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Biological Screening of *Boerrhavia diffusa* extract on Chemically Induced Hepatocellular Carcinoma with Reference to Biochemical Parameters

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ABSTRACT

The hepatoprotective activity of a *Boerrhavia diffusa* of 50 % ethanolic extract was studied using Swiss albino rats. The animals received a single intraperitoneal injection of N-nitrosodiethylamine 200mg/kg body wt followed by subcutaneous injection of CCl4 in a dose of 3 ml/kg body wt. The administration of *Boerrhavia diffusa* extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capability of extracts in respect with anticancer potency in comparison with the very much effective in preventing NDEA-induced multistage hepatocarcinogenesis possibly through antioxidant and antigenotoxic nature, which was confirmed by various liver injury and biochemical tumour markers enzymes and molecular events. *Boerrhavia diffusa* extract dose dependently and significantly the increase in serum hepatic enzyme levels after NDEA& CCl4 treatment compared to the toxin control group. The results of this study confirmed the antioxidant and hepatoprotective activity of the *Boerrhavia diffusa* extract against carbon tetrachloride& N-nitrosodiethylamine induced hepatotoxicity in Swiss albino rats.

Keywords: Carbon tetrachloride, N-nitrosodiethylamine, Hepatocellular carcinoma, Boerrhavia diffusa

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INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the elements in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and main compounds in synthetic drugs. Plants have long served as a useful and natural source of therapeutic agents. Almost all plants have medicinal values and their uses differ from place to place. Liver diseases pose an enormous health problem in spite of tremendous strides in modern medicine. There is hardly any drug that can effectively control inflammation, protect the liver from the damaging effects of hydrophobic bile acids which are retained in cholestatic disorders, promote protein synthesis, manifest antioxidative, anti-lipid peroxidative and antifibrotic properties, prevent fat from infiltrating the liver, enhance glucuronidation, decreases intestinal absorption and suppresses hepatic synthesis and storage of cholesterol, stabilize hepatocyte membranes, help the liver to replace damaged tissue and regenerate itself, promotes effective metabolism of drugs by maintaining levels of CYP or Cytochrome P450, protect the liver from damage and also regulate the liver enzymes. Because of the affordability, availability and accessibility of dependable hepatoprotective drugs in scientific/ conventional medicine, plants play an important role in the management of various liver disorders and in meeting the demands of primary health care in many developing countries. The hepatoprotective potentials of a *Boerrhavia diffusa* extracts are evaluated using carbon tetrachloride and NDEA In the traditional system of medicine, *B. diffusa* roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, and abdominal pain (Kirtikar and Basu, 1956), and as an antistress agent. The worldwide use of *B. diffusa* roots to treat liver disorders was validated when researchers demonstrated, in 1980 and 1991, that its root extract had antihepatotoxic properties (Chandan *et al*., 1991; Rawat *et al*., 1997).

MATERIALS AND METHOD

Animals

Healthy Swiss albino rats weighing 140 -160g and mice weighing 17-22g were used for this study. The animals were housed in polypropylene cages at controlled temperature, well ventilated with a 12-12 h light dark cycle. The rats and mice were fed with standard laboratory diet and water was provided *ad libitum.* The animals were maintained as per the CPCSEA guidelines and regulations and the study was approved by the institutional animals ethics committee at AIPR, Lucknow.(CPCSEA Registration No: 1146/ac/07/CPCSEA)

Plant Materials

The whole plant of *Boerrhavia diffusa* was collected from the National Botanical Research

Institute Garden, Lucknow in August-September 2011.The plant materials were authenticated and identified by Dr. Sayeeda Khatoon, chemo taxonomist and compare with Voucher specimens (NAB 98070) and voucher specimens (NAB 300697) was deposited in the NBRI herbarium and museum for future reference.

Preparation of plants extract(s)

The fresh plant materials of *Boerrhavia diffusa* was washed with distilled water and air –dried at 30± 2 C. The dried plant materials of *Boerrhavia diffusa* (1000g) was exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol (50% EtOH) and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour and then dried in lyophilizer under reduced pressure.

Efficacy of *Boerrhavia diffusa* on N-nitrosodiethylamine induced hepatocellular carcinoma in animal- in vivo model

Animals were randomized and grouped into experimental control rats. Group I rat were treated with 0.9% normal saline. Group II rats received single intraperitoneal injection of N-nitrosodiethylamine(200mg/kg body wt). Group III rats received subcutaneous injection of carbon tetrachloride(3ml/kg body wt) once a week for 6 weeks. Group IV rats received single intraperitoneal injection of N-nitrosodiethylamine(200mg/ kg body wt) followed by subcutaneous injection of carbon tetrachloride(3ml/kg body wt) as Group III. After 20-25 weeks hepatocellular carcinoma(HCC) was confirmed in Group IV(NDEA+CCL4) rats with the help of bio-chemical and histopathological studies and Group IV (NDEA+CCL4) rats were used for investigation of HCC experimentation.

Experimental Design

Effect of 50% EtOH extract of *Boerrhavia diffusa* on hepatocellular carcinoma

Group I- Normal Control

Group II- Chemical induced HCC (NDEA+CCL4)

Group III- 50% EtOH extract of *Boerrhavia diffusa* (100mg/kg.body wt p.o) in HCC rats

Group IV- 50% EtOH extract of *Boerrhavia diffusa* (200mg/kg.body wt p.o) in HCC rats

Group V- 50% EtOH extract of *Boerrhavia diffusa* (400mg/kg.body wt p.o) in HCC rats

Group VI- Cisplatin (6mg/kg. body wt i.p. weekly once for 3 weeks) 50%EtOH in HCC rats

Effect of 50% EtOH extract of *Boerrhavia diffusa* on hepatocellular carcinoma

The animals were treated with the test drugs and standard for 28 days. After completion of treatment, animals were anaesthetizes with anesthetic ether and blood was remove from the retro-orbital puncture with the help of capacity, collect, then after 30 min centrifuge at 3000 rpm

for 15 min then separate serum/plasma for biochemical estimation.

Estimation of tumour marker enzymes

- Determination of Serum glutamic oxaloacetic transaminase(SGOT)
- Determination of Serum glutamate pyruvate transaminase(SGPT)
- Determination of Serum alkaline phosphatase(SALP)
- Determination of Serum *Y* glutamyl transpeptidase(GGT) activity
- Determination of Serum bilirubin(SB)
- Determination of Serum total protein

Histological studies

At the end of each scheduled duration the control as well as treated rats were sacrificed by using cervical dislocation and the liver was dissected out and changes in liver weight and tumour incidence was noted and a part of liver tissue was immediately fixed in bouin's fluid for 24 hr and washed in running tap water to remove the color of bouin's fluid and dehydrated in alcohol in ascending and descending order, embedded in paraffin and cut at 5um in a rotary microtome. These sections were then deparaffinized in xylene and stained with hematoxylin-eosin using routine method. The sections were then stained with haematoxylin-eosin dye and mounted with Canada balsam. The histopathological slides were examined and photographs were taken with a digital stereomicroscope.

Statistical analysis

Data are expressed as mean ± SEM (standard error of mean).The difference among means has been analysed by unpaired student"s t-test

RESULTS AND DISCUSSION

Pharmacological Investigation

The present study was under taken to investigate the hepatocellular carcinoma effects of 50% EtOH extract of *Boerrhavia diffusa* with special emphasis on the molecular mechanisms involved in the curative activity against N-nitrosodiethylamine(NDEA) –induced hepatocellular carcinoma in rats.

General behavior and acute toxicity Studies

50% ethanolic extracts of selected plants *Boerrhavia diffusa* up to 2000mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc. during the period of observation.

Effect of the 50% ethanolic extracts of LAB on SGOT, SGPT, SALP and Bilirubin level(BL) in serum

50% ethanolic extracts of *Boerrhavia diffusa* at a dose of 100,200mg and 400mg (O.D x 28 days) were subjected for se effect by studying various biochemical parameters like SGOT, SGPT, SALP AND BL serum. The50% ethanolic extracts of *Boerrhavia diffusa* did not showed any significant effect on liver biochemical markers viz SGOT, SGPT, SALP AND BL levels(Table 1)

Table 1: Effect of the 50% ethanolic extracts of *Boerrhavia diffusa* (100, 200 and 400 mg) on SGOT (U/l), SGPT (U/l), SALP (U/l), and Bilirubin level (U/l), (BL) in serum of rat

Oral treatment (mg/kg, odx28days)	SGOT	SGPT	SALP	BL
Control (Normal rats)	196.0 ± 1.49	80.2± 1.11	232.3± 1.12	0.64± 0.08
Boerrhavia diffusa 100mg	196.1± 1.51	79.8± 1.43	232.1± 8.23	0.61± 0.07
Boerrhavia diffusa 200mg	197.3± 1.48	80.3± 1.54	231.2± 1.02	0.63± 0.12
Boerrhavia diffusa 400mg	196.2± 1.40	81.9± 1.01	232.6± 0.83	0.64± 0.06

Values are mean ± SEM of rats in each group

Effect of 50% ethanolic extract of *Boerrhavia diffusa* on SGOT, SGPT, SALP AND BL and GGT against NDEA+CCL4- induced Hepatocellular Carcinoma

It is clearly evident from the table 2 that NDEA+CCL4 caused significant elevation of liver serum markers. In the NDEA+CCL4 treated group, the level of SGOT (192.20 – 354.21, $p<0.001$), SGPT (83.21 – 371.53, $p<0.001$), SALP (234.14 -439.31, $p<0.001$), BL (0.72 – 1.26, $p<0.001$) and GGT (32.4 – 154.2, $p<0.001$). In contrast, the groups treated with *Boerrhavia diffusa* extract at dose of (100 – 400 mg/kg) once daily for 28 days prevented the cancer in a dose related manner. The range of protection in the serum marker were found to be SGOT (354.21–202.81, $p<0.05$ to $p<0.01$), SGPT (371.53–109.06, $p<0.05$ to $p<0.001$), SALP (439.31 – 249.96,$p<0.05$ to $p<0.001$), BL (1.26–0.82, $p<0.01$ to $p<0.001$) and GGT (154.2 – 78.1,$p<0.001$) respectively. The protection of cisplatin ranged for SGOT (354.21 – 198.32,$p<0.01$), SGPT(371.53 – 92.34, $p<0.001$),SALP(439.31 – 242.26,$p<0.001$),BL(1.26 – 0.78,$p<0.001$), GGT (154.2 – 52.4, $p<0.001$) respectively as shown in table 2. The histological observations also basically support the results obtained from serum enzyme assays (Figure.1)

An attempt has been made to investigate plants and plant product used to treat Hepatocellular carcinoma. An attempt has been made in scientifically validated experiment animal models to investigate a novel herbal drug based anticancer agents from *Boerrhavia diffusa* against hepatocellular carcinoma.

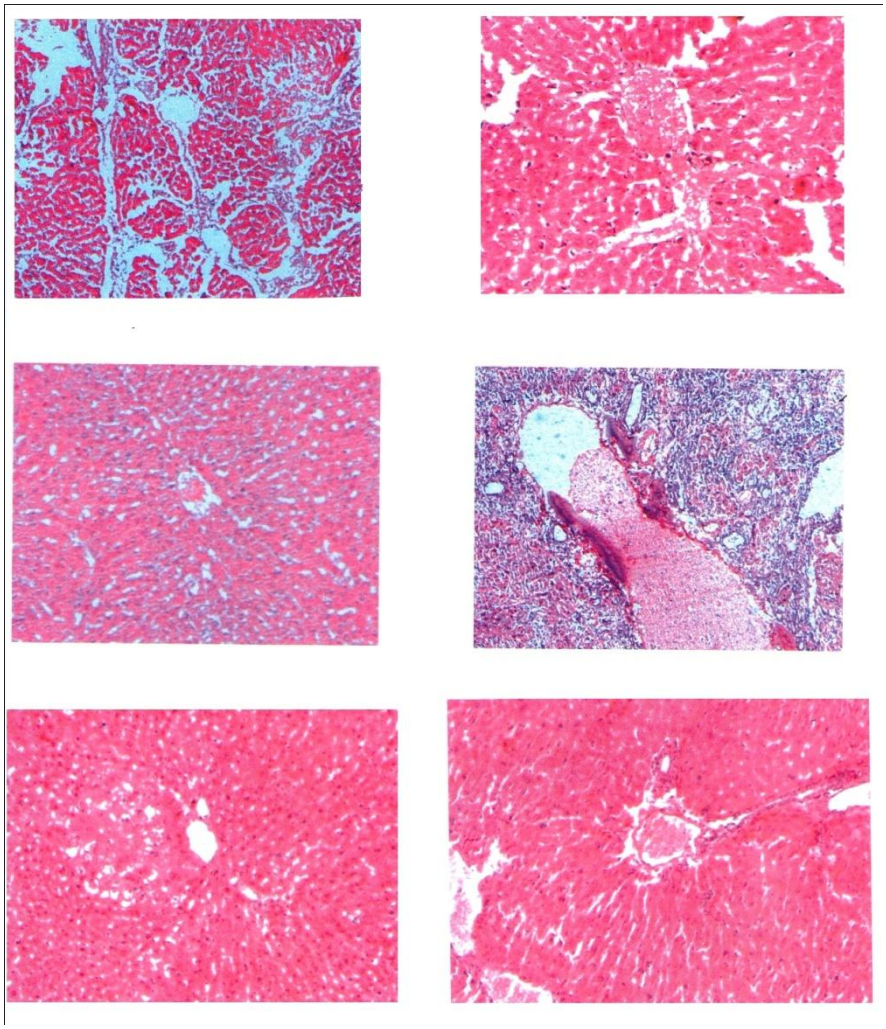

Figure 1 Histological observations also basically support the results obtained from serum enzyme assays

The 50% ethanolic extracts,The body weights were steadily increased after treatment with extracts and compared with standard cisplatin in hepatoma bearing animal which indicate, *Boerrhavia diffusa* extracts reduces the tumour incidence and change in energy metabolism and also shows anticancer potency. The administration of *Boerrhavia diffusa* extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capacity of extracts in respect with anticancer potency in comparison with the standard drug cisplatin.

The 50% ethanolic extract of the plant treatment showed significant dose dependent (100, 200 and 400 mg/kg) alteration in level of SGOT and SGPT in serum which were recuped back to near normal in HCC bearing animals which shows the anticarcinogenic activity of the plants. (table-2) .The levels of SGOT and SGPT in plants extract (100-400 mg/kg) in HCC bearing animals were found to be near to the levels of enzymes when treated with cisplatin. This result shows the antineoplastic effect of plant as with the standard.

Table 2:Effect of 50% ethanolic extract of *Boerrhavia diffusa* on SGOT (U/l), SGPT (U/l), SALP (U/l), and Bilirubin level (U/l), (BL) and Gamma glutamyl transpeptidase, GGT(U/l) in serum of rat .

Groups	Treatment	Dose	SGOT	SGPT	GGT	SALP	BL
I	Control	---	192.20± 1.64	83.21 ± 1.60	32.4± 0.54	234.14± 10.34	0.72± 0.02
II	NDEA + CCL4	200 mg/kg	354.21± 36.31z	371.53± 42.72z	154.2±12.8z	439.31± 28.31z	1.26± 0.08z
III	*Boerrhavia diffusa*	100 mg/kg	256.34±22.52a	246.38 ±34.29a	148.8±10.8	354.24±24.34a	0.91±0.07b
IV	*Boerrhavia diffusa*	200 mg/kg	218.51±36.31z	181.14 ±36.29b	121.4±9.6	299.28±21.54b	0.87± 0.06b
V	*Boerrhavia diffusa*	400 mg/kg	218.51±15.71b	109.06± 21.34c	78.1±7.7c	249.96 ±18.28c	0.82± 0.04c
VI	Cisplatin	6 mg/kg	198.32 ±10.78b	92.34 ± 23.48c	52.4± 8.1c	242.26 ±18.72c	0.78± 0.03c

Values are mean ± SEM of 6 rats in each group

P values: z<0.001 compared with respective control group

P values: a<0.05, b<0.01, c<0.001 compared with group II (NDEA+CCl4)

In the present investigation, the elevation in levels of ALP was observed in animal of hepatoma(NDEA and CCl4) induced hepatocellular carcinoma in the serum(Table 2).

In the present study, 50% ethanolic extract of the plants tested showed significant decrease in the levels of ALP or in other words it shows the potentiating the level of ALP back to normal and also likely show the level of this enzymes near to the level when treated with the standard drug cisplatin which indicates , the anti carcinogenic effects of plant in (NDEA and CCl4) treated hepatoma rats (Table 2) .

In groups treated with 50% ethanolic extract of the plant(100, 200 and 400 mg/kg) showed significant results, reducing the levels of these elevated levels in a dose dependent manner, indicates the restoring serum marker enzymes back to normal.(Table 2).

An increase in GGT activity in serum of hepatoma bearing animals (NDEA and CCl4) induced carcinogenesis in this study suggests its potential role as an indicator of carcinogen exposure and also reflects the toxic effects of drug on microsomal structure in liver cells. Recoupment of tumour marker enzyme (GGT) upon treatment with plant extracts of 50%ethanol showed a significant dose dependent decrease in the levels of GGT in comparisons with hepatoma bearing animals and it also shows the result same as comparisons with the standard drug cisplatin suggest a combinatorial therapy gives protective mechanism against abnormal cell growth by changing the permeability of membrane or affecting cellular growth (Table-2).

To prove the anticancer activity of *Boerrhavia diffusa,* histopathological studies were carried out. In the present investigation, noticeable changes were observed in the architecture of liver of cancer bearing animals. These indicates the presence of neoplastic conditions following NDEA and CCl4 administration. In drug treated animals, the NDEA and CCl4 damage was recovered due to anticancer potency of *Boerrhavia diffusa* .The regression of the tumours in liver may be due to the protective effect of *Boerrhavia diffusa.*

CONCLUSION

Recent studies on tumour inhibitory compounds of plant origin have yielded an impressive array of research on medicinal plant. The efficacy of *Boerrhavia diffusa* in experimental liver cancer described in the present investigation offer the potential for reaching on understanding of anticancer potency.The administration of *Boerrhavia diffusa* extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capability of extracts in respect with anticancer potency in comparison with the very much effective in preventing NDEA-induced multistage hepatocarcinogenesis possibly through antioxidant and antigenotoxic nature, which was confirmed by various liver injury and biochemical tumour markers enzymes and molecular events. Studies on molecular aspect of cancer therapy will give mechanistic information in cancer therapy and also critical balance should be there between the animal model and clinical research. This holds great promise for future research in human beings. The anticancer properties of *Boerrhavia diffusa* should provide useful information in the possible application in cancer prevention and cancer therapy.

REFERENCES

1. World Health Organization (WHO). Traditional medicine strategy 2002-2005, World Health Organization, WHO/EDM/TRM/2002.1, Geneva, 2001, pp.7
2. N.W. Tietz (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995.
3. Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.). Journal of Clinical Chemistry and Clinical Biochemistry. 8, 1970, 658.
4. Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft

für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) J Clinical Chemistry and Clinical Biochemistry. 10, 1972, 182 -92.

5. G. Szaszi. A kinetic photometric method for serum gammaglutamyl transpeptidase. Clinical Chemistry. 15 (2), 1969,124-136.
6. S. Reitman and S. Frankel. A colourimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 28 (1), 1957: 56-63.
7. H. Esterbauer and K.H., Cheeseman. Determination of aldehydic lipid peroxidation products: Malonaldhyde and 4-hydroxynonetal. Methods in Enzymology. 186, 1990, 407-421.
8. J. Sedlak and R.H. Lindsay. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue by Ellmans reagent. Analytical Biochemistry. 25 (1), 1968, 192-205.
9. P. Kakkar, B. Das and P. N. Viswanathan. A modified spectrophotometric assay of SOD. Indian Journal of Biochemistry and Biophysics. 21 (2), 1984, 130- 132.
10. D.G. Hafemann, R.A. Sunde and W.G. Houestra. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. Journal of Nutrition. 104 (5), 1974, 580-584.
11. W. H. Habig and W. B. Jakoby. Glutathione S-Transferases. The first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry. 249 (22), 1974, 7130-7139.
12. J. Bonaventura, W. A. Schroeder and S. Fang. Human erythrocyte catalase: an improved method of isolation and a revaluation of reported properties. Archives of Biochemistry and Biophysics. 150 (2), 1972, 606-617.
13. C. Weiler-Normann, J. Herkel and A.W. Lohse. Mouse models of liver fibrosis. Zeitschrift fur Gastroenterologie. 45(1), 2007, 43-50.
14. L. W. Weber, M. Boll and A. Stampfl. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical* Reviews in Toxicology. 33 (2), 2003, 105–136.
15. M.K. Manibusan, M. Odin and D.A. Eastmond. Postulated carbon tetrachloride mode of action: a review. Journal of Environmental Science and Health, Part C. 25 (3), 2007,

185–209.

16. J. Shi, K. Aisaki, Y. Ikawa and K. Wake. Evidence of hepatocytes apoptosis in rat liver after the administration of carbon tetrachloride. The American Journal of Pathology. 153 (2)**,** 1998, 515–525.
17. H. H. Mansour, H. F. Hafez and N. M. Fahmy. Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. International Journal of Biochemistry and Molecular Biology. 39 (6), 2006, 656-661.
18. C.S. Lee, J.H. Han, Y.Y. Jang, J.H. Song and E.S. Han. Differential effect of catecholamines and MMP+ on membrane permeability in brain mitochondria and cell viability in PC12 cells. Neurochemistry International. 40 (4), 2002, 361–369.
19. G. E. Arteel. Oxidants and antioxidants in alcohol-induced liver disease. Gastroenterology. 124(3)**,** 2003, 778–790.
20. B.P. Yu. Cellular defenses against damage from reactive oxygen species. Physiological Reviews. 74 (1), 1994, 139–162.
21. B. Halliwell. Antioxidant defense mechanisms: from the beginning to the end (of the beginning). Free Radical Research. 31 (4), 1999, 261–272.
22. J. Zhuge and A.I. Cederbaum. Depletion of S-adenosyl-l-methionine with cycloleucine potentiates cytochrome P450 2E1 toxicity in primary rat hepatocytes. Archives of Biochemistry and Biophysics. 466 (2*)*, 2007, 177–185.
23. T. Ozben. Oxidative stress and apoptosis: impact on cancer therapy. Journal of Pharmaceutical Sciences. 96 (9*)*, 2007, 2181–2196.