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Protective Effects of Aqueous Extract of *Sphaeranthus indicus* L on Paracetamol Induced Hepatic Damage in Rats

Sundari K * and Govindaraju G

Department of Biochemistry, PRIST University,Thanjavur, Tamilnadu, India

ABSTRACT

Aqueous extract of root of *Sphaeranthus indicus* L. was evaluated for hepatpprotective activity in rats. The plant extract 200 & 300 mg/kg body weight showed a remarkable hepatoprotective activity against acetaminophen induced hepatotoxicity as judged from the serum marker enzymes levels in liver tissues. Paracetamol overdose caused significant ($p<0.001$) increase in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), acid phosphatase, γ-Glutamyl transferase, Total Cholesterol and serum triglycerides, low density lipoprotein, total bilirubin with a reduction of total protein and high density lipoprotein . Treatment of rats with different doses of plant extract (200 & 300 mg/kg body weight) significantly ($P<0.001$) altered serum marker enzyme and lipid levels to near normal against acetaminophen treated rats. The activity of the extract at dose of 300 mg/kg was comparable to standard drug, silymarin (50mg/kg body weight). Results indicate the hepatoprotective properties of *Sphaeranthus indicus* L against acetaminophen induced hepatotoxicity in rats.

Keywords: *Sphaeranthus indicus*; acetaminophen, biochemical parameters; hepatoprotection

Corresponding author

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INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treatment or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence this organ is subjected to variety of diseases & disorders. Several hundred plants have been examined for use in a variety of liver disorders.

Sphaeranthus indicus Linn belongs to family Asteraceae. The plant is commonly known as Gorakmundi in Hindi. It is an annual spreading herb, which grows s approximately 15-30cm in heights. The plant is distributed throughout the plains and wet lands in India, Srilanka & Australia [1]. It is used indigenously in the Indian system of medicine as an anthelmintic [2]. The plant has a wide range of medicinal value and has been used in hemicranias, jaundice, leprosy, diabetes, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia, skin diseases and nerve tonic [3, 4]. Pharmacological activities such as immunomodulatory [5], antimicrobial [6, 7], antibacterial [8, 9], anxiolytic [4], wound healing action [10] were reported on this plant. Phytoconstituents isolated from this plant are eudesmanolides [11] isoflavonoids [12], 7-hydroxy eudesmanolides [13], sterol glycoside [14], essential oil (cadiene, ocimene, citral, p-methoxycinnamaldehyde, geraniol, eugenol and geranyl acetate) [15], and eudesmanolides [16]. The present study was undertaken to study the possible hepatoprotective role of ethanolic extract of roots of *Sphaeranthus indicus* L.

MATERIALS AND METHODS

Plant materials

The fresh roots of *Sphaeranthus indicus* L were collected during the month of February 2009 in the Thanjavur, Tamilnadu, India. It was botanically identified and authenticated. A voucher specimen (SIR-12) has been kept in our laboratory for future reference. The root was shade dried, powdered, sieved through 40 meshes and stored in a tightly closed container for future use.

Preparation of plant extract

The fresh dried powdered roots of *Sphaeranthus indicus* L were extracted (soxhlet) with water. These extracts were condensed using rotary vaccum evaporator followed by vaccum evaporator and stored in desiccators. The powder of all the extracts was suspended in appropriate solvent systems and was subjected to further analysis.

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Acute Toxicity Study

Acute toxicity study was carried out as per OECD guidelines 423 (Acute toxic class method) to determine the LD50. The dose of was fixed as 200 mg/kg & 300 mg/kg body weight.

Chemicals

Paracetamol was purchased from, CIPLA LTD., Vill. Juddikalan, Baddi, H.P. Silymarin was supplied by Panacea Biotech Ltd, New Delhi. All other chemicals and other bio chemicals used in the experiments were of analytical grade from different firms. The organic solvents were distilled before use.

Animals

Wistar male Albino rats weighing between 180-200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24 ± 2o under 12h light dark cycle and were fed ad libitum with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee.

Experimental designs

The experimental animals were divided into 5 groups of six rats each.

Group I: Control rats fed with standard diet.
Group II: Animals orally received paracetamol (1g kg^{-1} body weight) twice a week for 6 weeks.
Group III: Animals received 1g kg^{-1} body weight of paracetamol dissolved in glucose water orally along with 200 mg/kg body weight of aqueous extract of roots of *Sphaeranthus indicus* twice a week for 6 weeks.
Group IV: Animals received 1g kg^{-1} body weight of paracetamol dissolved in glucose water orally along with 300 mg/kg body weight of aqueous extract of roots of *Sphaeranthus indicus* twice a week for 6 weeks.
Group V: Animals received 50 mg/kg body weight of standard drug silymarin and 1g kg^{-1} body weight of paracetamol twice a week for 6 weeks and served as standard control.

Sample collection

At the end of the experimental period (45 days) the animals were sacrificed by cervical dislocation after an overnight fast. Blood sample of each group was collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37^0c.

Assessment of liver function:-

Biochemical parameters i.e., aspartate amino transferase (AST) (Reitman & Frankel, 1957) alanine amino transferase (ALT) (Reitman & Frankel, 1957), alkaline phosphatase (ALP) (King & king 1954),acid phosphatase,(King & king 1954), γ-Glutamyl transferase [19], total bilirubin (Mallay & Evelyn, 1937) & total protein [21], Total Cholesterol [22] and serum triglycerides [23], low density lipoprotein and high density lipoprotein [24] were analyzed according to the reported methods. The protocol was approved by Institutional Ethics committee constituted for the purpose (CPCSEA/265).

Statistical analysis:-

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnet's test was carried out and $P<0.001$ was considered as significant.

RESULTS

The results of Table 1 shows that administration of paracetamol caused a significant ($p<0.001$) increase in seni alanine aminotransferase (ALT), aspartate aminotransferase (AST),acid phosphatas(ACP) and alkaline phosphatase (ALP), γ-Glutamyl transferase,Total bilirubin after paracetamol intoxication in comparison with the control. However, animals pre-treated with aqueous extract of Sphaeranthus indicus significantly restored the enzymes below levels that can cause hepatic damage. Similarly, serum protein in paracetamol intoxicated animals was significantly reduced compared with the control and animals that were pre-treated with extract of Sphaeranthus indicus(Table 1).

Table 2 shows a significant ($p<0.001$) increase in total cholesterol, triglycerides and low density lipoprotein-cholesterol in paracetamol treated group compared with the control. Animals pre-treated with the extract significantly reduced the elevated lipid profiles in dose-dependent manner. Also, there was reduction in serum high density lipoprotein-cholesterol in paracetamol treated group compared with the control. Similarly, animals pre-treated with the extract significantly ($p<0.001$) reduced the elevated lipid profiles in dose-dependent manner.

DISCUSSION

Acetaminophen (paracetamol) a widely used antipyretic analgesic drug produces acute hepatic damage on accidental over dosage. It is metabolized in the liver to an active metabolite, N-acetyl-P-benzoquinone imine (NAPQI), by the cytochrome P-450 microsomal enzyme system with this resultant oxidative stress producing liver glutathione & glycogen depletion & hepatic necrosis [25, 26]. Paracetamol is relatively safe when taken at prescribed therapeutic doses. Paracetamol induced liver injury is commonly used as models for investigation into the efficacy of hepatoprotective drugs [27, 28]. The elevated serum liver enzymes such as ALT, AST & ALP in

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intoxicated rats can be attributed to the damage in the histostructural integrity of the liver cells (hepatocytes) [29].

Table 1: The effects of *Sphaeranthus indicus* L on biomarkers of hepatic damage

Group	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	ACP (IU/L)	GGTP (IU/L)	Total Protein (gm/dl)	Total Bilirubin (mg/dl)
Control	76.72 ± 3.36	70.48 ± 2.36	256.42± 4.91	10.77 ± 0.44	87.0 ± 1.2	9.62 ± 0.36	1.60 ± 0.14
Paracetamol	343.83 ± 5.64	297.47± 3.16	541.73± 4.51	32.73 ± 0.84	129.6 ± 2.7	3.87 ± 0.21	6.20 ± 0.63
AESI 200mg/kg + APAP	250.34 ± 3.19*	189.52± 4.17*	389.3± 3.92*	29.22± 0.64*	91.00± 1.02*	6.63± 0.17*	3.36 0.16*
AESI 300mg/kg + APAP	180.34± 2.97**	163.39 ± 3.17**	345.4 ± 4.74**	20.16 ± 0.30**	70.12 ± 1.03**	7.78 ± 0.96**	2.68 ± 0.35**
Silymarin 50mg/kg + APAP	112.25 ± 3.19	104.12 ± 2.68	276.45 ± 5.22	12.10 ± 0.20	55.3 ± 1.78	9.13 ± 0.26	1.97 ± 0.22

All values are in Mean ± SEM, P<0.001* significant, P<0.001** more significant Vs Control, N=6.

Table 2: The effects of *Sphaeranthus indicus* L on lipid profiles

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL - Cholesterol (mg/dl)	HDL - Cholesterol (mg/dl)
Control	93.60 ± 5.67	63.40 ± 4.46	25.33 ± 2.52	63.40 ± 4.46
Paracetamol	123.67 ± 5.11	89.00 ± 6.00	64.60 ± 3.28	25.60 ± 3.67
AESI 200mg/kg + APAP	101.60 ± 8.18*	58.40 ± 9.98*	40.12 ± 1.12*	30.10 ± 2.52*
AESI 300mg/kg + APAP	90.12 ± 2.27**	45.36± 2.10**	30.00± 2.12**	55.11 ± 2.12**
Silymarin 50mg/kg + APAP	58.12 ± 2.21	45.36 ± 2.11	25.22 ± 1.52	63.30 ± 4.00

All values are in Mean ± SEM, P<0.001* significant, P<0.001** more significant Vs Control, N=6.

It has been documented that covalent binding of N-acetyl-p-benzoquinone imine, an oxidation product of paracetamol, to sulphydryl groups of protein resulted in cell necrosis and lipid peroxidation with concomitant decrease in glutathione levels in the liver [30, 31]. In the assessment of liver damage by paracetamol, the determination of enzyme marker levels such as ALT and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can be therefore measured in serum as markers of hepatic damage. High levels of AST indicateliver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. The ALT catalysis the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury [32]. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [33]. Similarly, serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis; in presence of increasing biliary pressure has been reported by Muriel et al. [34].

In the present study, we have demonstrated the effectiveness of Sphaeranthus indicus by administration of paracetamol over dosage in rats, which is a known model for both hepatic injury and GSH depletion. Present results using the model of paracetamol-induced hepatotoxicity in the rats demonstrated that Sphaeranthus indicus at the different doses caused significant inhibition of ALT and AST levels. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [32]. Present results using the model of paracetamol-induced hepatotoxicity in rats demonstrated that Sphaeranthus indicus at different doses caused significant inhibition of serum ALP, ACP, and bilirubin levels. Effective control of bilirubin level acid phosphatase and alkaline phosphatase activity has been described to point towards an early improvement in the secretory mechanism of the hepatic cell. The abnormal high level of serum ALT, AST, ALP, ACP and bilirubin observed in our study (Table 1) are the consequences of paracetamol induced liver dysfunction and denoted the damage to the hepatic cells. Treatment with Sphaeranthus indicus reduced the enhanced level of serum ALT, AST, ALP, ACP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. A reduction in Total Serum Protein (TSP) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight. However, when Sphaeranthus indicus was given along with paracetamol, there was increase in TSP, though not significant indicating the hepatoprotective activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration.

γ-glutamyl transferase is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ- glutamyl transferase is a hepatic microsomal enzyme. Serum γ- glutamyl transferase is most useful in the diagnosis of liver diseases.Changes in γ-glutamyl transferase is parallel to those of amino transferases. The acute damage caused by paracetamol increased the γ-glutamyl transferase level but the same attains the normal after Sphaeranthus indicus L treatment due to its antioxidant activity.

Acute administration of paracetamol produced a marked elevation of the serum levels of SGOT, SGPT, ALP, ACP, total proteins and significant reduction in serum albumin in treated animals (Group II-V) when compared with control group (Group I). Treatment with Sphaeranthus indicus at a dose of 200 and 300 mg kg-' significantly reduced the elevated levels of the enzymes.

Treatment with Sphaeranthus indicus decreased the serum levels of ALT, AST towards near normal values which is an indication of stabilization of plasma membrane as well as repair of hepatic tissue. Likewise, serum total cholesterol, triglycerides and low density lipoprotein levels were significantly increased in paracetamol treated group. This biochemical aberration was corrected in animals that were administered both paracetamol and aqueous extract of Sphaeranthus indicus. Further work is therefore needed to elucidate the precise mechanism of action of the plant on fat breakdown. Similarly,

the significant reduction observed in serum values of High Density Lipoproteins (HDL-cholesterol) in paracetamol treated rats was elevated in experimental animals that received both the extract and overdose of paracetamol. The alteration in lipid profiles might result from accumulation of triglycerides, inhibition of bile acid synthesis from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels [35]. Therefore, suppression of cholesterol levels by the extract suggests that the bile acid synthesis was reversed.

CONCLUSION

In conclusion, the results of the present study suggest that *Sphaeranthus indicus* has a potent hepatoprotective action upon paracetamol- induced liver toxicity in rats. The hepatoprotective effect of *Sphaeranthus indicus* can be correlated directly with its ability to reduce activity of serum enzymes. The findings of this study suggest that S. indicus can be used as a safe, cheap and effective alternative chemopreventive and protective agent in the management of liver diseases.

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