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Combined effects of *Solanum trilobatum* and *Solanum melongena* against β-galactosamine induced hepatic damage in rats with reference to marker enzymes and antioxidants status

A.Purushothaman, Thabraz Ahmad, P.Meenatchi, M.N.Kathiravan, M.Shahjahan*

Post Graduate and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Chennai - 600 119, Tamil Nadu, (INDIA)

E-mail : nila_mugil@rediffmail.com; shajahanphd@yahoo.com

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ABSTRACT

In this study, methanol extract of *Solanum trilobatum* and *Solanum melongena* has been evaluated for hepatoprotective and antioxidant activity and against experimentally induced hepatic damage in rats. The hepatic damage was induced to rats using β- galactosamine (300mg/kg body weight i.p). The extract (250 mg/kg body weight) was administered orally for 7 days by gastric intubation to experimental animals. The activities of marker enzymes such as aspartate tranaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ-GT) in serum were studied. The status of antioxidants such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and lipid peroxidation in the liver of control and experimental animals were also evaluated and compared. Results of the study revealed the elevated levels of serum AST, ALT, ALP, LDH and γ-GT in β-galactosamine induced animals, which might be due to hepatic damage. Oral administration of the extract of ST and SM significantly ($p<0.05$) reduced the elevated levels of the above marker enzymes in serum. The enzymatic and non enzymatic antioxidants in liver were restored to normal values after the oral administration of the plants extract. From the results, it can be inferred that the combined extract of *Solanum trilobatum* and *Solanum melongena* positively modulated the hepatic marker enzymes and antioxidant activity in β-galactosamine induced liver damage. Histopathological examination of liver sections also proved the effect.

KEYWORDS

Solanum trilobatum;
Solanum melongena;
β-galactosamine;
Antioxidants;
Hepatoprotectives.

INTRODUCTION

The liver plays an astounding array of vital functions in the maintenance, performance and regulating homeostasis of the body. Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall

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health and well being. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol and prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease[1]. Steroids, vaccines and anti-viral drugs, which have been employed as a therapy for liver diseases, have potential adverse side effects especially when administered for long terms[2]. The use of the existing few hepatoprotectives in the therapy for the treatment of several liver diseases is still very tentative. In this context, natural products from plant sources, which are considered safe and devoid of toxicity, have gained importance as new and improved therapies[3].

Among the various plants associated with the life of Indians, one such family is Solanaceae that is economically very important, as several of its species are sources of food, fodder and drugs[4]. Traditionally, *Solanum trilobatum* Linn. (ST) has been in use in different parts of India for various ailments[5]. The bitter roots are used for consumption in the form of electuary, decoction or powder. The berries and flowers are administered for the treatment of cough. The decoction of various parts of this plant is used for chronic bronchitis[6, 7]. The alkaloids reported in the genus of Solanum include solanine, solasonine and solamarine[8]. The major alkaloids identified in the alcoholic extract of ST are β-solamarine[9, 10] and solasodine[11]. Reports have shown that the active principle sobatum isolated from ST possessed anti-tumor effect against chemically induced tumors[12], chemopreventive effect against cyclophosphamide[13] and radiation induced toxicity[14]. Earlier studies revealed the protective effect of ST against CCl_4 induced toxicity[15] and diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat[16].

Eggplant, *Solanum melongena* Linn. (SM), a common vegetable grown in the subtropics and tropics, is consumed throughout the world and contains a variety of phytochemicals such as phenolics and flavonoids that provide important health benefits[17]. *Solanum melongena* (Eggplant) fruit popularly known as aubergine (UK), melanzana, garden egg, bringal (India) and is one of the most important vegetable crops grown on over 1.7 million hectare worldwide[18]. Earlier studies have shown that *Solanum melongena* Linn. extract results in hypolipidemic activity in rats fed normal as well as high fat diets[19], suppresses the formation of blood vessels required for tumor growth and metastasis[20] and inhibits inflammation[21]. Extract from purple eggplant skin has been shown to possess a high capacity in the scavenging of superoxide radicals and inhibition of hydroxyl radical generation[22]. Various parts of the plant are useful in the treatment of inflammatory conditions, cardiac debility, neuralgia, ulcers of nose, cholera, bronchitis and asthma[23].

The present study aimed to determine hepatoprotective and antioxidant activities of *Solanum trilobatum* and *Solanum melongena* extract against β-galactosamine induced liver damage in rats.

EXPERIMENTAL

Plant materials

The aerial parts of *Solanum trilobatum* and fruit of *Solanum melongena* (pink variety) were collected from Sholinganallur, Chennai-600 119 Tamil Nadu, India in July 2009. The plants were identified and authenticated by Botanist, The IMPCOPS (Indian Medical Practitioner's Co-operative Pharmacy and Stores Ltd., Thiruvanmiyur, Chennai-600-041. India. The plant materials were cleaned, dried in shade and powdered. The powdered samples were stored in air tight container for further studies.

Chemicals and reagents

Dimethylsulfoxide (DMSO) was purchased from Southern India Scientific Corporation, Chennai, India. β- Galactosamine was purchased from Sigma Chemicals, USA. All other chemicals and reagents used were of Analytical Grade and highest purity.

Animals

The studies were performed on male albino rats of Wistar strain (120–150 g) obtained from the animal house facility of Mohamed Sathak A.J College of pharmacy, Sholinganallur, Chennai-600 119, India. The animals were housed in standard laboratory conditions 12±1 h day and night rhythm throughout the experimental period. The animals were housed in large spa-

cious polypropylene cages and they were given rodent pellet (supplied by Lipton India Ltd., Mumbai, India under the trade name Gold Mohur) and water ad libitum. The rats were acclimatized for at least 1 week before commencement of the experiment. During the course of the experiment the temperature remained around 27°C. The study has got the approval from the Institutional Animal Ethical Committee of Committee for the Purpose of Control and Supervision of Experiments on Animals (No. AJ/ IAEC/2010/02), Ministry of Environment & Forests (Animal Welfare Division), Government of India.

Preparation of the plants extract

500g each of shade dried, coarsely powdered plant materials (*Solanum melongena* and *Solanum trilobatum*) was charged in an aspirator bottle and allowed to soak in 90% methanol for 48 hours at room temperature. The extract was filtered and concentrated on a water bath to 20ml. The filtrate was again concentrated in a china dish and dried in vacuum desiccators at 4º C. The yield of the plant extract was about 10% since the yield was less, the procedure was repeated to get sufficient yield. The extract was dissolved in 10% DMSO for oral administration.

Induction of hepatic damage/liver damage

Liver damage was induced in animals using β-galactosamine as described previously[24] and the approval for the induction was got from the Institutional Animal Ethical Committee.

Experimental design

Animals were divided into four groups of 6 animals each.

- **Group I** served as control rats receiving 1ml of 10% DMSO as vehicle for the entire experimental period of 7 days.
- **Group II** rats served as induced animals (liver damage induced using β galactosamine (300mg/kg body weight i.p)
- **Group III** served as ST and SM co treated rats (animals induced for liver damage (as in Group II), and administered with 250mg/kg body weight/day of SM & ST extract dissolved in 1ml of 10% DMSO orally by gastric intubation, for the entire experimental period of 7 days.
- **Group IV** served as drug control rats receiving 250 mg/kg body weight per day of the ST and SM extract in 1 ml of 10% DMSO for 7 days by gastric intubation.

Animals were killed at the end of experimental period by cervical decapitation. Blood was collected and serum was separated. Liver tissues were excised from the animals and homogenate was prepared using 0.01 M Tris-Hcl buffer, pH 7.4 and were used for the assay of marker and antioxidant enzymes.

Biochemical assays

The activities of marker enzymes - aspartate aminotransferase, alanine aminotransferase[25], alkaline phosphatase[26], lactate dehydrogenase[27], *y*-Glutamyl transpeptidase[28] were assayed in the serum.

The protein content was determined by the method of Lowry et al.[25]. Lipid hydroperoxides were estimated by the method of Jiang et al.[26]. The activities of alanine aminotransferase and aspartate aminotransferase were measured by the method of King, 1965a[27]. Alkaline phosphatise was measured by the method of King, 1965b[28], the activities of lactate dehydrogenase[29] and *y* -Glutamyl transpeptidase[30] were assayed in the serum and liver tissue.

The levels of antioxidants - superoxide dismutase[31], catalase[32], glutathione peroxidase[33] and reduced glutathione[34] were also compared in the liver tissue of control and experimental animals.

Histopathological analysis

Formalin-fixed mammary liver tissues were paraffin embedded, sectioned (3 mm thickness) and placed on glass slides. Paraffin-embedded sections of tissue were deparaffinised, rehydrated with graded alcohol and stained with Harris' haematoxylin and eosin (Dako, Glostrup, Denmark) in a Leica Autostainer (Wetzlar, Germany).

Statisical analysis

Results were represented as mean±SD of six rats. The results were computed statistically (SPSS software package, version 16) using one-way analysis of variance (ANOVA). Post hoc testing was performed for intergroup comparison using Student-Newman-Keul multiple comparison test. Values of $p<0.05$ were considered significant.

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TABLE 1 : Effect of ST and SM extract on the levels of marker enzymes in serum of control and experimental animals.

Parameters	Group I (Control)	Group II (Induced)	Group III (Induced+ treated with ST+SM)	Group IV (Drug control)
AST (μ moles of pyruvate liberated/mg protein/min)	36.45±2.26	56.96±3.86[a*]	42.11±2.19[a*,b**]	34.88±2.26
ALT (μ moles of pyruvate liberated/mg protein/min)	26.35±1.21	40.64±2.77[a*]	33.85±1.78[a*,b**]	23.84±0.87
ALP (μ moles of p-nitrophenol liberated/mg protein/min)	46.07±1.62	107.65±7.42[a*]	62.83±5.54[a*,b*]	44.54±1.37
LDH (μ moles of pyruvate liberated/mg protein/min	78.83±4.33	130.38±8.61[a*]	94.67±5.69[a*,b*]	73.17±3.96
γ - GT (μ moles of p-nitro aniline formed/mg protein/min)	18.23±1.74	55.82±3.55[a*]	21.64±2.28[a*,b**]	19.96±1.88

Values are expressed as mean ± SD of six animals.

[a] Group II, III, IV compared with group I; [b] Group III compared with group II; * Statistical significance: p < 0.01. ** Statistical significance: p < 0.05

RESULTS AND DISCUSSION

Effect of ST and SM on serum marker enzymes of control and experimental animals

In untreated hepatic damage induced rats (group II), the activities of marker enzymes such as AST, ALT, ALP, LDH, and GGT were elevated to high levels in serum compared to control animals. These changes in serum marker enzymes were significantly ($p < 0.05$) reverted back upon ST and SM extract co administration at a dose of 250mg/kg body weight per day as shown in TABLE 1. The non toxic nature of combined ST and SM extract was witnessed from the maintenance of these marker enzyme activities in ST and SM extract administered animals (Drug control).

In recent years, plant based natural products (polyphenols, flavonoids, ahthocyanidins, terpenoids and steroids etc.) have received considerable attention due to their diverse pharmacological actions[36, 37]. A growing interest has been observed in the study of herbal drug for their potential benefits to human health. One of their main properties is antioxidant activity, which enables them to attenuate the development of tumor, inflammation and liver diseases. Antioxidants play an important role in inhibiting and scavenging free radicals and provide protection against infection and degenerative diseases. Realizing the fact, this investigation was carried out to evaluate combined hepatoprotective activity of *Solanum trilobatum* and *Solanum melongena* methanolic extract against β-galactosamine induced hepatic damage in rats. In the present investigation, β-galactosamine induced hepatotoxicity was evidenced by biochemical measurements and histopathological changes. Increased level of serum AST, ALT, ALP, LDH and GGT indicated deterioration in the hepatic architecture and functions. Histopathological observations of liver tissue of control and experimental rats were consistent with β-galactosamine induced hepatic damage. Co administration of ST and SM extract was found to revert back the normal condition to the hepatic musculature as shown by biochemical and histological findings. The stimulation of hepatic regeneration was considered as the possible hepatoprotective mechanism of standardised herbal extracts. Such stimulation was known to cause the liver to become more resistant to hepatotoxin induced liver injuries[37]. Likewise, activation of the functions of the reticuloendothelial system was also considered as some possible hepatoprotective mechanisms, which could reduce the hepatotoxicity of beta galactosamine[38].

Effect of ST and SM on lipid peroxidation of control and experimental animals

The effect of ST and SM on the levels of lipid peroxidation in control and experimental animals was shown in Figure 1 and was found to be significantly increased ($p < 0.05$) in hepatic damage induced animals when compared with the control rats. These changes were significantly ($p < 0.05$) reverted back to near normal upon ST and SM administration. No significant changes were observed between control and drug control animals.

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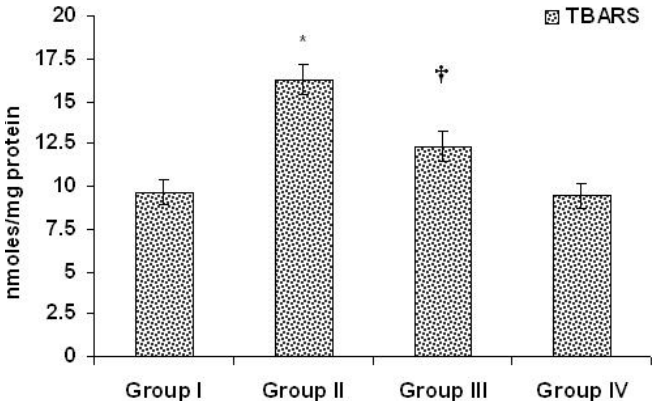

Figure 1 : The levels of lipid peroxidation estimated as thiobarbituric acid reactive substances in the liver tissue of control and experimental animals (mean ± SD; n= 6). (group I, control; group II, β-galactosamine induced; group III, induced + ST+SM treated; group IV, control+ ST+SM).
***Comparisons are made between groups I and II; † Comparisons are made between groups II and III; Statistical significance (*, † p<0.05)**

Previous studies have shown that the phytoconstituents of ST possess antioxidant potential[39], in addition to its ability to inhibit free radical generation[40]. The presence of quercetin and β-sitosterol in ST could also support its role in modulating the antioxidant defenses both enzymatically and non-enzymatically. The phenolic compounds and flavoinds of eggplant fruit implicated in both inhibition of hydroxyl radical generation and superoxide scavenging activity, which could contribute to hepotoprotective activity[41].

Effect of ST and SM on liver antioxidant status of control and experimental animals

TABLE 2 shows the levels antioxidants such as SOD, CAT, GPx and reduced glutathione (GSH) in control and experimental animals. A significant decrease in the activities of SOD, CAT, GPx and the levels of GSH were observed in the induced (group II) animals compared to control animals. In treated (group III) animals, the activities of SOD, CAT and GPx and the level of GSH were significantly increased ($p < 0.05$) compared to induced (group II) animals.

Antioxidants act as the primary line of defence against reactive oxygen species (ROS) and suggest their usefulness in estimating the risk of oxidative damage. In the present study, there was a decrease in the activities of antioxidant enzymes SOD, CAT, GPx and GSH in β-galactosamine induced rats. This might be due to the excessive production of free radicals in cancerous condition. Several studies have shown that decreased antioxidant system results in oxidative stress, which dysregulates the cellular functions[42, 43]. SOD and CAT are the two major enzymes that are directly involved in the elimination of ROS[44]. SOD is an important antioxidant enzyme that catalyses the dismutation of superoxide radicals[45], while CAT is a hemoprotein that catalyses the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals[46]. The decrease in the activities of these enzymes may be due to increased production of free radicals such as superoxide anions during inflammatory reactions by the phagocytic cells[47].

GPx is an important defence enzyme against oxidative damage and this in turn requires reduced glutathione as a cofactor. GPx works together with glutathione in the decomposition of hydrogen peroxide or other organic hydroperoxides to non-toxic products at the expense of GSH[48]. The decreased activity of GPx in liver of hepatic damage induced rats obtained from

TABLE 2 : Effect of ST and SM extract on the levels of antioxidants in liver of control and experimental animals.

Parameters	Group I (Control)	Group II (Induced)	Group III (Induced+ treated with ST+SM)	Group IV (Drug control)
Superoxide dismutase (unit min^{-1} mg $protein^{-1}$)	6.45±0.46	$4.37±3.86^{a*}$	$5.11±2.19^{a*,b**}$	6.34±2.26
Catalase (μmol H_2O_2 utilised min^{-1} mg $protein^{-1}$)	56.35±3.21	$40.64±2.77^{a*}$	$48.85±1.78^{a*,b**}$	58.84±2.87
Glutathione peroxidase (μmol of GSH consumed min^{-1} mg $protein^{-1}$)	7.28±0.86	$5.65±0.42^{a*}$	$6.28±0.74^{a*,b*}$	7.41±0.77
Reduced glutathione(μg/mg protein)	6.53±0.43	$4.38±0.61^{a*}$	$5.67±0.69^{a*,b**}$	6.44±0.56

Values are expressed as mean ± SD of six animals.
[a] Group II, III, IV compared with group I; [b] Group III compared with group II; * Statistical significance: p < 0.01. ** Statistical significance: p < 0.05.

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the present investigation indicates the weak free radical defence system against oxidative stress.

The decreased activity of antioxidant enzymes observed in induced rats might be due to high concentration of ROS. The decline in SOD activity leads to down regulation of H_2O_2. Since H_2O_2 is the substrate for the enzymes CAT and GPx, they were also found to be decreased[49].

Administration of ST and SM extract caused the activities of these enzymes to return to normal levels. This may be due to hepatoprotective property of the drug indicating the protective role on tissue damage. Earlier reports have shown that the active principle sobatum isolated from ST possessed anti-tumor effect against chemically induced tumors[50], chemopreventive effect against cyclophosphamide and radiation induced toxicity[51]. Shah Jahan et al have reported the protective effect of ST against carbon tetrachloride induced toxicity[15].

Solanum melongena L. contains a variety of phytochemicals such as phenolics and flavonoids that provide important health benefits. Studies have shown that administration of SM extract results in potential hepatoprotective action against cytotoxicity of tert-butyl hydroperoxide (t-BuOOH) in human hepatoma cell lines (HepG2), suppresses the formation of blood vessels required for tumor growth and metastasis[52], and inhibits inflammation[53]. Nasunin, an anthocyanin isolated from the skin of purple eggplant fruit and other phenolic compounds of the extract implicated in both inhibition of radical generation and hepatoprotecion.

Effect of ST and SM on histopathology of control and experimental animals

Histopathological examination of liver sections from control (group I) animals revealed normal architecture with normal liver parenchyma and cells with granulated cytoplasm and small uniform nuclei radially arranged around central vein (Figure 2a). The liver of section of induced (group II) animals revealed loss of architecture with with hepatocytes looking large, pale and having pleomorphic nuclei (Figure 2b), whereas ST and SM treated (group III) animals had comparatively less altered foci and hepatocytes maintaining near normal architecture (Figure 2c). Drug control (group IV animals also exhibited similar architecture indicating the non-toxic nature of the ST and SM extract (Figure 2d).

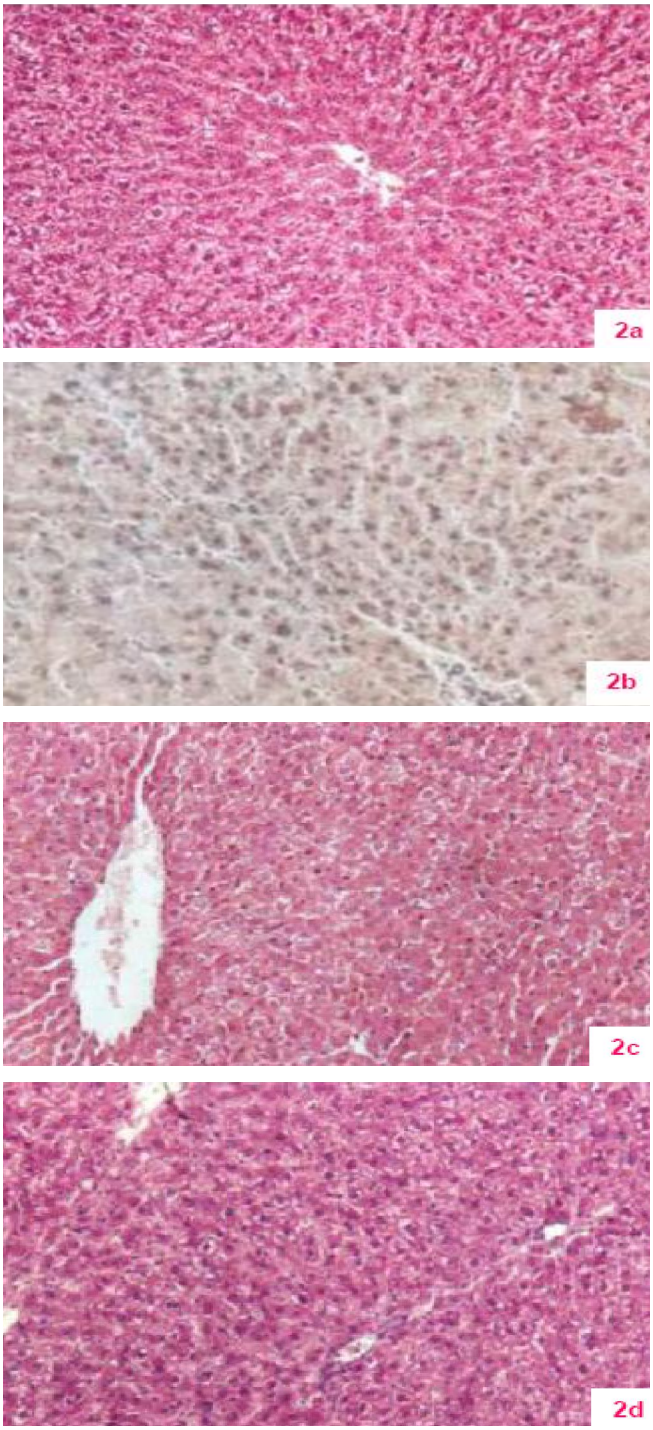

Figure 2 : Histopathological examination of liver sections stained with haematoxylin and eosin (100X) Control (group I) rat shows normal architecture of liver (2a). Rats induced with β-galactosamine (group II) showing hepatocytes with loss of architecture, necrosis, fatty changes and are looking pale (2b). Group III rats, induced and treated with ST+SM extract (250 mg/kg body weight) show normal cellular architecture with distinct hepatic cells and sinusoidal spaces. Treatment with extract only (group IV) exhibit almost normal histology (2d).

Conflict of interest statement: We declare that we have no conflict of interest; Source of financial support: Nil

CONCLUSIONS

In conclusion, our current investigation revealed the antioxidant and hepatoprotective effects of *Solanum trilobatum* and *Solanum melongena* against β-galactosamine induced hepatic damage in rats. The flavonoids present in ST and SM must have acted synergistically and contributed to the profound hepatoprotective effects.

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