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Protective role of sphaeranthus amaranthoides on hepatic marker enzymes

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Protective role of sphaeranthus amaranthoides on hepatic marker enzymes

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Abstract - The ethanolic extract of *Sphaeranthus amaranthoides* at an oral dosage 500mg/kg body weight exhibited a significant protection against D-galactosamine induced liver damage. *Sphaeranthus amaranthoides* showed hepatoprotective activity by reducing a D-galactosamine induced alteration in biochemical changes, that was evident by examination of the levels of hepatic marker enzymes. The plant extract may interfere with free radical formation or it may exert antioxidant activity which may result in hepatoprotective action.

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I. Introduction

Liver, a vital organ of the body plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. The liver has a paramount importance in the maintenance and regulation of the homeostasis of the body. Liver can also detoxify the xenobiotics and antibiotics(Ahsan et al., 2009). Any hepatic damage leads to the distortion of these metabolic functions. Unfortunately, the conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes have serious side effects on the other organs. Herbal drugs or their extracts are prescribed for treatment of liver diseases widely, though their biological active compounds are unknown (Gupta et al., 2005). Herbal drugs speed up the natural healing process of liver. Thus growing interest on the herbal plants continues due to their effectiveness and safety in treatment of liver diseases.

The plant sphaeranthus amaranthoides (asteraceae) is called as sivakaranthai in Tamil. It is weed in paddy feild. This plant reported to possess a variety of medicinal properties(swarnalatha et al 2009). Sphaeranthus amaranthoides posses the antimicrobial activity, anti dioarrheal activity(swarnalatha et al 2009). *Sphaeranthus amaranthoides* also showed wound healing acativity (swarnalatha et al 2009).

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Anticancerous activity of sphaeranthus amaranthoides was reported from the whole plant (swarna latha et al 2011). The phytochemical analysis of ethanol extracts of the plant revealed the presence of flavonoides, phenolics, tannins, steroids and glycosides(swarna latha et al 2009). The current pharmcological study determines the efficacy of hepatoprotective activity of *Sphaeranthus amaranthoides* againest D-galactosamine induced hepatotoxicity. Hepatitis induced by D-galactosamine (D-galn) have been reported to show many metabolic and morphological changes in the liver of experimental animals which is similar to the viral hepatitis. The mode of action of is attributed to peroxidation of endogenous lipid and loss of plasma lipid membrane integrity(Ananham *et al* 1998, Kucera *et al* 2006).

II. Materials and Methods

a) Plant material

The plant *Sphaeranthus amaranthoides* was collected from the Sengottai, Tirunelveli, Tamil Nadu, India. The plant material was identified and authenticated by Mr. V.Chelladurai, Retired Research officer-botany, Central Council For Research In Ayurveda and Sidha (C.C.R.A.S). Govt. of India, Tirunelveli. The Collected plant material was free from diseases and also free from contamination of other plants.

b) Preparation of Plant extract

About 1 Kg of powdered material was taken in a clean, flat bottomed glass container and Soaked in petroleum ether to remove the pigments. Then the plant material is transferred into 2.75lt of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper no.42. The filtrate (ethanol extract) obtained was evaporated using rotary evaporator under reduced pressure. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol. The extract was transferred to a closed container for further use and protection. The extract obtained was 14 %(w/w) of dry powder. This extract is given to the rats by mixing in a Tween 80.

c) Experimental animals

The experimental animals were divided into four groups of six animals each. Group 1 served as control, group II animals were intraperitoneally injected with D-galn (500mg/kg, dissolved in saline for 1day) for induction of hepatitis as described by Deaciue *et. al*., (1993). Group III animals were pretreated with plant extract alone for 21 days(500mg/kg). Group IV animals were orally pretreated with plant extract(500mg/kg per day, for 21 days, dissolved in Tween 80) and then intraperitoneally injected with D-galn (500mg/kg per day) for one day.

d) D-galactosamine induced hepatotoxicity

D-galactosamine was obtained from SRL laboratories. Animals of the test groups were given the plants extracts in Tween 80 prior to the administration of D-galactosmine for 21 days. The control group received normal diet alone The biochemical parameters were determined 24 h after the D-galactosamine challenge or administration

e) Assessment of liver function

Rats of all groups were anaesthetetized by diethyl ether, 24h after the administration of hepatotoxin. The blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and analyzed for various biochemical parameters:

Alanine aminotransferatse, aspartate amino-transferase and acidific transferase were assayed by the method of (king J 1965) he enzyme activity was expressed as μ moles ofpyruvate liberated per min/mg protein. ALP was assayed by the method of (Reitman S and Frankel SA, 1957). The enzyme activity is expresed as IU/l for plasma and μmoles of phenol liberated per min/mg protein for tissue LDH was assayed according to method of Rosalki and Rau (32), and its activity is expressed as IU/l for plasma and μmoles of pyruvate liberated per min/mg protein for tissue. The assay of GGT was carried out by the method of (Malloy E and Evelyn K, 1987)

f) Statistical analysis

Results of the biochemical estimations are reported as mean S.E.M.Total variation, present in a setof data was estimated by one-way analysis of variance (ANOVA), Student's t-test was used for determining significance (Woolson,1987).

III. Results and Discussion

Intraperitoneal administration of D-galacto-samine led to significant increse the serum enzymes level by 2-3 fold as compared to the normal control group ($p<0.001$). The results for hepatoprotective marker enzymes are shwon in the table. Pretreatment of the rats with ethnolic extract of *sphaeranthus amaranthoides* at 500mg/kg body weight induced significant decrease in the serum enzymes levle($P<0.001$),($P<0.01$).when compared with D-GalN treated rats.

The liver marker enzymems (ALT, AST, ACP, ALP, GGT and LDH) are cytoplasmic in nature upon injury these enzymes enter in to the circulatory system due to the altered permeability of membrane (Zimmerman and Seeff, 1970). D-GalN is a well-established hepatotoxicant that induces a diffuse type of liver injury morphologically and functionally closely resembling human viral hepatitis (Decker and Keppler 1972). GalN causes the hepatic injury with spotty hepatocyte necrosis and marked elevation portal and parenchymal infiltration (Keppler and Decker, 1969). Galactosamine also causes depletion of uridine diphosphate (UDP) by increase the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration(Decker et al 1973, Elmofty et al 1975). This results in the disruption of the plasma membrane causing leakage of the enzymes from cell, (Naik and Panda, 2007) which leads to elevation in serum levels of ALT, AST, ACP, ALP, GGT and LDH is consistent with a number of earlier reports (Muntae et al 2000, Wills and Asha 2006 and Zhou et al 2008). The increase in plasma LDH level indicates the necrosis of hepatocytes (Quintero et al 2002). Further intense galactosamination of membrane structure is thought to be responsible for loss of activity of ionic pumps. The impairment in the calcium pump with consequent increase in the intracellular calcium is considered to be responsible for cell death(Tsai et al., 1997).

The hepatocellular damage leads to the raise in the ALT which is followed by AST raise in the serum (Rao et al., 1989). It has been shown that pretreatment with plant extract exerts its protective action against D-GalN induced liver injury by impairment of D-GalN mediated lipid peroxidation, either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself (Naik and Panda, 2007). In the present study the structural integrity of the hepatocellular membrane was preserved by *sphaeranthus amaranthoides* as evidenced by the decrease in the markder enzyme lelves when compared with the D-GalN treated rats.. This concludes that the crude ethanolic extracts of *sphaeranthus amaranthoides* showed a potent protective effect on D-galN induced acute liver toxicity.

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Effect of *S.amarathoides* on the levels of hepatic marker enzymes

Parameters	Group I Control	Group II D-GalN intoxicated	Group II *S.amaranthoides* treated	Group IV D-GalN+ *S.amaranthoides* treated
ALT	114.2 ± 12.9	274.4 ± 26.9***	115.9 ± 12.31^{NS}	138.2 ± 12.9***
AST	86.9 ± 9.1	189.9 ± 19.3***	86.8 ± 9.10^{NS}	95.4 ± 9.23***
ACP	14.9 ± 2.3	36.5 ± 2.9***	15.1 ± 1.09^{NS}	16.6 ± 2.31***
ALP	110.5 ± 9.9	226.2 ± 22.7***	110.4 ± 9.17^{NS}	117.8 ± 9.91***
GGT	6.1 ± 0.29	11.20 ± 0.78***	6.21 ± 0.48^{NS}	7.27 ± 0.49***
LDH	220.0 ± 22.1	378.8 ± 36.7***	222.1 ± 18.9^{NS}	227.4 ± 24.9

Values are expressed as mean ± SD (six animals in each group).
As compared with respective controls (comparisons are made between Group II and Group I; Group III and Group I, Group IV and Group II) statistical analysis by *students t-test* ***P<0.001, ^{NS}–Non significant.
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