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Research Article

COMPARITIVE STUDY OF HEPATOPROTECTIVE ACTIVITY OF *ACANTHOSPERMUM HISPIDUM* PLANT EXTRACT AND HERBAL NIOSOMAL SUSPENSION AGAINST ANTI-TUBERCULAR DRUG INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: Compare the hepatoprotective activity of *Acanthospermum hispidum* ethanolic extract (AHEE) and herbal niosomal suspension (HNS) against hepatotoxicity.

Methods: AHEE and HNS were investigated against hepatotoxicity produced by administering a combination of four anti-tubercular drugs (ATDs) isoniazid (27 mg/kg), rifampin (40 mg/kg), pyrazinamide (66 mg/kg), and ethambutol (53 mg/kg) for the period of 28 days by oral route in rats. AHEE (400 mg/kg) and HNS (400 mg/kg) were administered along with 1 hr prior administration of ATDs once daily to five groups (six animals per group) of Albino Wistar rats weighing about 150-200 g. Silymarin was used as a standard drug (100 mg/kg p.o.). Liver biomarkers such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin, and total protein were elevated indicating the induction of hepatotoxicity in experimental animals.

Results: The AHEE and HNS prevented the hepatotoxic effects of the combination of isoniazid, rifampin, pyrazinamide, and ethambutol on the above serum parameters. Histopathological studies of liver and liver biomarker estimations also supported the hepatoprotective effect of AHEE and HNS.

Conclusion: The protective effect of HNS was found to be significant when compared to standard and AHEE.

Keywords: Hepatoprotective, *Acanthospermum hispidum*, Isoniazid, Silymarin, Herbal niosomal suspension.

INTRODUCTION

Hepatotoxicity as injury to the liver that is allied with diminished liver function. The drug-induced liver injury may account for as many as 10% of hepatitis cases in overall adults, in adults over 50-year-old hepatitis case 40% and 25% cases of severe liver failure. A higher risk of hepatotoxicity has been reported in Indian patients than in their Western counterparts [1,2]. The risk of hepatotoxicity based on data from four prospective Indian studies was 11.5% compared with 4.3% in Western publications [3]. In Western countries, paracetamol represents the first cause of all liver failures, but it accounts only for 25-40% cases of fulminant hepatic failure [4]. Anti-tubercular drugs (ATDs) are the second common cause of drug-induced hepatotoxicity. AT drugs are the commonest agents causing serious, clinically significant drug-induced acute liver failure in India. The most common used ATDs such as Isoniazid, rifampicin, pyrazinamide, and ethambutol are reported to be hepatotoxic [5-13]. The underlying mechanism of ATD-induced hepatotoxicity and the factors predisposing to its development are not clearly understood [14].

Acanthospermum hispidum, (family Compositae) commonly known as Palleru (Telugu) is found in India. The plant is used in traditional medicine for the treatment of constipation, fever, jaundice, malaria, stomachache [15], and viral infections [16]. The plant has been reported for its hepatoprotective activity [17], antimicrobial activity [18], antiplasmodial activity [19], antidiarrheoal activity [20], antitumor activity [21], antidiabetic activity, and anthelmintic [22]. So, the present study was aimed at formulating a niosomal suspension of *A. hispidum* ethanolic extract (AHEE) and comparing its hepatoprotective effect with AHEE and Silymarin (standard drug) in ATD-induced hepatotoxicity in rats.

METHODS

Drugs

Silymarin was procured from Sigma-Aldrich Pvt. Ltd, (India). Isoniazid, rifampicin, pyrazinamide, and ethambutol were procured from Lupin Ltd. India and all other chemicals and reagents used were of analytical grade, procured from standard deviation fine chemicals Ltd, (India). Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, and total protein estimation kits were procured from Kamineni Life Sciences Pvt. Ltd. (India).

Animals

Albino Wistar rats of both the sexes (150-180 g) obtained from animal house of Sree Vidyanikethan College of Pharmacy were used. The animals were housed under standard environmental conditions (22±5°C with 12 hrs of light/dark cycle) and fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum.* All animal experimental protocols were approved by Institutional Animal Ethical Committee (SVCP/IAEC/I-020/2013-2014).

Plant material

The plant *A. hispidum* was collected from Tirumala hills, Andhra Pradesh, India. The taxonomical identification and authentication of the plant was done by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S. V. University, Tirupathi, Andhra Pradesh, India. The voucher specimen was deposited at the department for future reference. The aerial parts of the plant were dried under the shade, powdered and passed through 40-mesh sieve.

Extraction of plant material

The 500 g of dried powder was extracted in soxhlet apparatus using ethanol as a solvent. The extract was concentrated on a rotary flash

evaporator to semisolid consistency and then dried over a water bath. The yield of the extract obtained was 72 g.

Preparation of niosomal suspension

Herbal niosomes are prepared by ether injection method using nonionic surfactant and cholesterol. Cholesterol and surfactant were dissolved in 10 ml diethyl ether mixed with 10 ml ethanol containing weighed quantity of herbal extract as tabulated in table. The resulting solution was slowly injected using syringe at a rate of 1 ml/minute into 20 ml of hydrating solution phosphate buffer (pH 7.4). The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60-65°C. As the lipid solution was injected slowly into the aqueous phase, the differences in temperature between phase cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes [23,24].

Acute toxicity studies

Acute toxicity studies were performed for AHEE and herbal niosomal suspension (HNS) according to OECD guidelines 423. Ten mice were selected for the study and oral administration of AHEE and HNS at a dose of 5, 50, 300, 2000 mg/kg given at 48 hrs interval simultaneously. In this toxic study, animals were observed for any changes in consumption of food and water, body weight, behavioral changes, and mortality rates [25,26].

Study protocol

Hepatotoxicity was induced by using isoniazid (H) (27 mg/kg, p.o), rifampicin (R) (40 mg/kg, p.o), pyrazinamide (Z) (66 mg/kg, p.o) and ethambutol (E) (53 mg/kg, p.o) for 28 days and silymarin (100 mg/kg, p.o) was used as the standard. The oral doses of ATDs were extrapolated from daily human dose using the conversion table based on body surface area [27].

Experimental procedure

Experimental animals were randomly divided into 5 groups, each group containing 6 animals and the treatment schedule for 28 days as follows.

Group I: Control (0.9% normal saline 1 ml/kg, p.o),

Group II: Toxic control (ATDs - H, R, Z, E, p.o.),

Group III: Silymarin (100 mg/kg, p.o) + administration of ATDs after 1 hr,

Group IV: Ethanolic extract of *A. hispidum* (400 g/kg, p.o) + administration of ATDs after 1 hr and

Group V: HNS (400 g/kg, p.o) + administration of ATDs after 1 hr. On 29th day, blood is collected for estimation of the liver biomarker enzymes. On an equivalent day, the liver is removed and keeps in 10% formalin solution for processing of histopathological studies.

Estimation of biochemical parameters

SGOT and SGPT were estimated by Reitman and Frankel method; ALP was estimated by kind King's method. Total bilirubin and total protein were estimated by Jendrassik and Grofs method and cholesterol oxidase/peroxidases method, respectively [28-31].

Histopathological studies

The livers from rats were fixed in 10% neutral formalin solution, dehydrated in alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analyses.

Statistical analysis

The results are presented as mean ± standard error of mean (n=6 in each group). Analyses were performed using One-way ANOVA followed by Dunnett's multiple for the difference between the control and treatment groups.

RESULTS

Acute toxicity studies

The ethanolic extract of *A. hispidum* and HNS were found to be safe since no animal died even at the dose of 2000 mg/kg when administered orally, and the animals did not show any gross behavioral changes.

Biochemical parameters

Animals treated with ATDs (toxic control) showed significantly elevated levels of SGOT, SGPT, ALP, total bilirubin, and total protein levels when compared to control group. *A. hispidum* and HNS 400 mg/kg given with 1 hr prior administration of ATDs showed significant decreased serum diagnostic liver enzymes when compared to toxic control (Table 1).

Histopathological studies of liver

Hepatic control group animals showed significant liver cell necrosis compared to normal control group. HNS 400 mg/kg showed protective effect on the hepatocellular necrosis and their lobular structure was normal when compared to *A. hispidum* extract (Figs. 1-5).

DISCUSSION

Hepatotoxicity of ATDs is a serious adverse drug reaction because it causes significant morbidity and mortality. Isoniazid, rifampicin, pyrazinamide, and ethambutol are potentially hepatotoxic, when given

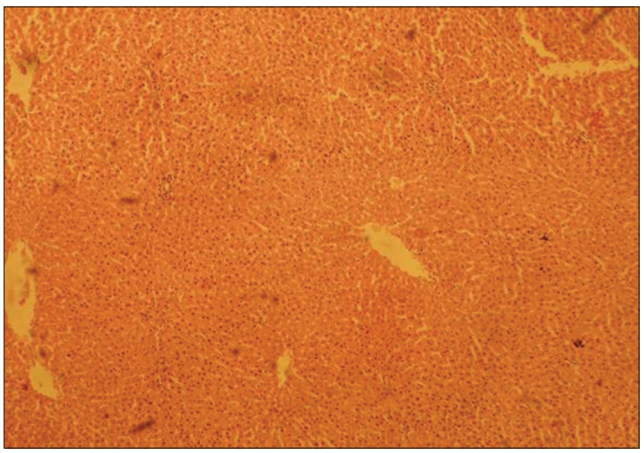

Fig. 1: Control (normal saline 1 ml/kg)

Table 1: Estimation of biochemical parameters in rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total blilirubin (mg/dl)	Total protein (mg/dl)
Control	35.02±0.0654	36.87±0.3180	45.20±0.3416	0.3325±0.0090	5.283±0.1078
Toxic control (ATDs)	171.8±2.027$^{\#}$	183.6±0.888$^{\#}$	122.8±0.8891$^{\#}$	1.257±0.0117$^{\#}$	3.30±0.10
Silymarin+ATDs	83.92±0.2386***	82.62±0.7947**	93.90±0.7765***	0.438±0.0069**	4.252±0.0166***
Plant extract (400 mg)+ATDs	124.2±1.356*	139.3±0.9062*	134.1±0.5498**	0.640±0.0088*	3.385±0.0133*
Herbal niosomal suspension (400 mg)+ATDs	112.6±2.823**	72.08±0.8076**	156.1±1.427***	0.399±0.0036*	4.642±0.0153**

Data are expressed as mean±SEM (n=6), One-way ANOVA Dunnet's multiple comparison tests; $^{\#}$p≤0.05 versus control (Group I); *p≤0.05 versus toxic control (Group II); **p≤0.01 versus toxic control (Group II); ***p≤0.001 versus toxic control (Group II), SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase, SEM: Standard error of the mean, ATDs: Anti-tubercular drugs

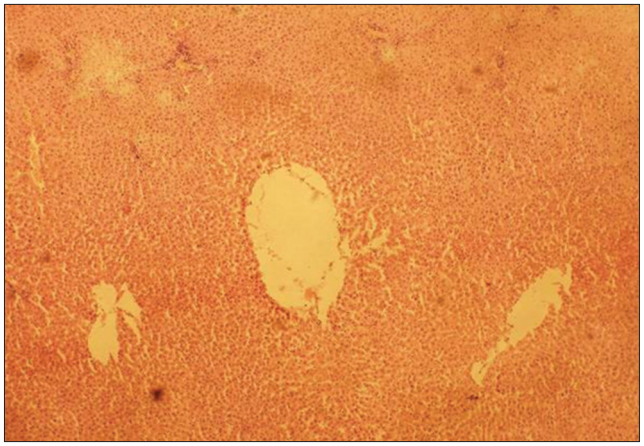

Fig. 2: Toxic control (anti-tubercular drugs)

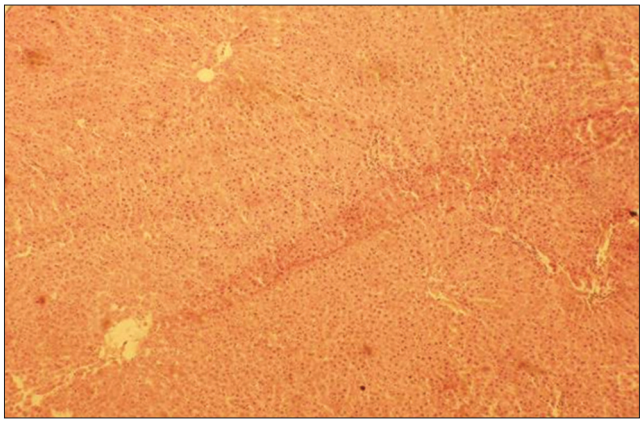

Fig. 3: Standard (silymarin-100 mg/kg) + anti-tubercular drugs

in combination their toxic effects are increased [32]. In the present study, the combination of ATDs was used to induce hepatotoxicity in rats [12].

As shown in Table 1, daily administration of ATDs (HRZE) for 28 days result in hepatic injury as confirmed by elevated levels of serum diagnostic enzymes such as SGOT, SGPT, and ALP levels. In toxic control animals observed depletion may be due to increased utilization of ATDs. At the time of hepatic injury, these enzymes leak out from liver into the blood circulation due to liver tissue damage. The treatment of AHEE and HNS, the levels of these liver marker enzymes in serum were near to normal, this may be a consequence of the stabilization of plasma membrane, as well as repair of hepatic tissue damage caused by ATDs. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increases the bilirubin release [33]. The treatment of AHEE and HNS restored the level of bilirubin to near normal may be due to the inhibitory effect on mitochondrial enzymes responsible for the metabolism of ATDs. ATDs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of the hepatic antioxidant defense system. The depletion of antioxidant defenses or rise in free radical production deteriorates the pro-oxidant, antioxidant balance, leading to oxidative stress-induced cell death.

Histopathological observation shows AHEE and HNS have reduced heavy hemorrhage and hepatocellular necrosis. The treatment with AHEE and HNS normalized the ATDs induced histopathological changes, therefore, it is suggested that hepatoprotective activity of AHEE and HNS against ATDs induced hepatotoxicity might be due to its property of reducing oxidative stress.

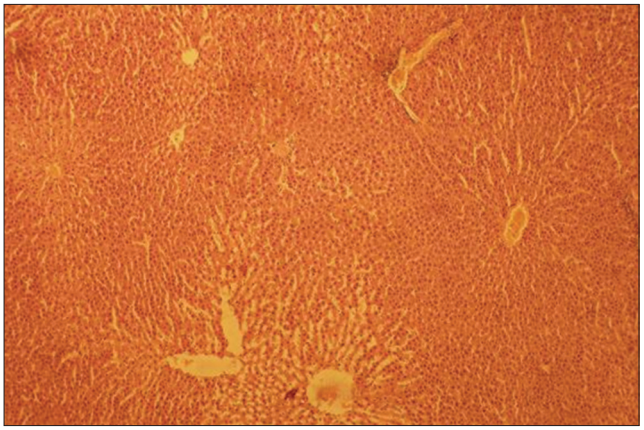

Fig. 4: Group VI - (*Acanthospermum hispidum* ethanolic extract 400 mg/kg) + anti-tubercular drugs

Fig. 5: Group V - (herbal niosomal suspension 400 mg/kg) + anti-tubercular drugs

CONCLUSION

Results obtained from the analysis of antioxidant parameters, biochemical parameters, and histopathological studies; it is clear that AHEE and HNS show hepatoprotective activity at the dose of 400 mg/kg as compared to toxic control. Compared to AHEE - HNS show better results in the management of drug-induced liver toxicity.

REFERENCES

1. Singh J, Garg PK, Tandon RK. Hepatotoxicity due to antituberculosis therapy. Clinical profile and reintroduction of therapy. J Clin Gastroenterol 1996;22(3):211-4.
2. Dutt AK, Moers D, Stead WW. Short-course chemotherapy for tuberculosis with mainly twice-weekly isoniazid and rifampin. Community physicians' seven-year experience with mainly outpatients. Am J Med 1984;77(2):233-42.
3. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. Chest 1991;99(2):465-71.
4. James LH. Drug induced liver disease. Adv Gastroenterol 2000;84(5):1275-312.
5. Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha DR, Dhali GK. Mitochondrial oxidative stress and permeability transition in isoniazid and rifampicin induced liver injury in mice. J Hepatol 2006;45(1):117-26.
6. Pal R, Rana SV, Vaiphei K, Singh K. Isoniazid-rifampicin induced lipid changes in rats. Clin Chim Acta 2008;389(1-2):55-60.
7. Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. Respirology 2006;11(6):699-707.
8. Tahaoglu K, Ataç G, Sevim T, Tärün T, Yazicioglu O, Horzum G, *et al.* The management of anti-tuberculosis drug-induced hepatotoxicity. Int J Tuberc Lung Dis 2001;5(1):65-9.

9. Santhosh S, Sini TK, Anandan R, Mathew PT. Hepatoprotective activity of chitosan against isoniazid and rifampicin-induced toxicity in experimental rats. Eur J Pharmacol 2007;572(1):69-73.
10. Harish R, Shivanandappa T. Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*. Food Chem 2006;95:180-5.
11. Khatri A, Garg A, Agrawal SS. Evaluation of hepatoprotective activity of aerial parts of *Tephrosia purpurea* L. and stem bark of *Tecomella undulata*. J Ethnopharmacol 2009;122(1):1-5.
12. Saraswathy SD, Suja V, Prema G. Effect of Liv.100 against antitubercular drugs induced hepatotoxicity in rats. Indian J Pharmacol 1998;30:233-8.
13. Bello B, Wudil AM. Protective Effect of *Allium sativum* against liver injury induced by anti-tubercular drugs in rats. Br J Pharmacol Toxicol 2012;3:89-92.
14. Anand AC, Seth AK, Paul M, Puri P. Risk factors of hepatotoxicity during anti-tuberculosis treatment. Med J Armed Forces India 2006;62:45-9.
15. Mshana NR, Abbiw DK, Addae Mensah I, Adjanohoun E, Ahyi MR, Ekpere JA, *et al*. Traditional Medicine and Pharmacopoeia: Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana. Accra: Organization of African Unity/Scientific, Technical & Research Commision; 2000. p. 101-2.
16. Summerfield A, Keil GM, Mettenleiter TC, Rziha HJ, Saalmüller A. Antiviral activity of an extract from leaves of the tropical plant *Acanthospermum hispidum*. Antiviral Res 1997;36(1):55-62.
17. Edewor TI, Olajire AA, Olaniyan LE. Effect of oral administration of ethanolic leaf extract of *Acanthospermum hispidum on* carbon tetrachloride induced acute liver injury in rats. Res J Med Sci 2007;1(1):39-41.
18. Fleischer TC, Ameade EP, Sawer IK. Antimicrobial activity of the leaves and flowering tops of *Acanthospermum hispidum*. Fitoterapia 2003;74(1-2):130-2.
19. Sanon S, Azas N, Gasquet M, Ollivier E, Mahiou V, Barro N, *et al.* Antiplasmodial activity of alkaloid extracts from *Pavetta crassipes* (K. Schum) and *Acanthospermum hispidum* (DC), two plants used in traditional medicine in Burkina Faso. Parasitol Res 2003;90(4):314-7.
20. Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdurahman EM. Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. J Ethnopharmacol 2005;101(1-3):27-30.
21. Deepa N, Rajendran NN. Anti-tumor activity of *Acanthospermum hispidum* DC on Dalton ascites lymphoma in mice. Natl Prod Sci 2007;13(3):234-40.
22. Roy A. Study on anthelmintic and antidiabetic activity of leaves of *Acanthospermum hispidum* dc. Int J Pharm Chem Sci 2013;2(3):1324-7.
23. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes – non-ionic surfactant vesicles. J Pharm Pharmacol 1985;37(12):863-8.
24. Yadav JD, Kulkarni PR, Vaidya KA, Shelke GT. Niosomes: A review. J Pharm Res 2011;4(3):632-6.
25. OECD Guidelines for the Testing of Chemicals Revised Draft Guideline 423: Acute Oral Toxicity: Paris: Organisation for Economic Co-Operation and Development; 2000.
26. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D. Acute and subacute toxicity studies of *Aegle marmelos* Corr. an Indian medicinal plant. Phytomedicine 2007;14(2-3):209-15.
27. Ghosh MN. Fundamentals of Experimental Pharmacology. Kolkata: Hilton and Company; 2007.
28. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Am J Clin Pathol 1957;28:56-63.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265-75.
30. Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol 1954;7(4):322-6.
31. Mallay HT, Evelyn KA. Estimation of serum bilirubin level with the photoelectric colorimeter. J Biol Chem 1937;119:481-4.
32. Vijaya Padma V, Suja R, Shyamala Devi CS. Hepatoprotetive effect of Liv. 52 on anti-tubercular drug induced hepatotoxicity in rats. Fitoterapia 1998;6:520.
33. Yuen MF, Kato T, Mizokami M, Chan AO, Yuen JC, Yuan HJ, *et al.* Clinical outcome and virologic profiles of severe hepatitis B exacerbation due to YMDD mutations. J Hepatol 2003;39(5):850-5.