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Research Article

SCREENING OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *BOERHAAVIA DIFFUSA* AND *ANISOCHILUS CARNOSUS*

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ABSTRACT

Objective: Alcoholic and aqueous extracts of *Boerhaavia Diffusa* and *Anisochilus Carnosus* (Alc.EBD & Alc.EAC and Aq.EBD & Aq.EAC) were screened for hepatoprotective and antioxidant activity at the doses of 150mg and 300mg/kg body weight of Alc.EBD and Aq.EBD, 200mg and 400mg/kg body weight of Alc.EAC and Aq.EAC.

Methods: Thioacetamide (TAA) induced hepatotoxicity in rats was used to screen hepatoprotective activity. Antioxidant activity was evaluated by DPPH radical scavenging activity, xanthine oxidase inhibition assay and nitric oxide radical scavenging assay.

Results: The extracts shows hepatoprotective and antioxidant activity in a dose dependent manner.

Conclusion: The alcoholic extracts of the plants shows potent activity when compared to an aqueous extracts in both the plants.

Keywords: *Boerhaavia Diffusa, Anisochilus carnosus*, Hepatoprotective, Antioxidant, Thioacetamide, DPPH, Xanthine oxidase, Nitric oxide.

INTRODUCTION

Liver injury may be either acute or chronic. Acute liver injury may present with non-specific symptoms of fatigue and abnormal LFTs, or with jaundice and acute liver failure. Chronic liver injury is defined as hepatic injury, inflammation and/or fibrosis occurring in the liver for more than 6 months. In the early stages patients can be asymptomatic with abnormal LFTs. With more severe liver damage, however the presentation can be with jaundice, portal hypertension or other signs of cirrhosis. Any cause of liver damage can produce acute liver failure, provided it is sufficiently severe. Acute viral hepatitis is the most common cause world-wide, whereas paracetamol toxicity is the most frequent cause in the UK. Acute liver failure occurs occasionally with other drugs, or from Amanita phalloides (mushroom) poisoning, in pregnancy, in Wilson's disease, following shock and, rarely, in extensive malignant disease of the liver. In 10% of cases the cause of acute liver failure remains unknown and these patients often labeled as having non-A-E viral hepatitis or cryptogenic acute liver failure [1].

The term 'exogenous antioxidant' refers to numerous vitamins, minerals and other phytochemicals to protect against the damage caused by reactive oxygen species (ROS). The ROS such as superoxide anion radical, hydrogen radical have been implicated in the pathophysiology of various clinical disorders, including aging, cancer and atherosclerosis. These are highly reactive species and capable of damaging nucleus and cell membranes by reacting with various vital intracellular molecules like DNA, protein, carbohydrates and lipids. Free radicals and other reactive oxygen species are derived either from normal metabolic process in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air-pollutants and industrial chemicals. The inhibition/quenching of free radicals can serve as facile model for evaluating the activity of hepatoprotective agents [2].

Boerhaavia Diffusa belongs to the family Nyctaginaceae, which is commonly known as Horse-purslane, Hogweed and Pig weed in English. Atikimamidi, Atima mamidi, Punarnava in Telugu. It is a diffuse herb with shout root-stocks. Leaves are thick-chartaceous in unequal pairs, ovate or elliptic-oblong, subfleshy. Anthocarps club-shaped, 5-ribbed, glandular hairy, top rounded; seeds erect. It is commonly distributed weed along roadsides, fields and waste places throughout the Chittoor district of Andhra Pradesh, India. The whole plant is used for the treatment of jaundice, dyspnoea, constipation, arthritis, anemia, cardiac diseases and liver diseases.

Anisochilus Carnosus belongs to the family Lamiaceae, which is commonly known as thick-leaved lavender in English. Saugudu ganapa, ritchu-rodda and karpuravalli in Telugu. It is an annual erect herb,stems guadrangular, sparsely pubescent, brownish from prolonged exposure to sun. Leaves fleshy, broadly ovate, deeply crenate, obtuse or acute, base rounded, verrucose above, and pubescent beneath. It is commonly distributed in rock crevisces on hills. On the way from Papanasam to Kumaradhara theertham (tirumala), dhanambanda area in Talakona. The whole plant used as diaphoretic, stimulant, expectorant, liver disorders, cough and cold. Leaf used for cough, dropsy, indigestion and sores in the leg fingers [3].

Antibacterial activity of *Boerhaavia Diffusa* L.leaves[4] , Chemopreventive action of *Boerhaavia Diffusa* on DMBA-induced skin carcinogenesis in mice[5], anti-ulcer activity of *Anisochilus Carnosus* leaf extract in pylorus ligated rats[6] has been reported. A detailed literature reviews indicated that, the hepatoprotective and antioxidant activity of alcoholic and aqueous extracts of stem and leaves of *Boerhaavia Diffusa* and leaves of *Anisochilus Carnosus* has not been clinically evaluated so for. In the present study, the hepatoprotective and antioxidant activity of alcoholic and aqueous extracts of *Boerhaavia Diffusa* and *Anisochilus Carnosus* is reported.

MATERIALS AND METHODS

Plant material

The stem and leaves of *Boerhaavia Diffusa* and leaves of *Anisochilus Carnosus* were collected from Sri Venkateswara University campus, Tirumala gardens of Chittoor district of Andhra Pradesh and the same were authentified by Assistant Professor, Dr.K.Madhava Chetty, Department of Botany, S.V.University, Tirupati, AP. Voucher specimens were deposited at department of pharmacognosy for further reference.

Extraction and Phytochemical screening

The shade dried plant materials were reduced to moderately coarse powder and extracted successively with alcohol and distilled water using Soxhlet apparatus after defatting. The prepared extracts were subjected to identify the presence of various phytoconstituents [7].

Experimental animals

Wistar albino rats weighing between 200-250gm were obtained from Venkateswara Enterprises, Bangalore, Karnataka, India. The

animals were maintained on the suitable nutritional and environmental conditions throughout the experiment as per the rules and regulations of the Institutional animal ethics committee. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional animal ethical committee (1423/PO/a/11/CPCSEA).

Acute toxicity studies

Acute toxicity studies were performed for the extracts of Alc.EBD & Alc.EAC and Aq.EBD & Aq.EAC using different [8]. For the hepatoprotective studies, the amount of dose administered was adjusted on the basis of observation during the toxicity studies.

Thioacetamide induced hepatotoxicity

Rats were divided into eleven groups, each group consisting of six animals.

Group I: Control group animals received normal saline 2ml/kg.

Group II: Toxic control animals received thioacetamide 50mg/kg body weight subcutaneously for 21 days.

Group III: Standard drug group animals received Silymarin 50mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. subcutaneously one hour after the respective assigned treatments every 72 hours.

Group IV: Treated animals received Alc.EBD 150mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group V: Treated animals received Alc.EBD 300mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group VI: Treated animals received Alc.EAC 200mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group VII: Treated animals received Alc.EAC 400mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group VIII: Treated animals received Aq.EBD 150mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group IX: Treated animals received Aq.EBD 300mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group X: Treated animals received Aq.EAC 200mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

Group XI: Treated animals received Aq.EAC 400mg/kg p.o. for 21 days and simultaneously administered TAA 50mg/kg b.w. s.c. every 72 hours.

At the end of experimental period, the animals were sacrified by cervical decapitation. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2500rpm for 15 min and analyzed for various biochemical parameters [9].

In Vitro Antioxidant Activity

DPPH radical scavenging activity

One mM solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) in methanol was prepared and 1ml of this solution was added to 3ml of various concentrations (50,100,250,500 and 1000µg/ml) of plant extracts of *Boerhaavia Diffusa* and *Anisochilus Carnosus* and to the reference compound ascorbic acid. After 30 min, absorbance was measured at 517nm. All the tests were performed in triplicate and the percentage scavenging of the DPPH free radical was calculated using the following equation[10-12].

$$\text{Percentage Inhibition} = \frac{C-T}{C} \times 100$$

Where, C=Absorbance of control, T=Absorbance of Test sample/standard

From the calculated percentage inhibition, the IC_{50} was calculated by,

$$IC_{50} = \frac{X-Y}{M}$$

Where, X=50, Y=Intercept and M=slope.

Xanthine Oxidase inhibitory (XOI) activity

The xanthine oxidase inhibitory activity was assayed using xanthine as the substrate. The extract and the standard drug allopurinol (1mg/ml) were prepared by dissolving in dimethyl sulphoxide (not exceeding more than 5% of total volume) initially and then made up to the required volume with potassium dihydrogen phosphate buffer (P^H 7.5). The assay mixture consisted of 1ml of extract at different concentrations (5, 10, 25, 50 and 100µg/ml), 2.9 ml of potassium dihydrogen phosphate buffer (P^H 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1U/ml in potassium dihydrogen phosphate buffer, P^H 7.5, prepared immediately before use). After pre-incubation at 25ºC for 15 min, the reaction was initiated by the addition of 2ml of substrate solution (150µM xanthine in phosphate buffer, P^H 7.5). The assay mixture was incubated at 25ºC for 30 min; the reaction was then stopped by the addition of 1N HCl. The absorbance was measured at 290nm against blank. Allopurinol (5-100µg/ml), a known inhibitor of xanthine oxidase, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1mmol of uric acid /min at 25ºC. XOI activity was expressed as the percentage inhibition of XO. The IC_{50} values were calculated from the percentage inhibition[13].

Nitric oxide radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological P^H interacts with oxygen to produce nitrite ions, which was measured by Griess reagent. Four ml extract solutions at different concentration (50,100,200,400 and 800µg/ml) mixed with 1.0ml of 25mM sodium nitroprusside solution and incubated at 37ºC for 2 hrs. Two ml of incubated solution was mixed with 1.2ml Griess reagent and absorbance was measured at 570nm. The experiment was performed in triplicate and the percentage scavenging activity was calculated. The IC_{50} values were calculated from the percentage inhibition [14].

Statistical Analysis

Experimental results were expressed as mean±SEM. Statistical analysis was performed with one way ANOVA followed by Dunnett's test using GraphPad InStat 3.

RESULTS

The acute toxicity study of both extracts revealed no mortality when administered orally up to a dose of 6.4g/kg body weight. At this dose there were no behavioral changes.

The results of thioacetamide induced hepatotoxicity of Alc.EBD & Alc.EAC and Aq.EBD & Aq.EAC is given in table-1. The results shows that, the higher dose of Alc.EBD and Alc.EAC shows the significant hepatoprotective activity ($p<0.01$) against TAA induced hepatotoxicity in rats. Also the results was comparable with that of the standard drug silymarin, which has significant at $p<0.01$.

Table 2 & 3 shows the percentage inhibition and IC_{50} values of Alc.EBD & Alc.EAC and Aq.EBD & Aq.EAC respectively by DPPH radical scavenging activity. From the results obtained, the IC_{50} of Alc.EAC is 163.88µg/ml, where as the other doses shows less significant than that of Alc.EAC.

In xanthine oxidase inhibition assay the significant IC_{50} value was obtained for Alc.EAC, i.e., 77.26µg/ml. The results of XOI assay is given in table-4 & 5.

The results of nitric oxide radical scavenging assay is given in table 6 & 7. The results show the IC_{50} value of Alc.EAC is 250.28µg/ml. This is more significant than that of other doses of both aqueous and alcoholic extracts.

Table 1: Effect of Alc.EBD & Alc.EAC and Aq.EBD & Aq.EAC on Biochemical Parameters against TAA induced Hepatotoxicty in Rats

Groups		SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
I	Control	97.78±2.64	42.21±2.41	122.20±2.50	0.75±0.041	0.11±0.010
II	TAA	181.05±2.18	113.13±2.56	319.56±3.06	1.37±0.044	0.38±0.022
III	Standard	124.93±1.75**	57.37±2.51**	168.43±2.41**	0.81±0.070**	0.13±0.011**
IV	Alc.EBD 150mg	171.81±2.27*	104.00±2.35*	307.16±3.07*	1.20±0.052	0.32±0.013*
V	Alc.EBD 300mg	147.82±1.99**	83.10±2.16**	231.12±2.58**	0.92±0.047**	0.15±0.006**
VI	Alc.EAC 200mg	153.86±1.87**	90.08±2.18**	265.01±2.33**	1.13±0.053*	0.20±0.011**
VII	Alc.EAC 400mg	140.28±1.78**	75.46±1.94**	199.55±2.21**	0.86±0.047**	0.17±0.011**
VIII	Aq.EBD 150mg	177.16±2.78	106.36±2.18	310.47±2.96	1.35±0.051	0.36±0.014
IX	Aq.EBD 300mg	166.97±1.57**	101.83±2.84*	274.91±2.12**	1.17±0.046*	0.30±0.013*
X	Aq.EAC 200mg	171.42±2.34*	105.52±1.95	307.00±3.25*	1.31±0.042	0.31±0.013*
XI	Aq.EAC 400mg	156.21±1.99**	87.95±2.26**	230.20±2.34**	1.12±0.062*	0.24±0.021**

Each value represents the mean±SEM, n=6. * p<0.05, ** p<0.01, Statistical significant test for comparison was done by ANOVA , followed by Dunnett's test. Groups III to XI are compared with group II.

Table 2: DPPH radical scavenging activity of Alc.EBD and Alc.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Alc.EBD	Alc.EAC	Ascorbic Acid
50	32.01±0.77	33.99±0.51	34.86±0.86
100	47.20±1.63	51.02±1.02	54.54±0.67
250	55.32±1.21	58.21±0.56	65.13±2.16
500	69.85±1.49	72.20±1.39	75.09±1.13
1000	80.63±1.43	82.19±1.00	83.15±1.79
IC_{50} (µg/ml)	226.12	163.88	81.23

Values represent the mean±SD, n=3. IC_{50} values were determined by linear regression analysis

Table 3: DPPH radical scavenging activity of Aq.EBD and Aq.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Aq.EBD	Aq.EAC	Ascorbic Acid
50	30.74±1.08	33.48±1.52	35.37±0.98
100	44.30±0.65	48.11±0.69	53.39±1.72
250	49.81±0.94	52.25±0.65	64.41±0.73
500	64.08±0.91	67.14±1.33	77.16±0.89
1000	76.35±1.30	77.31±1.07	84.85±1.55
IC_{50} (µg/ml)	309.29	239.82	87.59

Values represent the mean±SD, n=3. IC_{50} values were determined by linear regression analysis.

Table 4: Xanthine Oxidase Inhibition Assay of Alc.EBD and Alc.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Alc.EBD	Alc.EAC	Allopurinol
5	04.22±0.49	06.19±0.80	09.74±1.13
10	12.06±0.84	14.22±0.91	21.18±0.90
25	20.62±0.57	23.08±1.38	44.73±0.55
50	42.34±0.44	45.02±0.57	84.47±0.58
100	52.22±0.58	57.41±0.82	90.90±1.34
IC_{50} (µg/ml)	86.65	77.26	37.76

Values represent the mean±SD, n=3. IC_{50} values were determined by linear regression analysis

Table 5: Xanthine Oxidase Inhibition Assay of Aq.EBD and Aq.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Aq.EBD	Aq.EAC	Allopurinol
5	02.89±0.73	05.07±0.29	10.22±1.09
10	10.93±0.71	12.18±0.53	21.42±1.21
25	18.07±0.97	20.47±0.30	44.93±0.84
50	38.25±1.07	41.96±0.56	84.45±0.53
100	51.19±0.59	53.52±1.04	91.06±1.00
IC_{50} (µg/ml)	89.79	84.23	37.51

Values represent the mean±SD, n=3. IC_{50} values were determined by linear regression analysis.

Table 6: Nitric Oxide Radical Scavenging Assay of Alc.EBD and Alc.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Alc.EBD	Alc.EAC	Curcumin
50	25.05±0.78	29.00±1.12	41.91±0.31
100	35.33±0.56	40.11±0.30	54.10±0.59
200	47.24±0.78	55.20±0.49	59.31±0.33
400	57.18±0.57	64.73±0.37	69.38±0.47
800	72.31±0.83	79.00±0.78	88.91±0.78
IC_{50} (µg/ml)	355.07	250.28	84.98

Values represent the mean±SD, n=3. EC_{50} values were determined by linear regression analysis

Table 7: Nitric Oxide Radical Scavenging Assay of Aq.EBD and Aq.EAC

Concentration (µg/ml)	Percentage Inhibition (%)		
	Aq.EBD	Aq.EAC	Curcumin
50	22.34±0.87	25.43±0.49	42.40±0.54
100	30.85±1.10	34.17±0.65	53.11±0.91
200	41.86±0.46	47.28±0.23	61.09±1.31
400	54.32±0.92	62.37±0.33	70.00±0.53
800	67.84±1.10	75.18±0.35	89.04±0.58
IC_{50} (µg/ml)	424.82	328.04	77.86

Values represent the mean±SD, n=3. EC_{50} values were determined by linear regression analysis.

DISCUSSION

A variety of chemicals have been used for the evaluation of hepatotoxicity in rats. This includes thioacetamide, carbon tetrachloride, paracetamol, alcohol, liquid paraffin, etc. The present study was carried out with the use of thioacetamide for the screening of hepatoprotective activity of Boerhaavia Diffusa and *Anisochilus carnosus*. Also, in the numerous methods of antioxidant assay, DPPH radical scavenging activity, xanthine oxidase inhibition assay and nitric oxide radical scavenging assay was followed for the evaluation of antioxidant activity of the reported plants. It was concluded that the plants of *Boerhaavia Diffusa* and *Anisochilus Carnosus.*

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