Hepatoprotective and Antioxidant Effects of *Ecbolium viride, Gendarussa vulgaris* and *Sphaeranthus amaranthoides* in NASH Induced Hepatotoxicity in Albino Wister Rats

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Abstract

Non-alcoholic steatohepatitis (NASH) caused due to over-nutrition, decreased physical activity with disproportionate high-fat food intake, obesity, type II diabetes mellitus (T2DM), and the metabolic syndrome characterized by steatosis, lobular inflammation and progressive pericellular fibrosis. This hepatic disease cannot be remedied by chemicals its addition may lead to complication. Hence herbal drug is the possible remedy.The hepatoprotective effect of *Ecbolium viride, Gendarussa vulgaris* and *Sphaeranthus amaranthoides* were monitored by estimating the Triglycerides(TG),Total cholesterol(TC),Low density lipoprotein(LDL), High density lipoprotein (HDL), Aspartate transaminases (AST), Alanine transaminases (ALT), Alkaline phospatases (ALP), and Lactate dehydrogenases (LDH).Plant extracts singly or in combination at a dose of 2g/kg significantly prevented the increase in serum level inliver. Theantioxidant enzymes following D-Galactosamine and diet induced hepatoxicity in albino rats was reversed by plant extracts.

Keywords: *Ecbolium viride, Gendarussa vulgaris, Sphaeranthus amaranthoides,* NASH, Hepatoprotection, D-Galactosamine.

Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a range of disorders associated with fatty liver, which occur in the absence of evident infection or significant consumption of alcohol.(Angulo, 2002) Non-alcoholic steatohepatitis (NASH) is a part of the spectrum of NAFLD. (Angulo, 2007) characterized by steatosis, lobular inflammation and progressive pericellular fibrosis. NASH is now regarded as the most common cause of abnormal liver function tests worldwide. In USA, the estimated prevalence of NAFLD is 20%-30% and that of NASH is 3.5%-5% (McCullough*et al.,* 2005), while in Asia, the prevalence of NAFLD is reported to be 12%-24% (Fan *et al.,* 2007). Owing to the increased trend of due to over-nutrition, decreased physical activity with disproportionate high-fat food intake, obesity, type II diabetes mellitus (T2DM), and the metabolic syndrome (Angulo, 2007; Fan *et al*., 2007 and Younossi, 2011; Williams, 2006)the prevalence of NAFLD worldwide has witnessed a substantial increase over the past decades and is likely to further increase in the near future (Williams, 2006). Long-standing NASH may progress to hepatic fibrosis, cirrhosis or even hepatocellular carcinoma - HCC, (Day, 2002; Fan *et al.,* 2007; Harrison *et al.,* 2003; Powell *et al.,* 1990) which causes major deaths worldwide.

Animal models of NASH can provide critical information leading to understanding its molecular mechanisms. Furthermore, animal models of NASH are an important platform for the testing of the therapeutic potency of certain agents against NASH (Larter, 2007; Fan*et al.,* 2001).The present study was conducted with the objective of evaluating the efficacy of the *Ecbolium viride*, *Gendarussa vulgaris* and *Sphaeranthus amaranthoides* in protecting the liver against diet induced NASH in rats.

Materials and Methods

Plant material and extraction

Ecbolium viride, Gendarussa vulgaris and *Sphaeranthus amaranthoides*were procured fromKarapattu and Melthaniyalam pattu in Villupuram District and Palayangkottai, Thirunelveli District in Tamilnadu.and authenticatedby using standard books such as Gamble and Mayuranathan. Theplant materials were dried, reduced tomoderately coarse powder and then 20g plant material was extracted in 200 mL of hydroalcoholic solution (70% ethanol) by maceration method.

Selection and Acclimatisation of Animals

Albino rats of wistar strains weighing between 180-220 g were produced from animal experimental laboratory and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25 ± 2 °C) and 12 hrs dark/ light cycle with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance (S.Subhashini/Ph.D/MU01/2011/1518/IAEC/KMCP/59 /2012-13).

Treatment Protocol

The acclimatized animals were divided into eight groups of each six animals, designated as follows.

Group I

Served as normal control and receive normal diet and water for 8 weeks.

Group II

HFD induced rat control received HFD (71% fat, 11% carbohydrates and 18% proteins) for 8 weeks.

Group III

HFD and D-galactosamine (250 mg/kg) induced rat control received HFD (71% fat, 11% carbohydrates and 18% proteins) for 8 weeks.

Group IV

Standard control received HFD and D-galactosamine (250 mg/kg) along with silymarin 750 mg/kg orally for 8 weeks.

Group V

The treatment control received HFD and D-galactosamine (250 mg/kg) along with extract of *E. viride*at a dose of 2 g/kg orally for 8 weeks.

Group VI

The treatment control received HFD and D-galactosamine (250 mg/kg) along with extract of *G. vulgaris*at a dose of 2 g/kg orally for 8 weeks.

Group VII

The treatment control received HFD and D-galactosamine (250 mg/kg) along with extract of *S. amaranthoides*at a dose of 2 g/kg orally for 8 weeks.

Group VIII

The treatment control received HFD and D-galactosamine (250 mg/kg) along with extract of *E. viride* + *G. vulgaris* + *S. amaranthoides*at a dose of 2g/kg orally for 8 weeks.

Hepatoprotective Activity

Chemicalsusedare high fat induced diet, Silymarin, D-Galactosamine,extracts of selected plants such as*E. viride, G. vulgaris* and *S. amaranthoides*. The experimental animals were kept at animal house under the control of Department of Pharmacology, K. M. College of Pharmacy, Uthangudi, Madurai. Animal house maintained at an ambient temperature of 25 ^{o} C and 45-55% relative humidity, with 12 hrs, of each of dark and light cycles. Animals were fed with pellet diet and water ad-libitum.

Maintenance and use of animals as per the experimental design was approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals. (S. Subhashini/Ph.D/MU01/2011/1518/IAEC/KMCP/59/2012-13).

Biochemical Estimation

On the 57 th day, 24 hrs after the administration of Silymarin and three extracts, animals from each group were humanely sacrificed using Ketamine HCL and 4 mL of blood was withdrawn by cardiac puncture and allowed to clot for 30 mins at room temperature. The serum was separated by using refrigerated centrifuge and used for the assay of lipid profile with marker enzymes viz Triglycerides(TG), Total cholesterol(TC), Low density lipoprotein(LDL), High density lipoprotein (HDL), Aspartate transaminases (AST), Alanine transaminases (ALT), Alkaline phospatases (ALP), and Lactate dehydrogenases (LDH). The livers were dissected out immediately, washed with ice-cold saline and 10% homogenates

with phosphate buffer solution (PH 7.4) were prepared. Liver homogenate was used for the assay of Lipid peroxidation (Lpo) while some fraction of homogenates were centrifuged at 7000 rpm for 10 min at 4 $^{\circ}$C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx). Some portion of liver from each group was aseptically excused and stored in 10% formalin for histopathological studies.

Statistical Analysis

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul's multiple range tests. The values are represented as Mean $\pm$ SEM. Probability value of $P<0.01$ was determined to be statistically significant.

Results:

Table 1 Effect of Extracts of E. viride, G. vulgaris and S. amaranthoides and SilymarinPre-treatment on Biochemical Parameters of the Rats in Toxicated with NASH (HFD).

Group. No.	Treatment Dose (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL-C (mg/dl)	LDH (U/L)
I	Normal control 10 mL/ kg normal saline	43.36± 1.47	29.09± 1.49	22.67± 1.27	80.25 ± 4.45	86.50 ± 3.08	95.88 ± 2.70	46.60 ± 2.76	306.40± 18.48
II	NASH control (HFD)	*a 04.99± 2.36	*a91.49 ± 1.05	*a141.0 4± 2.21	186.90 ± 6.48*(a)	148.80± 5.74*(a)	*a172. 33± 2.88	24.40± 1.40*(a)	445.17± 24.18*a
III	HFD + D-Galactosamine (250 mg/kg)	*a 12.63± 2.55	*a 102.52 ± 1.45	*a 152.16± 2.30	190.85 ± 6.55*(a)	155.82 ± 5.85*(a)	*a176. 40± 2.96	22.22 ± 1.23*(a)	456.20± 27.30*a

IV	Standard control HFD + Silymarin 750 mg/kg	*b 59.04 ± 1.19	*b39.56± 1.06	*b54.4± 1.68	126.80 ±5.06*(b)	110.70 ± 4.60*(b)	*b120 .17± 1.99	40.90 ± 1.86*(b)	366.08± 15.75*b
V	Treatment control HFD + *E. viride* 2 g/kg	*b 66.64± 1.44	*b 50.82± 2.72	*b64.83 ± 2.28	140.82 ± 5.52*(b)	130.91 ± 4.75*(b)	*b138 .24± 3.04	36.23 ± 1.10*(b)	388.18± 16.10*b
VI	Treatment control HFD+*G. vulgaris* 2 g/kg	*b 62.91± 0.65	*b 46.94± 0.97	*b 59.49± 1.92	134.70 ± 5.30*(b)	121.80 ± 4.65*(b)	*b 29.95 ± 1.20	38.53 ± 1.26*(b)	379.20± 15.68*b
VII	Treatment control HFD + *S. amaranthoides* 2 g/kg	*b 65.55± 0.58	*b48.25± 0.88	*b62.20 ± 2.45	138.26 ± 5.65*(b)	126.42 ± 4.98*(b)	*b132 .20± 1.45	34.15 ± 1.05*(b)	74.20± 15.68*b
VIII	Treatment control HFD + *E. v+G. v+S. a* 2 g/kg	49.03 ± 1.17	*b37.36± 1.03	*b45.4± 1.62	110.25 ±3.06*(b)	100.67 ± 5.61*(b)	*b118 .21± 3.89	42.75 ± 1.46*(b)	345.05± 14.65*b

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmann keul's multiple range tests.
- *a - values are significantly different from Normal control at P< 0.01.
- *b - values are significantly different from NASH control (G2) at p< 0.01.

Enzymic Antioxidant Studies

Table 2.Effect of Extracts of *E. viride*, *G. vulgaris* and *S. amaranthoides* with SilymarinPre-treatment on Biochemical Liver Homogenate Parameters of the Rats in Toxicated with NASH (HFD)

Group. No.	Treatment Dose (mg/Kg)	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein

I	Normal control 10 mL/kg normal saline	130.23±2.37	288.39± 2.38	1.09± 0.05	3.87± 0.14
II	NASH control (HFD)	*a67.19±1.62	*a190.71±2.66	*a0.37±0.02	*a7.35±0.40
III	HFD + D-Galactosamine (250 mg/kg)	*a64.05±1.35	*a186.60±2.42	*a0.30±0.01	*a 7.68±0.46
IV	Standard control HFD + silymarin 750 mg/kg	*b86.03±1.81	*b258.42±1.90	*b0.83±0.02	*b4.48±0.16
V	Treatment control HFD + *E. viride* 2 g/kg	*b95.51±1.98	*b228.03±1.78	*b0.56±0.02	*b5.25±0.28
VI	Treatment control HFD+*G. vulgaris* 2 g/kg	*b92.66±1.80	*b235.73±2.65	*b0.72±0.02	*b4.80±0.22
VII	Treatment control HFD + *S. amaranthoides* 2 g/kg	*b90.42±1.75	*b230.40±2.20	*b0.70±0.02	*b4.68±0.18
VIII	Treatment control HFD + *E. v*+*G. v*+*S. a* 2 g/kg	*b76.04 ± 1.83	*b210.32±1.73	*b0.70±0.02	*b4.35±0.16

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmann keul's multiple range tests.
- *a - values are significantly different from Normal control at P< 0.01.
  *b - values are significantly different from NASH control (G2) at p< 0.01.

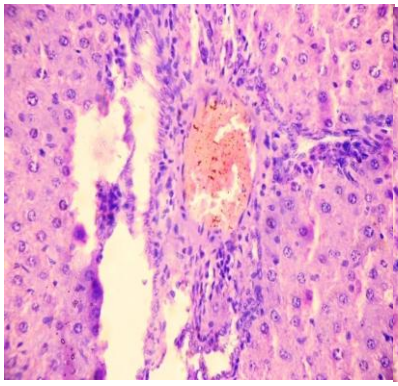
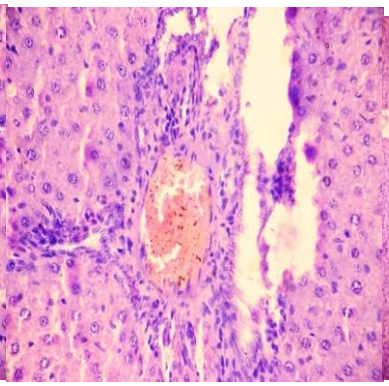
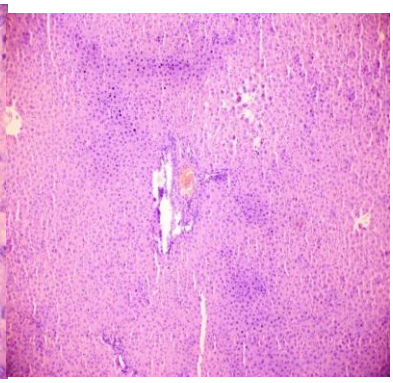

Fig 1 **Fig.2** **Fig. 3**

Discussion

Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver (Chandan *et al.*, 2007). Hence significant rise in the transaminases levels could be taken as an index of liver damage. In our study the rise in AST, ALT, ALP, LDH levels induced by HFD and D-galactosamine administration were significantly reduced by extracts of *E. viride*, *G.vulgaris* and *S. amaranthoides* and silymarin pre-treatment suggesting that its hepatoprotective activity might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes.

In this study, treatment with the plant extracts of singly and in combination and silymarin (control) probably ameliorated oxidative liver injury through its antioxidant effect which further reduced hepatic injury as shown as biochemical findings. In addition, several reports showed that hepatic antioxidant defense system associated with antioxidant enzymes such as SOD, CAT and GPx are disrupted in D-Gal and HFD treated rat.In rats, treated with D-Gal and HFD, neutrophil infiltration into the liver cells increased with the formation and progression of liver injury and that reactive oxygen species, such as superoxide radical, released from activated neutrophils infiltrating into the liver of D-Gal and HFD treated rats caused the extension of liver cell necrosis .Rats treated with D-Gal and HFD showed that, neutrophil infiltration into liver cells occurs at an early stage of the injury (Keppler *et al.*, 1969). Accordingly, the reactive oxygen species of scavenging action of the plant extracts and silymarin if administered after D-Gal and HFD intoxication may contribute to the attenuation of disruption of hepatic antioxidant defense system in D-Gal and HFD treated rats. Therefore, it may be possible that post administration of the plant extracts and silymarinexerts a preventive effect on liver injury progression in D-Gal and HFD treated rats through its indirect antioxidant action to the system in addition to its direct antioxidant action

to maintain antioxidant defense systems by scavenging ROS and inhibiting lipid peroxidation. D-Gal and HFD induced-hepatotoxicity(Fig.2)(NASH) has striking resemblance with the human viral hepatitis. The present study confirms the Hepatoprotective efficacy of extracts of *E. viride*, *G. vulgaris* and *S. amaranthoides* and silymarin (Positive control) against D-Gal and HFD induced hepatitis in rats.

D-Gal is reported to produce intensive inflammatory infiltration in the liver parenchyma and peripheral areas. In our study also D-Gal administration showed severe hepatotoxicity with heavy infiltration of inflammatory cells around portal tract and in the liver parenchymal cells (Fig.1). Treatment with extracts of *E. viride*, *G. vulgaris* and *S. amaranthoides* and silymarin for 8 weeks protected the rat livers from D-Gal and HFD induced histopathological changes (Fig.3) and (Tables 1 and 2).

Conclusion

It may be stated that the present investigation has complimented the pharmacological potentials of the three plants. Their combination had an antagonistic effect which was comparable to control. Further studies on human clinical trials may help to cure NASH disease among humans.

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