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RESEARCH ARTICLE

HEPATOPROTECTIVE PROPERTY OF *SCHUMANNIANTHUS VIRGATUS* (ROXB.)ROLFE AGAINST D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN WISTAR RATS.

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Abstract

The rhizomic extract of *Schumannianthus virgatus* Roxb.)Rolfe is used by Kani tribe of Kerala to treat liver disorders. In the present study, the potential effectiveness of crude and ethanolic extract of *Schumannianthus virgatus* on D-galactosamine induced hepatotoxicity was evaluated. Pretreatment with crude (SV) and ethanolic extract (SVEF) significantly attenuated the elevation in biochemical parameters, such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase(ALP) ,total bilirubin and the malondialdehyde concentrations in liver tissue. Pretreatment with SV and SVEF significantly restored the reduction of catalase activity and glutathione (GSH) content. The histopathalogical studies also supported the protective effect of the extract. The overall data indicated that *Schumannianthus virgatus* possesses hepato protective effect against D-GalN induced hepatic damage, and the main mechanism involved in the protection could be associated with its strong capability to reduce the intracellular level of reactive oxygen species by enhancing the level of both enzymatic and non-enzymatic antioxidants .

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Introduction:-

The liver is the most important organ which regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders.The available synthetic drugs used to treat liver disorders in this condition also cause further damage to the liver. Hence, herbal drugs have become increasingly popular and their use is widespread. In view of severe undesirable side effects of synthetic agents, there is increasing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines, that are claimed to have hepatoprotective activity(Madhavi et al.,2009) .

Schumannianthus virgatus Roxe.Rolfe, belonging to the family Marantaceae is locally known as 'Malamkoova'.It is an erect herb of 4cm height with tuberous root stock. It is distributed in South India and Sri Lanka. In Kerala, the plants are abundant in Western Ghats.It is used by tribal healers of Kerala to treat jaundice. Various pharmacological effects like diuretic properties, anti bacterial, antifungal and anti viral properties have been reported for this plant (Goel et al.,2002).D-Galactosamine is a well-established hepatotoxicant, inducing liver injury which closely resembles human viral hepatitis in its morphologic and functional features and, therefore, it is very useful for evaluation of hepatoprotection (Keppler et al.,1968)

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The present investigation is to evaluate the hepatoprotective effect of *S.virgatus* rhizome on D-galactosamine (D-GalN) induced liver damage in Wistar rats.

Materials and Methods:-

Chemicals and instruments:-

Solvents of analytical grade were purchased from Merck India Pvt., Ltd., Mumbai, India. All the other chemicals including paracetamol were purchased from Sigma Aldrich, USA. Biochemical kits were purchased from Coral Clinical System, Goa, India. Rotary evaporator was from Buchi R-215, Switzerland and Spectrophotometer Agilent 100 UV-Vis from Germany.

Plant Collection:-

The fresh rhizomes of the plant *Schumannianthus virgatus* were collected from Kulathupuzha, Kollam, Kerala. The plant was identified and authenticated by the plant taxonomist of the institute. A voucher specimen has been deposited at the herbarium of the Institute [TBGT 86803]

Preparation of the plant extract:-

Freshly collected rhizomes were washed under running tap water and distilled water to remove adhering dust and then shade dried and powdered. The powder(100 g) was then extracted with 1000 ml ethanol, using a Soxhlet's apparatus and the solvent removed by evaporation, at low temperature, under reduced pressure in a rotary evaporator. The crude extract was referred to as SV . For administration , the plant extract, SV was suspended in 0.5% Tween-80 to required concentrations.

Serial fractions of *S.virgatus* rhizome:-

Rhizome powder was first extracted with hexane using Soxhlet apparatus, powder was then dried and again extracted with chloroform and finally with ethanol to get the following fractions. a) Hexane fraction (SVHF), b) Chloroform fraction (SVCF), c) Ethanolic fraction (SVEF).As the ethanol fraction showed potent *in vitro* antioxidant activity, ethanol fraction was selected for evaluating hepatoprotective activity.

Animals:-

Wistar male albino rats (150-200 g) and Swiss albino male mice, obtained from the Institute Animal House were used for the study. All animals were housed under standard conditions and fed commercial rat feed (Lipton India Ltd,Mumbai,India) and boiled water *ad libitum*. All animals were acclimatized for one week before starting the experiment. All the experiments were done, according to NIH guidelines, after getting the approval of the Institutes Animal Ethics Committee (No:B-01/12/2011/03-B).

D-galactosamine induced hepatotoxicity:-

D-galactosamine induced hepatotoxicity was carried out according to the procedure of Lin et al.(1995). Wistar rats were divided into nine groups of six animals each. Group I, the normal control group was given a single daily dose of 0.5% Tween-80 for 5 days and injected with a single dose of saline (10 mL/kg, i.p) on the 6th day. Group II, D-Galactosamine(D- GalN) control group was given a single daily dose of 0.5% Tween-80 for five days and injected with D- GalN (400 mg/kg in saline, i. p) on the 6th day.

Groups III, IV and V, the drug treated groups were administered SV reconstituted in 0.5% Tween- 80 at doses 50,100 and 150 mg/kg, p.o., Groups VI, VII and VIII, the drug treated groups were administered SVEF reconstituted in 0.5% Tween- 80 at doses 50,100 and 150 mg/kg, p.o. respectively for a period of 5 days and on the 6th day,they were dosed with D- GalN as Group II. Group IX, the standard group was administered a single daily dose of Silymarin (100 mg/kg, p.o.) for 5 days and on the 6th day, dosed with D-GalN as group II. On the 7th day, 24 h after D- GalN treatment, all the animals were sacrificed by carbondioxide inhalation and blood samples were collected from the carotid artery for evaluating the above mentioned biochemical parameters (estimation of plasma markers of hepatic injury) and liver tissue slices were collected for histopathological studies and antioxidant assays (estimation of malondialdehyde (MDA), assay of catalase (CAT), determination of reduced glutathione (GSH).).

Estimation of malondialdehyde:- Malondialdehyde in the rat liver was estimated by the modified procedure of Ohkawa et al.(1979). 1 ml of liver homogenate (10%w/v) from each group was mixed with 100 µl of 8.1% SDS and 600 µl of 20 % acetic acid solution and kept for 2 min at room temperature. Then 600 µl of 0.8% solution of TBA was added, heated at 95^0C for 60 min in water bath and cooled with ice cold water at 4^0C. The mixture of n-butanol

and pyridine (15:1 v/v) were added, shaken vigorously and centrifuged at 10,000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. Lipid peroxidation was expressed as n mol/g wet liver.

Assay of Catalase:-
Catalase in the rat liver was assayed according to the method of Aebi, et al., To 0.9 ml of phosphate buffer (0.01M, pH-7.0) 0.1 ml of liver homogenate (10% w/v) and 0.4 ml of H_2O_2 (0.2 M) were added. After 60 sec, 2 ml of dichromate - acetic acid reagent (5%) was added. The tubes were kept in boiling water bath for 10 min and the colour developed was read at 620 nm. Standard H_2O_2 in the range of 2-10 µl were taken with blank containing reagent alone. CAT activity was measured proportionately to the rate of H_2O_2 reduction. Dichromate in acetic acid was converted to perchromic acid and then chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. Absorbance values were compared with a standard curve generated from known catalase .

Determination of reduced glutathione (GSH):-
Homogenized rat liver sample (10% W/V) from the entire group (0.2 ml) was mixed with 1.8 ml of 1 mM EDTA solution. To this 3.0 ml precipitating reagent (1.67 g of met phosphoric acid, 0.2 g of EDTA disodium salt and 30 g sodium chloride in 1 l distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2 ml of the supernatant, 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB (5,5'dithio-bis (2-nitro benzoic acid)) reagent were added and absorbance was read at 412 nm. Absorbance values were compared with a standard curve generated from known GSH (Ellman, 1959).

Histopathological investigations:-
Seven micrometre thick paraffin sections of buffered formalin- fixed liver samples were stained with haematoxylin-eosin for photomicroscopic observations of the liver histological architecture of the control and treated rats.

Statistical Analysis:-
All the data were expressed as mean ± SD. The significance of difference among the group was assessed by using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism version 5.00 for Windows, GraphPad software, San Diego, California USA. $P \leq 0.01$ was considered statistically significant.

Results and Discussion:-

D-Galactosamine induced hepatotoxicity:-
Galactosamine administration in rats disrupts the membrane permeability of the plasma membrane, causing leakage of the enzymes from the cell, which leads to elevation in levels of serum enzymes (Mitra et al., 2000). It is found from this study that the levels of AST, ALT, ALP, GGT, total cholesterol, bilirubin and triglycerides increased significantly in the group treated with D-GalN compared to normal control and it indicates hepatic damage.Results are shown in Table:1. Among the doses studied, SV at a dose of 100 mg/kg b.w., p.o and SVEF at a dose of 50 mg/kg b.w., p.o were more effective and provided maximum protection against D-Galactosamine intoxication. The results obtained were almost comparable to silymarin, the standard drug used in the study. D-GalN hepatotoxicity is considered as an experimental model of acute hepatitis and it does not affect other organs (Jaishree and Badami, 2010). D-GalN has great liver specificity because hepatocytes have high levels of galactokinase and galactose-1-uridyltransferase, and it disrupts the synthesis of essential uridylate nucleotides. Depletion of these nucleotides ultimately impairs the synthesis of protein and glycoprotein, leads to progressive damage of cellular membranes, resulting in change in permeability of the cellular membrane, and finally with enzyme leakage from the cells(Hemalatha, 2008).Pretreatment with SV/SVEF restored the serum enzyme levels to normal.This effect is in agreement with the fact that serum levels of transaminases return to normal with healing of the liver parenchymal cells and healing of hepatocytes as reported by Gupta et al.,(2012). The results of the present study is in accordance with the previous study conducted in *Tridax procumbens* by Ravikumar et al. (2005).

Table 1:- Effect of *S.virgatus* crude extract (SV) / ethanolic fraction (SVEF) on serum markers of hepatic injury after D-Galactosamine administration.

Treatment groups	Parameters						
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (U/L)	SB (mg/dl)	TC (mg/dl)	TGL (mg/dl)
Normal control	48.70±3.67	50.04±4.39	62.23±4.15	11.73±1.18	0.31±0.06	50.87±2.77	56.88±6.74
D-GalN (400 mg/kg)	135.60±6.61 ***	167.45±6.2***	136.27±6.88 ***	32.07±5.03 ***	4.70±0.81 ***	98.69±4.87 ***	126.34±6.70 ***
D-GalN + SV (50 mg/kg)	82.29±5.98***	102.51±6.98 ***	66.91±7.02 ns	24.31±4.09 **	0.77±0.09 ns	69.0±7.07 **	79.83±12.5 **
D-GalN + SV (100 mg/kg)	52.14±3.90ns	102.51±6.98 **	60.61±3.13 ns	12.83±3.3 ns	0.39±0.03 ns	60.43±4.60 ns	59.30±6.30 ns
D-GalN + SV (150 mg/kg)	53.31±5.80 ns	71.58±2.14**	67.19±3.61 ns	21.92±3.2.*	0.49±0.084ns	69.73±4.60 **	77.05±5.60 **
D-GalN + SVEF (50 mg/kg)	53.17±5.80 ns	63.30±9.48 ns	67.50±7.19 ns	14.58±3.6 ns	0.35±0.07 ns	55.76±2.05 ns	59.72±1.45 ns
D-GalN + SVEF(100 mg/kg)	66.86±7.60**	96.17±5.42***	82.27±7.36 **	23.77±3.2**	0.45±0.05 ns	70.8±3.90***	71.32±3.81 *
D-GalN + SVEF(150 mg/kg)	79.99±4.23***	99.60±4.73***	90.73±3.58 ***	22.96±4.0 *	0.67±0.04 ns	68.65±7.10 **	81.99±5.10 ***
D-GalN + Silymarin(100 mg/kg)	54.18±4.55 ns	66.75±10.25 *	59.48±5.23 ns	14.08±4.4 ns 59**	0.28±0.03 ns	64.97±4.14 *	56.76±3.60 ns

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D-GalN +	79.99±4.23*	99.60±4.73*	90.73±3.58	22.96±4.0 *	0.67±0.04	68.65±7.10	81.99±5.1

SVEF(150 mg/kg)	**	**	***		ns	**	0 ***
D-GalN + Silymarin(100 mg/kg)	**54.18±4.55 ns**	**66.75±10.25 ***	**59.48±5.23 ns**	**14.08±4.4 ns 59****	**0.28±0.03 ns**	**64.97±4.14 ***	**56.76±3.6 0 ns**

Values are expressed as mean ± SD of six values, one way ANOVA followed by Dunnet's multiple comparison test,* $P \leq 0.05$,** $P \leq 0.01$,*** $P \leq 0.001$,ns= not significant compared to normal control.

In the present study, hepatic GSH and CAT levels are reported to be decreased in liver of rats treated with hepatotoxicant D-GalN. Oxidative stress has been reported as one of the major causes of D-GalN induced liver damage. Excessive production of free radicals generated in response to D-GalN intoxication can damage macromolecules such as lipids and this in turn decreased liver GSH and CAT. Previous reports have revealed that D-GalN provoke serious oxidative insult due to the reactive oxygen species (ROS) in the rat liver leading to apoptosis and necrosis (Sun et al., 2003).Increased level of MDA is detected in rats treated with D-GalN which is an indication of enhanced lipid peroxidation. Zhou et al. (2008) reported that, treatment with D-GalN decreased antioxidative enzyme activities. Pretreatment with SV/SVEF effectively increased the levels of CAT activity and GSH and reduced the levels of MDA (Fig:1). CAT is a hemeprotein , an enzyme predominantly located in peroxisomes that catalyzes the dismutation of toxic hydrogen peroxide (Masaki et al., 1998). The tripeptide gamma-glutamylcysteinylglycine or GSH is the major non enzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cells (Meister and Anderson, 1983).GSH protects cells against free radicals, peroxides and other toxic compounds. Tissue levels of GSH often decrease upon elevation of local oxidative stress. Deficiency of GSH within living organisms can lead to tissue disorder and injury. The studies herein have shown that SV/SVEF could efficiently scavenge excessive free radicals against oxidative stress by enhancing the levels of CAT and GSH. The results are in line with reports of Najmi et al.(2005).

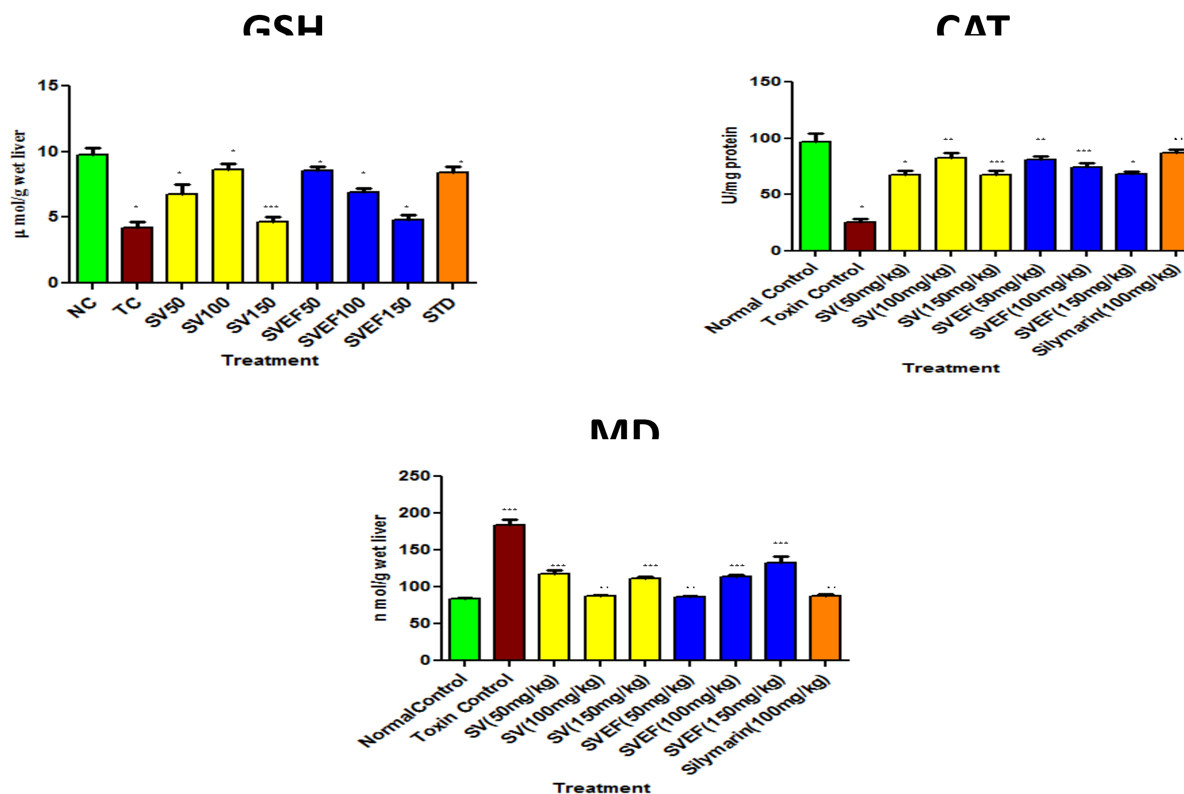
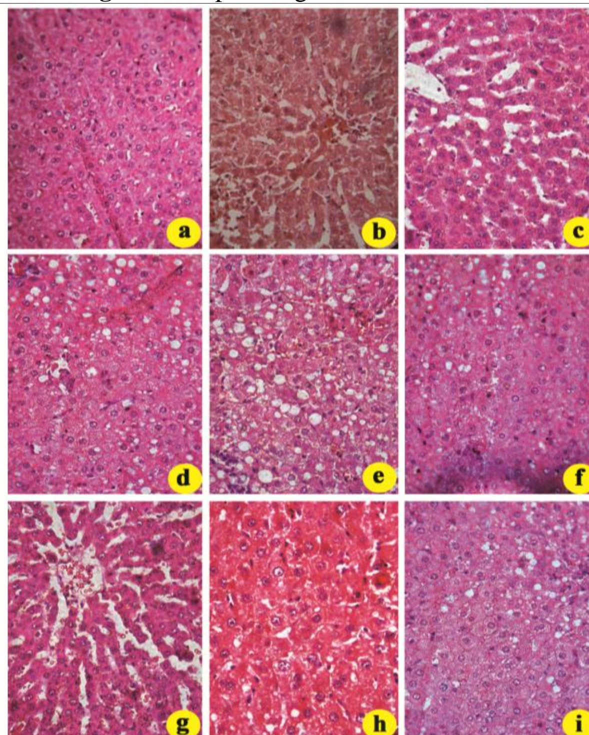


Fig 1:- Effect of *S.virgatus* crude extract (SV) / ethanolic fraction (SVEF) on hepatic GSH,CAT,MDA after D-Galactosamine administration

Values are expressed as mean ± SD of six values, one way ANOVA followed by Dunnet's multiple comparison test, comparison test,* P≤ 0.05 ,** P≤ 0.01,*** P≤ 0.001, ns= not significant compared to normal control.

The histological observations support the results obtained from serum enzyme assays(Fig:2). Liver sections from normal control rats showed central vein surrounded by hepatic cord of cells, distinct hepatic cells with well preserved cytoplasm and sinusoidal spaces, While the histological architecture of D-galactosamine treated liver sections showed massive fatty changes, ballooning degeneration and the loss of cellular boundaries, nuclear pycnosis and karyolysis. However administration of extracts significantly normalized these defects in the histological architecture of the liver.

Fig.2:- Histopathological observation



It is found out from the present study that SV / SVEF exhibited strong hepatoprotective activity. Statistically significant changes were also observed in the biochemical parameters of the group which was administrated with SV / SVEF. Even though SV/SVEF were found to be hepatoprotective, SVEF at a dose of 50 mg/kg was more effective in ameliorating the hepatic damage induced by D -GalN. Therefore the present study provides experimental evidence for the traditional claim on the hepatoprotective effect of the rhizome of *Schumannianthus virgatus*.

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