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Hepatoprotective and antioxidant effect of *Sphaeranthus indicus* against acetaminophen–induced hepatotoxicity in rats.

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Abstract
The flower heads of *Sphaeranthus indicus* Linn (Asteraceae) a traditional Indian medicinal plant is commonly used to nourish and improve the liver conditions. This study was designed to evaluate the hepatoprotective and antioxidant effect of aqueous (AQS) and methanolic (MES) extract of flower heads of *Sphaeranthus indicus* on Acetaminophen (APAP)-induced heptotoxicity in rat's *in-vivo*. Activities of liver marker enzymes ,glutamate-oxaloacetate transaminase (SGOT) glutamate pyruvate transaminase (SGPT), acid phosphatase (ACP) and alkaline phosphatase (ALP) bilirubin and total protein at an oral dose of MES (300mg/kg) showed a significant hepatoprotective effect in comparison with the same dose of aqueous extract. This fact was also confirmed by studying the liver histopathology of treated animals. As Regards the antioxidant activity, MES exhibited a significant effect showing increasing levels of superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxides (GP_X) by reducing malondialdehyde (MDA) levels.

Keywords: Acetaminophen, antioxidant activities, hepatocellular damage, hepatoprotection, *Sphaeranthus indicus*

INTRODUCTION

The pharmaceutical imbalance between remedies that protect the liver and have antioxidant properties and drugs that induce hepatotoxicity has prompted and accelerated research into plants used in folk medicines to treat liver diseases and boost liver functions.

Sphaeranthus indicus Linn. (Asteraceae) commonly in Hindi known, as Gorakhmundi is an annual spreading herb, distributed through out the plains and wetlands of India, Sri Lanka and Australia [1, 2]. The entire plant is reportedly used in the ayurvedic system of medicines in the treatment of epilepsy and mental disorders [3]. It reportedly used to cure piles, hepatitis [4] and have protection against immunosupression [5]. Literature reports on the ariel parts of this plant revealed the presence of an essential oil glucosides, and eudesmanoids[6] an alkaloid sphaeranthine and an isoflavone 5,4'-dimethoxy-3'-prenylbiochanin 7-o-β-galctoside with some interesting sesquiterpene[8,9,10] and a new flavone glycoside[11] from the stem have been isolated from this herb. In this study, we have investigated the ability of flower heads extracts of *Sphaeranthus indicus* to protect liver against acetaminophen induced hepatocellular damage and oxidative stress in rats *in-vivo.*

MATERIALS AND METHODS:

Plant materials: Dried flower heads of *Sphaeranthus indicus* (SI) were procured from local drug market of Meerut U.P, India and were identified by Dr. H.B Singh, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, India. A voucher specimen was deposited at the herbarium of our Pharmacognosy laboratory.

Extraction: Flower heads was shade dried for a week and powdered mechanically (Sieve No. 10/44). About 250g of the powder was thougherly extracted with methanol for 36h using soxhlet apparatus. The solvent was distilled off at low temperature under reduced pressure using rotary vacuum evaporator (Buchi flawil Switzerland). The yield was 22.5% w/w. Another 250g of powdered material was completely extracted in boiling distilled water for

30min. kept for 3 days with intermittent shaking filtered and concentrated using rotary vacuum evaporator to obtain the aqueous extract. The yield was 20.9% w/w. The residue of both extracts made into a suspension in water and propylene glycol (4:1) containing Tween-80 (0.08%) at the concentration of 200mg/ml separately.

Animals: Thirty pathogen free male albino rats (four weak) of either sex weighing 180±20g respectively were used for the study. They were housed in specific standard laboratory conditions and were kept in temperature 25±2^{0}C control environment, in a relative humidity 55-62%, with regular 12h light/12h dark cycle. All animals were fed with standard rat chow diet, water *ad libitum* and received humane care in accordance approval of Institutional ethics committee rules.

Hepatoprotective activity: Rats were divided into five groups, with six animals in each group. Group I, the normal control group animals were administered p.o., a single daily dose of 0.5% Tween-80 (1ml) on all five days. Group II, the APAP control animals were administered a single daily dose of 0.5%Tween 80 (1ml) p.o., on all the 5 days and on second and third day they were administered APAP (2g/kg p.o.,). Group III and IV animals were administered AQS and MES suspensions respectively (300mg/kg p.o.,) on all five days and a single dose of APAP (2g/kg p.o.,) on days second and third, 30 min after of each extracts. Group V animals were administered Silymarin, the known hepatoprotective compound (Sigma Chemical Company USA), at a dose of 100mg/kg p.o., on all 5 days and single dose of APAP (2g/kg p.o.,) on days 2 and 3, 30 min after silymarin administration. The blood was withdrawn through retro-orbital plexus of rats on 5th day. Microscopic observation of liver was also done.

Assessment of antihepatotoxic activity:

Assessment of antihepatotoxic activity was done by determining SGPT and SGOT enzyme activity. The enzyme assay was carried out by Reagent Kits maintained by Biocon India Ltd Bangalore and the procedures were essentially those described in the literature available with kits. Estimations were made on Auto-analyser; Reitman and Frankel method (1957) was used for determining the enzyme activity in the supernatant of various groups.

On fifth day, animals were sacrificed and blood was collected directly through retro-orbital plexus serum was separated after coagulating at 37^{o}C for 30 min and centrifuging at 1200–1500 rpm for 15–20 min. Serum was analyzed for various biochemical parameters, i.e. SGOT, SGPT ACP,ALP SB and total protein [12, 13, 14,15]

Determination of antioxidant enzyme activity: The liver was perfused with 0.86% cold saline to remove all the red blood cells. Then it was suspended in 10% (w/v) ice cold 0.1M phosphate buffer (pH 7.4) cut into small pieces, and required quantity was weighed and homogenized using a Teflon homogenizer. The homogenate was used for estimation of enzymic and non-enzymic antioxidants like SOD, CAT, GPx, [16, 17, 18] and lipid peroxidation level [19].

Statistical analysis: All Data were expressed as mean S.D. and analyzed with one-way analysis of variance (ANOVA). Dunnett's t test was used to calculate statistical significance. $P<0.05$ and $P<0.01$ were considered statistical significance using SPSS software

Table.1: Effect of Sphaeranthus indicus flower heads on- Acetaminophen induced heptotoxicity in Rats

Treatment	SGPT (U/mL)	SGOT (U/mL)	ACP (KA unit)	ALP (KA unit)	Bilirubin (mg/dl)		Total protein
					Total	Direct	
Normal Control	34.41± 6.12	45.21± 8.15	4.80±.0.41	10.77± 0.44	0.59± 0.01	0.25±0.01	5.46± .24
Toxic control (APAP)	128.44± 7.14*	115.23± 61.10*	11.46± 0.79*	32.73±0.84*	2.49±0.08*	0.82±0.03*	1.97±0.67*
AQS (300mg/kg) + APAP	87.42± 19.21Ψ	98.41± 34. 01Ψ	10.44± 1.62Ψ	26.42± 3.97Ψ	1.88±0.02Ψ	0.51± 0.00Ψ	4.44±0.73Ψ
MES (300mg/kg) + APAP	43.11± 7.72Ψ	62.48± 18.11Ψ	14.11± 1.24Ψ	12.16± 4.34Ψ	0.63± 0.01Ψ	0.32± 0.01Ψ	5.91±0.36Ψ
Silymarin (100mg) + APAP	39.11± 5.71Ψ	46.94± 10.91Ψ	5.61± 0.40Ψ	9.21±1.14Ψ	1.01±0.01Ψ	0.24±0.01Ψ	5.98±0.54Ψ

Values were expressed as mean ± S.D of six animals in each group statistical analysis ANOVA followed by Dunnet *t*-test.
* $P<0.05$ as compared with group 1
Ψ $P<0.05$ as compared with group 2

Table.2 Effect of *Sphaeranthus indicus* flower heads on liver antioxidant enzymes and lipid peroxidation Acetaminophen intoxicated rats

Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
Normal Control	3.09 ± 0.34 [a]	38.40± 4.01[a]	51.40 ± 2.05[a]	1.43 ± 0.20[a]
Toxic control (APAP)	1.76 ± 0.17	20.64± 1.24	32.07 ± 1.54	2.78 ± 0.15
AQS Extract (300mg/kg) + APAP	2.54 ± 0.23[b]	27.11± 2.68[b]	36.79 ± 2.40[b]	1.92 ± 0.26[b]
MES extract (300mg/kg) + APAP	2.83 ± 0.18[b]	34.98± 1.73[b]	45.98 ± 4.95[b]	1.52 ± 0.29[b]
Silymarin (100mg/kg) + APAP	3.06 ± 0.71[b]	39.48± 1.79[b]	51.94 ± 5.04[b]	1.34 ± 0.14[b]

Values were expressed as mean ± S.D, (n=6) statistical analysis ANOVA followed by Dunnet *t*-test.
a. Significantly different from the control Group (P<0.01)
b. Significantly different compared with Acetaminophen -intoxicated group (P<0.05)
[AQS] Aqueous extract of *Sphaeranthus indicus*
[MES] Methanol extract of *Sphaeranthus indicus*

RESULTS: Serum activities of transaminases, SGPT, SGOT and ACP, ALP, serum bilirubin and total protein are given in Table.1 where the single dose of APAP significantly elevated SGPT, SGOT activities when compared to normal animals. (Table.1).

Treatment of AQS & MES (300mg/kg) extracts 1h prior to APAP administration significantly protected the elevation of marker enzymes, serum bilirubin and ACP and ALP activities. Reduced activities of enzymic and non-enzymic antioxidants and enhanced activities of lipid peroxidation were seen in the APAP-treated group (Table.2), whereas standard silymarin and the drug treated group showed significant ($p<0.01$) rise in antioxidant level with reduction in lipid peroxidation level when compared with the standard drug, silymarin (Table. 2).

Histopathology of Group II animals shows patches of liver cell necrosis with inflammatory collections around central vein where as drug treated group showed absence of cell necrosis but with minimal inflammatory conditions around the central vein. The MES (300mg/kg, p.o)-treated group showed minimal inflammatory conditions with near normal architecture possessing higher hepatoprotective action (Figure C).

DISCUSSION: In the present study the methanolic extract of *Sphaeranthus indicus* was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in liver function markers and also serum bilirubin concentrations and by preventing liver histopathological changes in rats induced with

hepatotoxicity. Moreover, the methanolic extract of *Sphaeranthus indicus* enhanced the activities of antioxidant enzymes (SOD, CAT, and GPx) and diminished the amount of lipid peroxides against acetaminophen-induced hepatotoxicity in these animals, suggesting the reduction of oxidative stress in this scenario plays a role in mechanism of its hepatoprotective effect. Acetaminophen at therapeutic doses is primarily metabolized and detoxified by glucuronidation and sulphation and subsequently followed by renal excretion [20]. However when acetaminophen is taken in a toxic doses, the compound is converted to a toxic form N-acetyl-p-benzo-quinone imine (NAPQI). This is an electrophilic intermediate, oxidized by cytochrome P_{450} and converted to a highly reactive and toxic metabolite as in the case of acetaminophen over dose [21]. NAPQI can rapidly react with the glutathione (GSH) and lead to a 90% total hepatic GSH depletion in the cells and mitochondria, which can result in hepatocellular death and mitochondrial dysfunction [22]. In addition NAPQI can increase the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide, and nitro oxide and peroxynitrite, respectively. Excess level of ROS and RNS can attack biological molecules such as DNA, protein and phospholipids, which leads to lipid peroxidation, nitration of tyrosine, and depletion of anti oxidant enzymes (SOD, CAT, GPx) that further results in oxidative stress[23]. NAPQI can also induce DNA stand breaks and promote apoptosis and necrosis in

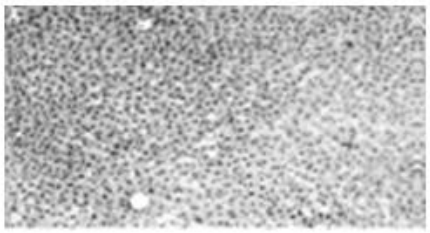

Figure A: Normal Control

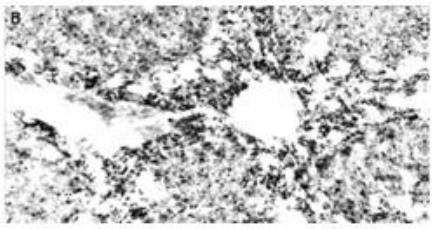

Figure B: Toxic Control

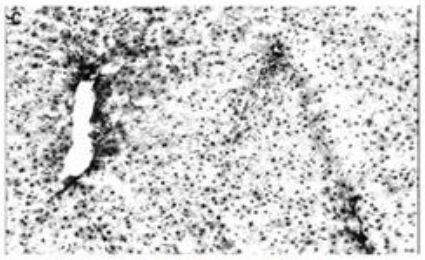

Figure C: MES Treated

Figure D: AQS Treated

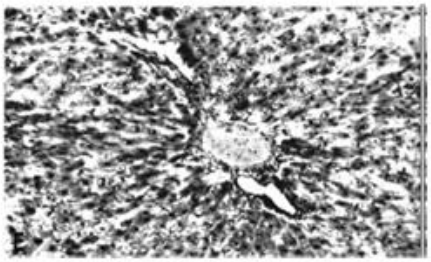

Figure E: Silymarin Treated

acetaminophen-induced heptotoxicity [24]. In the present study, the data suggested that high dosage of acetaminophen in the liver could lead to decreased level of anti oxidant enzymes (SOD, CAT, GPx) and present a significant level of hepatotoxicity in the course of treatment. However, the methanol extract of *Sphaeranthus indicus* could raise the level of SOD, CAT, and GPx against the -induced oxidative stress mediated by ROS and RNS. Furthermore, the level of MDA was increased in the group receiving acetaminophen administration, but pretreatment with the methanol extract of *Sphaeranthus indicus* reduced the amount of MDA. This result indicated the decreasing the formation of lipid peroxidation is also one of the event of preventing the oxidative toxicity by acetaminophen. In conclusion the present study has demonstrated that methanolic extract of *Sphaeranthus indicus* has hepatoprotective effect against acetaminophen-induced hepatotoxicity in rats. Interestingly the more active hepatoprotective compound of *Sphaeranthus indicus* appears to exist in methanolic extract and not more in aqueous fraction, which could include flavonol and flavonoid .The enhanced level of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanism of *Sphaeranthus indicus* methanolic extract in preventing the development of liver damage induced by acetaminophen.

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