammals. *Biochem Physiol* 1993; 106:477-87.
29. Gupta M, Mazumder UP, Kumar ST, Gomathi P and Ramanathan S. Antioxidant and Hepatoprotective Effects of *Bauhinia racemosa* against Paracetamol and Carbon Tetra-chloride Induced Liver Damage in Rats. Iranian J Pharmcol Ther 2004; 3:12-20.
30. Gupta YK, Sharma M and Chaudhary G. Pyrogallol induced hepatotoxicity in rats: A model to evaluate antioxidant hepatoprotective agents. Methods Find Exp Clin Pharmacol 2002; 24:497-500.
31. Boll ML, Weber WD, Becker E and Stamfl A. Mechanism of CCl_4 Induced hepatotoxicity. Hepatocellular damage by reactive CCl_3O. J Biosci 2001; 56:649-55.
32. Friedman M and Bayer SO. The Mechanism underlying hypercholesterolemia Induced by Triton WR 1339. Ame J Physiol 1957; 190:439–45.

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