



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Study on Anticancer Activity of *Boerhaavia Diffusa* on Wistar Albino Rats Treated with Diethyl Nitrosamine

P. S. Bedi^{1*}, Ashish K. Sharma², Najmeddin, Zayed M Ellali³

1. Department of Chemistry, Wollega University, Nekemte, Ethiopia.

2. Department of Biotechnology, S.B.S.PGI., Balawala, Dehradun, India.

3. Department of Chemistry, Faculty of Science, Agilat, Zawia University, Zawia, Libya.

ABSTRACT

In present investigation the anticancer properties of *Boerhaavia diffusa* were studied. Carcinogenicity was induced by DEN i.e. nitrosamine (@ 200 µl/Kg body weight). In a single dose DEN challenged animals were given *Boerhaavia diffusa* extract (@200mg/Kg body weight) daily and named them as test group. For control group no DEN and no treatment was given. Negative control was given only DEN, to check its carcinogenicity with control and test group. After eight weeks the blood samples were collected to study the effect of carcinogen and plant extract of *Boerhaavia diffusa*. Various biochemical parameters like : Superoxide dismutase (SOD), Glutathione (GH), Alkaline phosphatase (ALP), Acid phosphatase (ACP), SGOT, SGPT, Triglycerides, Albumin, Catalase (CAT), Lipid per oxidation (MDA), Total proteins (TP), Globulin, Albumin-Globulin ration, Bilirubin, Creatinine, Total cholesterol, HDL, LDL, HDL/LDL ratio and body weight gain were studied. The results of the present study showed that there was a significant decrease in body weight gain among all the groups. Significant increase in SOD, MDA, GH, ALP, ACP, SGPT, SGOT etc. and CAT, TP, Albumin, Globulin, Total Cholesterol, HDL, Triglycerides were significantly decreased as compared with control group. The test group had shown significant hepato protective and anti carcinogenic activity against DEN. The biochemical changes were also supported by histopathological lesions. The present study concludes that plant extract of *Boerhaavia diffusa* have shown hepato protective and anti liver cancer activity.

Keywords: Anticancer, Hepatoprotective, Diethyl nitrosamine, *Boerhaavia diffusa*, biochemical parameters and histopathology.

*Corresponding Author Email: drbedips@gmail.com

Received 10 January 2018, Accepted 30 January 2019

Please cite this article as: Bedi PS *et al.*, Study on Anticancer Activity of *Boerhaavia Diffusa* on Wistar Albino Rats Treated with Diethyl Nitrosamine. American Journal of PharmTech Research 2019.

INTRODUCTION

Cancer has emerged as an important health hazard globally. The impact of this disease is more in developing countries where we have limited resources to fight with it. Many cancer patients in these countries depend on many forms of alternative medicine for treatment and palliation. Many types of herbal, natural products are tried by cancer patients. Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age. Cancer causes about 13% of all deaths. According to American Cancer Society, 7.6 million people died from cancer in the world during 2007 (Boyle and Levin 2008 and Siegel *et al.*, 2017). Cancer rate could increase by 50% to 15 million new cases in the year 2020. This will be mainly due to steadily aging population in both developed and developing countries and also to current trends in smoking prevalence and the growing adoption of unhealthy lifestyle. The report also reveals that cancer has emerged as a major public health problem in developing countries, matching its effects in industrialized nations (Jemal *et al.*, 2007). The alkylating agent DEN is a well studied liver carcinogen which on administration continuously to rats, produces a well characterized dose response to liver tumor incidence (Druckrey *et al.*, 1969; Peto *et al.*, 1991; Verna *et al.*, 1996; Kang *et al.*, 2007 and R Tolba *et al.*, 2015) .

Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue. Herbal medicines are in great demand in the developed countries due to their higher safety margins and lesser costs. *Boerhaavia diffusa* is a plant having numerous therapeutic uses. The whole plant or its specific parts (leaves, stem and roots) are known to have medicinal properties and have a long history of used by indigenous and tribal people (Shweta and Verma 2017).

MATERIALS AND METHOD

Present study was performed on Wistar albino rats obtained from the institute (SBSPGI, Dehradun). The animals were housed in a clean polypropylene cages kept in institute animal house and fed with commercially available feed and water ad-libitum. The litter was changed after every five days. Prior permission from the institute animal ethical committee for the use of experimental animals was duly obtained and all the rats were labeled.

Preparation of herbal extract of *Boerhaavia diffusa*

Boerhaavia diffusa was bought from the herbal medicine shop in Dehradun and water extract was prepared in the laboratory. This water extract was used to treat the rats given DEN.

Experimental design

A total of 36 animals were equally divided in to three groups (N=12 in each group).

Treatment Schedule

Single dose of DEN 200 µl/ Kg body weight was given to each rat of group II and group III. The rats of group III were given plant extract 200mg/Kg body weight from day one for eight weeks.

Body weight of animals

Weekly body weight of all the experimental animals in different groups was taken till the end of the experiment. First day of the experiment was considered as zero day. At the end of experiment all the animals were anaesthetized by sprinkling chloroform in a closed jar. The animals were kept in it for 1-2 minutes.

Table 1: Distribution and details of the treatment

Group	N	Type	Treatment
I	12	Control	No Treatment
II	12	Negative Control	Diethyl nitrosamine (200 µl/ Kg body weight)
III	12	Test	Diethyl nitrosamine (200 µl/ Kg body weight) + Herbal extract (200mg/Kg body weight)

Dissection and sample collection

After eight weeks of treatment all the rats were sacrificed. The anaesthetized animals were dissected from the ventral side. Blood samples were collected by heart puncture. A small part of liver and kidneys were preserved in 10% formalin solution for histopathological studies. The rest of the liver and kidneys were homogenized with chilled phosphate buffer (pH 7.0).

Tissue homogenate

Liver was cut in to small pieces and was homogenized in 25 ml of phosphate buffer by using glass homogenizer. The homogenate so obtained was centrifuged at 10000 rpm for 10 minutes. Supernatant was collected for further tests. Same procedure was repeated for kidney. Tissue homogenate of liver and kidneys were prepared and subjected for assay of superoxide dismutase (SOD) by following the method of (Mishra and Fridovich 1972), Catalase activity (CAT) by (Beers and Seezers 1952), Protein by (Lowery *et al.*, 1951), lipid peroxidation product- malonaldehyde (MDA) by (Esterbauer and Cheeseman 1993).

Serum Collection

The blood was collected after heart puncture and 1 ml was taken in tubes containing heparin (anti coagulant) and rest of the blood was kept in slant for clotting. Clotted blood samples were

centrifuged at 5000 rpm for 10 minutes for serum collection. The serum samples were then aliquoted in vials and stored at -20°C. Serum samples were subjected for biochemical studies viz. SGOT, SGPT, Alkaline phosphatase, Acid phosphatase, Bilirubin, Total proteins, Albumin, Cholesterol, HDL- cholesterol, Triglycerides, Creatinine etc. by using Bayer's Auto-pack kits procured from local market.

Histopathological examination

Histopathological lesions were also studied to find out histo anatomical changes in organs like liver and kidney. The samples stored in 10% formalin were sent to Division of pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly, for histopathological examination.

Statistical analysis

The results were statistically analyzed using one way analysis of variance (ANNOVA).The analysis was performed with the help of statistical software package.

RESULTS AND DISCUSSION

In present study the results revealed that in control (rats) group the mean values of body weight on zero day was 206.92 ± 1.654 and body weight in treated groups i.e. 202.50 ± 0.870 and 202.16 ± 1.153 in II and III groups respectively. The body weight in these groups was found to be lesser than the control group on zero day itself. The body weight was raised to 216.43 ± 1.397 and decreased to 201.06 ± 0.969 in groups I and III respectively, however, mean body weight was reduced to 190.90 ± 0.953 in group II by the end of the experiment (i.e. eighth week). The body weight was decreased in the DEN treated group as compared to the other groups. The difference in total body weight gain was significantly different among all the groups.

Ramjee *et al.*, (1992) reported that aflatoxin has been directly related to under weight status in children in Benin and Togo. Bedi *et al.*, (1996) also reported decrease in body weight in Guinea fowl fed on aflatoxin B₁. In present study body weight in DEN treated rats significantly decreased in eight weeks. However, there was an increase in body weight in control group and negligible change in the treatment group.

Biochemical investigations in liver homogenate

MDA level

In rats fed on normal diet the mean value of MDA was 8.77 ± 0.208 nmol/L. The values were raised in treated groups to 13.24 ± 0.206 and 9.860 ± 0.201 nmol/L in groups II and III respectively (Figure 1). There was significant increase in MDA level in liver tissue in DEN treated group as compared to

the control and group III. The use of *Boerhaavia diffusa* has shown the positive response by lowering the MDA level in liver tissue as compared to DEN treated group.

Catalase activity

The Catalase activity in liver samples of control group was 0.756 ± 0.017 U/mg protein but there was significant decrease in Catalase activity in treated groups i.e. 0.515 ± 0.015 and 0.616 ± 0.029 U/mg protein in II and III groups respectively (Figure 1). There was significant decrease in Catalase level in liver tissue in the DEN treated group as compared to the control and other group as well. The group treated with the *Boerhaavia diffusa* extract has shown a significant increase in Catalase activity in liver homogenate as compared to DEN treated rats but the level was found significantly lower than the control group.

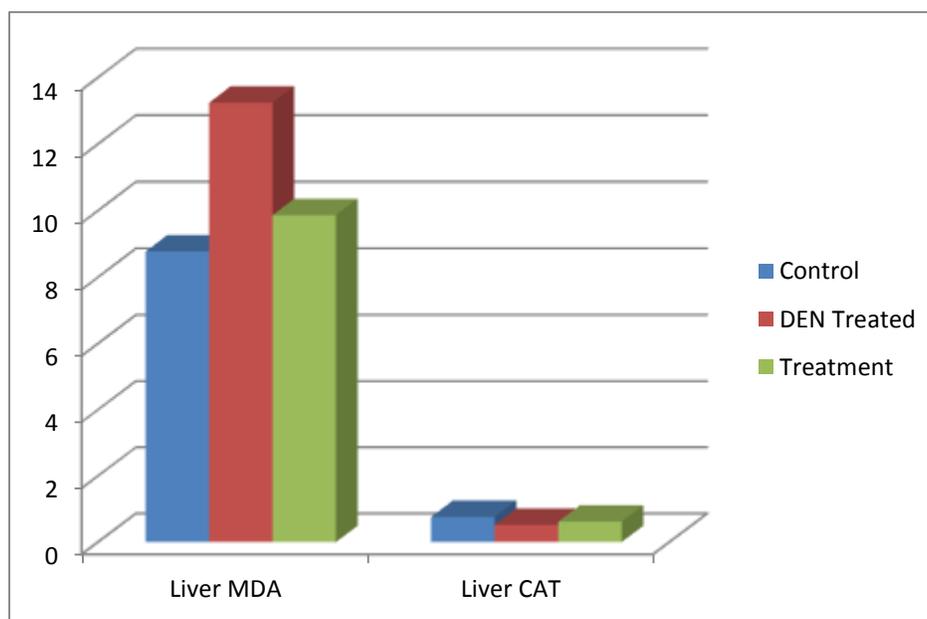


Figure 1 : Effect of *Boerhaavia diffusa* extract on MDA and Catalase activity of liver

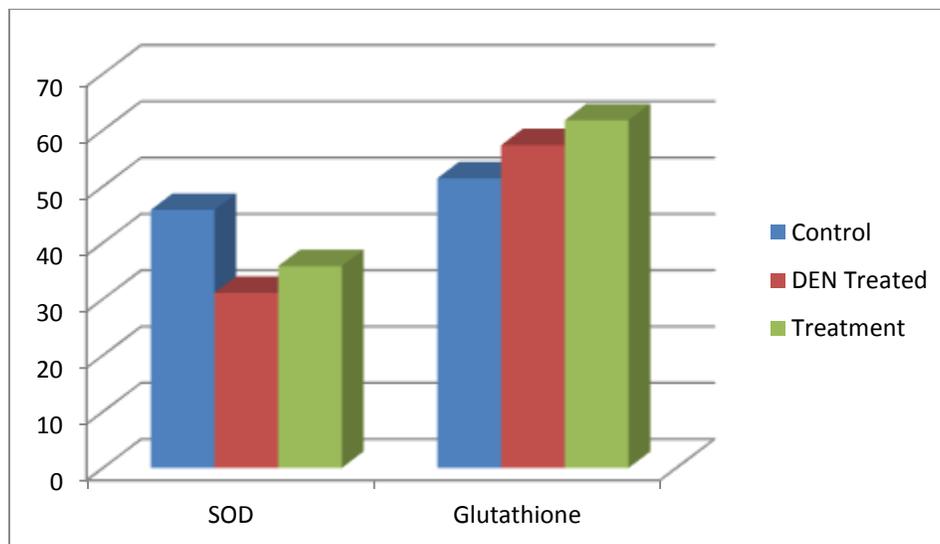
SOD activity

The mean of SOD in liver samples of control group was 45.82 ± 0.439 U/mg protein, which was significantly decreased in treated groups i.e. 31.07 ± 0.188 and 35.83 ± 0.239 U/mg protein in II and III groups respectively (Figure 2). The SOD levels in liver tissue decreased significantly in the DEN treated group as compared to the control and other group as well. The treatment with *Boerhaavia diffusa* extract has shown a significant increase in SOD level in liver tissue as compared to DEN treated rats but, its level was found to be lower than control group.

Table 2: Comparison of means \pm SE of weekly body weight (g) changes in different experimental groups

Groups/Weeks	Zero	1	2	3	4	5	6	7	8
Control (I)	206.92 ^a	207.33 ^a	208.08 ^a	209.00 ^a	210.08 ^a	211.34 ^a	212.92 ^a	214.62 ^a	216.43 ^a
Negative	± 1.65	± 1.66	± 1.61	± 1.63	± 0.56	± 1.54	± 1.45	± 1.43	± 1.40
Control (II)	202.51 ^a	202.50 ^b	203.62 ^b	204.62 ^b	202.25 ^b	199.62 ^b	196.87 ^b	193.97 ^b	190.90 ^b
Treatment (III)	± 0.87	± 0.87	± 0.97	± 0.91	± 0.98	± 0.99	± 0.81	± 0.75	± 0.95
	202.16 ^a	202.16 ^b	203.29 ^b	204.29 ^b	203.79 ^b	202.49 ^c	202.09 ^c	201.49 ^c	201.06 ^c
	± 1.15	± 1.15	± 1.20	± 1.19	± 1.14	± 1.10	± 1.13	± 0.98	± 0.97

Figures with different superscripts differs significantly ($P \leq 0.005$ and $P \leq 0.01$)

**Figure 2: Effect of *Boerhaavia diffusa* extract on SOD and Glutathione activity of liver****Glutathione activity**

Glutathione is a common enzyme found in nearly all the living organisms. The glutathione activity in liver samples of control group was 51.44 ± 0.226 U/mg protein but there was increase in glutathione activity in treated groups i.e. 57.32 ± 0.299 and 61.74 ± 0.232 U/mg protein in groups II and III respectively (Figure 2). There was significant increase in glutathione level in liver tissue in the DEN treated group as compared to the control and the glutathione level was found significantly higher in group III as compared to group II.

Zeliha *et al.*, (2009) treated adult female Wistar rats with DMBA and the novel organo-selenium compounds [1-isopropyl-3-Methylbenzimidazole-2-Selenone (Se I) and 1, 3 di-p-Methoxybenzyl pyrimidine-2- Selenone (Se II)] with the determined doses. They concluded that the Se I and Se II fully or partially restored enzyme activity. Lipid peroxidation was found to be decreased in Se I and Se II treated groups consequently. In the present study the results shows that *Boerhaavia diffusa* could not revert the changes in levels of glutathione in liver.

Biochemical investigations in kidney homogenate

MDA level

In rats fed on normal diet the mean value of MDA was 1.25 ± 0.008 nmol/L. The mean value of MDA in treated groups raised to 2.30 ± 0.146 and 1.25 ± 0.036 nmol/L in II and III respectively. The MDA level in kidney tissue in the DEN treated group increased significantly as compared to control and other (Figure 3). The treatment with extract of *Boerhaavia diffusa* showed the significant decrease in MDA level in kidney tissue as compared to DEN treated group.

Catalase activity

The Catalase activity in kidney samples of normal group was 0.75 ± 0.139 U/mg protein but Catalase activity in treated group was 0.42 ± 0.004 and 0.55 ± 0.03 U/mg protein in groups II and III respectively (Figure 3). There was significant decrease in Catalase activity in kidney tissue in DEN treated group as compared to the control group. Whereas, the extract of *Boerhaavia diffusa* significantly increased the Catalase activity in kidney homogenate as compared to group II.

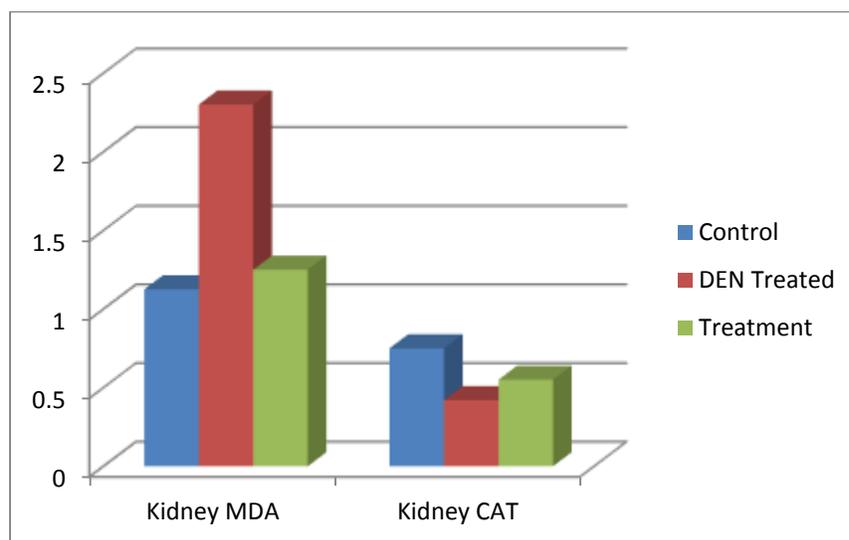


Figure 3: Effect of *Boerhaavia diffusa* extract on MDA and Catalase activity of kidney

SOD activity

The mean value of SOD in kidney samples of the control group was 46.12 ± 0.47 U/mg protein. The values decreased in treated groups i.e. 27.73 ± 0.211 and 35.17 ± 0.196 U/mg protein in groups II and III respectively (Figure 4). There was significant decrease in SOD level in kidney tissue in DEN treated group as compared to the control and treatment group as well. Whereas the treatment with extract of *Boerhaavia diffusa* showed significant increase in SOD level in kidney tissue as compared to DEN treated group but, level was found lower than control.

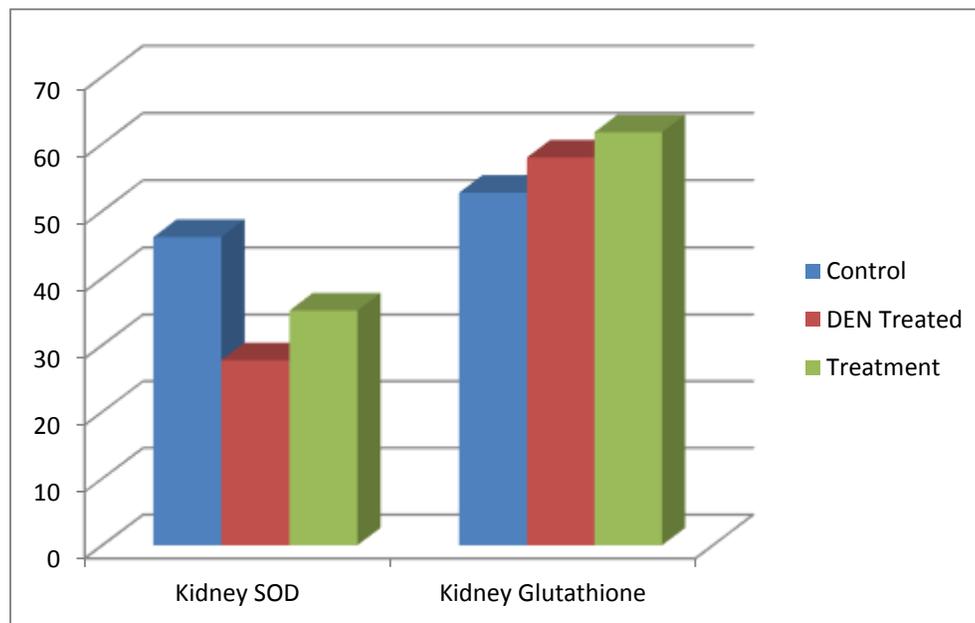


Figure 4: Effect of *Boerhaavia diffusa* extract on SOD and Glutathione activity of kidney

Glutathione activity

In rats fed on normal diet the mean value of glutathione was 52.71 ± 0.130 nmol/L. The mean values of glutathione in treated rats were 57.94 ± 0.092 and 61.72 ± 0.162 nmol/L in groups II and III respectively (Figure 4). There was significant increase in glutathione level in kidney tissue in the DEN treated group as compared to the control and other group as well. Whereas, the treatment of extract of *Boerhaavia diffusa* showed the significant increase in glutathione level in kidney tissue than the other groups.

Liver function tests

SGPT and SGOT levels

The mean SGPT and SGOT levels of control group were 158.83 ± 1.906 and 253.6 ± 3.916 U/L respectively. The mean value of SGOT raised in treated groups to 468.82 ± 3.521 , 365.74 ± 3.963 and SGPT levels raised to 377.58 ± 4.296 and 315.34 ± 9.755 in group II and III respectively (Figure 5). There was significant elevation in levels of SGOT and SGPT enzymes in DEN intoxicated rats when compared with that of control group. The SGOT and SGPT levels were significantly lowered in the group treated with the extract of *Boerhaavia diffusa* as compared to DEN treated group.

Alkaline and Acid phosphatase levels

The mean values of alkaline phosphatase (ALP) and acid phosphatase (ACP) in control group was 194.74 ± 10.0 and 53.52 ± 0.518 U/L. The level of ALP and ACP was significantly raised to 370.74 ± 4.803 , 312.88 ± 5.801 , 83.80 ± 0.752 and 69.135 ± 1.502 U/L in groups II and III respectively. Treatment with extract of *Boerhaavia diffusa* has shown the significant decrease in ALP and ACP

levels as compared to DEN treated group, but level was found to be higher than the control group (Figure 5). Sadeghi *et al.*, (2008) evaluated the hepato protective activity of hydro-alcoholic extract of *Cichorium Intybus* using CCl₄ induced liver injury in rats. The leaf extract at oral dosage 200, 400, and 500 mg/Kg body weight exhibited significant protective effect against CCl₄ induced hepatotoxicity. Levels of serum markers such as ALT, AST, ALP were significantly increased in CCl₄ treated rats. Simultaneously, *Cichorium Intybus* extract significantly suppressed mainly the increase in plasma activities of AST, ALT and ALP concentrations which are considered as markers of liver functional state. The results of the present study confirmed the hepatoprotective activity of the *Boerhaavia diffusa* extract.

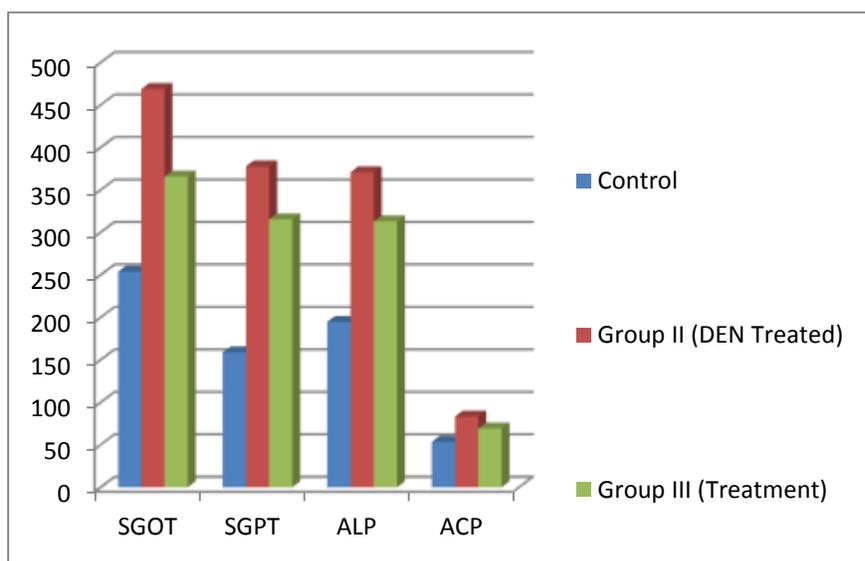


Figure 5: Effect of *Boerhaavia diffusa* extract on SGOT, SGPT, Alkaline phosphatase and Acid phosphatase activity

Total Proteins

In control rats on normal diet the mean value of total proteins was 6.16 ± 0.079 U/L. The mean values decreased to 2.93 ± 0.098 and 2.915 ± 0.561 U/L in groups II and III respectively. There was significant decrease in total proteins level in DEN treated group as compared with control group (Figure 6). Treatment with the extract of *Boerhaavia diffusa* has also shown the significant decrease in total proteins level.

Albumin and Globulin levels

In control rats on normal diet the mean values of albumin and globulin were 3.64 ± 0.93 and 2.51 ± 0.076 U/L respectively. These were decreased to 1.78 ± 0.64 , 1.64 ± 0.805 , 1.15 ± 0.046 and 1.26 ± 0.05 U/L in group II and III respectively (Figure 6). There was significant decrease in albumin and globulin levels in DEN treated group. The albumin, globulin ratio in control group was

1.46±0.074 U/L it was significantly higher in group II i.e. 1.56±0.051 and marginally lower in group III i.e. 1.45±0.052 as compared with control group.

Bilirubin

The mean values of Bilirubin in control group were 0.078±0.002 U/L, which was also significantly raised to 1.06±0.012 and 0.084±0.019 U/L in group II and III respectively (Figure 6). There was no significant difference in control group and the group treated with extract of *Boerhaavia diffusa*.

Creatinine

In control rats fed on normal diet the mean value of Creatinine was 0.70±0.008 U/L. This level was raised to 2.70±0.064 and 1.33±0.044 U/L in group II and III respectively (Figure 6). The test was done to evaluate kidney function. The results showed that the kidney function was abnormal as the Creatinine level was increased in the blood of treated groups due to decreased excretion of the Creatinine in urine.

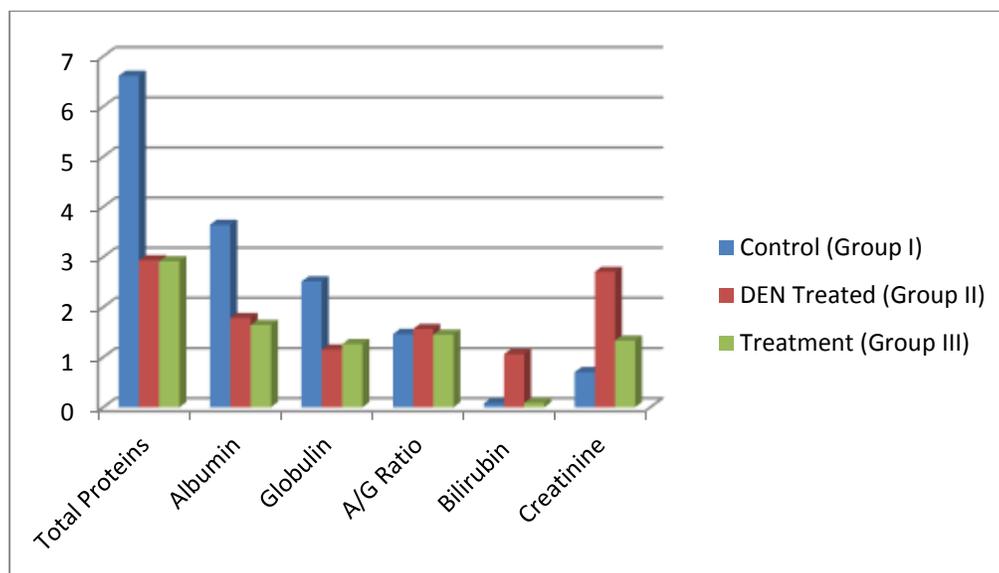


Figure 6: Effect of *Boerhaavia diffusa* extract on Total proteins, Albumin, Globulin, A/G ratio, Bilirubin and Creatinine levels

Lipid Profile

In rats on normal diet the mean values of total cholesterol, HDL-cholesterol and LDL-cholesterol were 541.87±2.957, 224.63±6.399 and 228.66±7.572 U/L respectively. The levels of total cholesterol and HDL-cholesterol were significantly decreased to 441.50±5.951, 491.865±13.440, 158.60±5.590, 197.005±5.573 in groups II and III respectively. Whereas, the level of LDL-cholesterol was significantly increased to 257.30±3.966 and 282.745±9.493 U/L in groups II and III respectively (Figure 7). The ratio of LDL/HDL was found to be 1.28±0.060, 1.56±0.04 and

1.42±0.27 in control and treatment groups respectively, which was significantly higher in group II and III as compared to control group.

The mean value of triglycerides was 116.08±1.549 U/L in control group. The values of triglycerides were increased significantly to 90.5±1.296 and 100.79±5.205 U/L in groups II and III respectively (Figure 7). Treatment with extract of *Boerhaavia diffusa* showed significant decrease in triglycerides level as compared to control group, but the level was found to be significantly higher than the DEN treated group.

Guptha BHMRK *et al.*, (2018) reported that rats given ethanolic extract of *Chloroxylon swietenia* showed the higher values of total cholesterol, LDL, VLDL and triglycerides in treated animals than in control animals whereas, the level of HDL was significantly reduced. Necrosis or membrane damage releases the enzyme in to circulation therefore it can be measured in serum. High levels of SGPT and SGOT indicate liver damage. ALT catalyses the conversion of alanine to pyruvate and glutamate and it is released in a similar manner. Therefore SGPT is more specific to liver and is thus a better parameter for detecting liver injury (Williamson *et al.*, 1996). Pandey and Kumar (2007) reported that aqueous extract of *Ganoderma lucidum* reduced the level of SGOT, SGPT and ALP by 58.8%, 44.4% and 66.0% respectively.

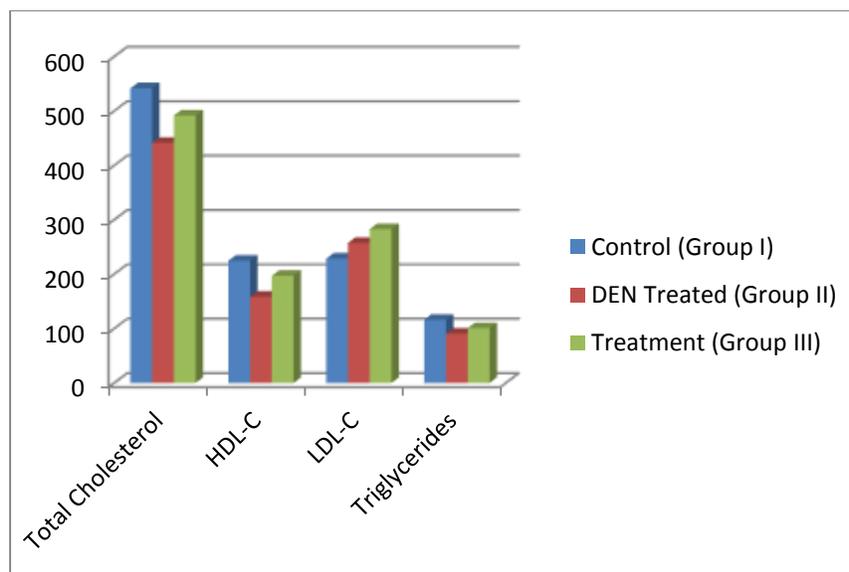


Figure 7: Effect of *Boerhaavia diffusa* extract on Total Cholesterol, HDL-C, LDL-C and Triglycerides

Pathological Examination

Gross Pathology

Gross pathological studies also provide supportive evidence for biochemical analysis. The carcinogen mediated changes in color and size of liver of rats treated with extract of *Boerhaavia*

diffusa after DEN intoxication than those observed in rats treated only with DEN. Cysts were observed on liver of rats of group II (Figure 8).



Figure 8: Liver with cyst formation in rat treated with DEN

Rats treated with extract of *Boerhaavia diffusa* have not shown any cyst formation, but enlargement and paleness in liver was observed (Figure 9). In group II results showed more liver enlargement, paleness and proliferation as well.



Figure 9: Liver with paleness and enlargement in rat treated with the extract of *Boerhaavia diffusa*

Kidney and heart were found to be normal in all groups. There was no enlargement or change of color in kidneys and heart of rats from all the three groups. M Halim Eshrat (2003) reported that the rats suffering with diabetes showed increase in weight of kidneys which was decreased in the treatment group given aqueous extract of *A. augusta* and *C. indica*. In the present study there was prominent enlargement in liver of rats treated with DEN with cyst formation. These finding suggests that DEN has carcinogenic effect on vital organ.

Histopathology

Histopathological studies of liver showed nodular formation of distinct focal areas of hepatocytes. These were lacking sinusoids. Blood vessels showed engorgement in hepatic lobules. Bile ducts were dilated and some hepatocytes also showed vacuolations in group II rats.



Figure 10: Liver with treatment group showing degenerated hepatocytes with enlarged nuclei along with hepatochromic cytoplasm and hyperplasia of biliary cells

However, degenerated hepatocytes with enlarged nuclei along with hypo chromatic cytoplasm and hyperplasia of biliary cells were seen along with dilated bile ducts and focal vacuolar degeneration in liver cells of group III rats (Figure 10).

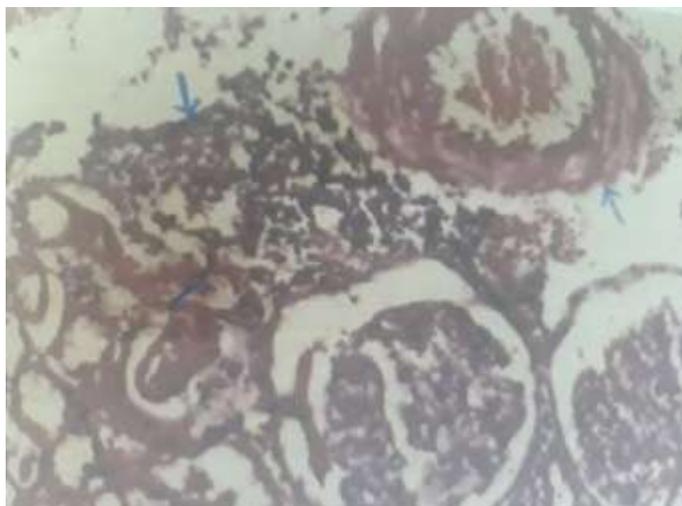


Figure 11: Kidney of treatment group showing periglomerular and perivascular mononuclear infiltration

In the kidneys of group II rats, renal tubules looked dilated at cortico-medulary junction areas, glomeruli looked hypertrophied. Kidneys of group III showed that the pelvis was dilated along with mononuclear infiltration, periglomerular and perivascular mononuclear infiltration were also seen

(Figure 11). The histopathological changes observed in liver and kidney explains the carcinogenicity caused by DEN in group II rats.

CONCLUSION

In the present study the extracts of *Boerhaavia diffusa* prevented the weight loss and have shown positive effect on growth of rats. *Boerhaavia diffusa* could bring drastic positive changes in liver functions, lipid profile and kidney functions by significantly reducing the effects of DEN. Gross and histopathological examination also revealed that the extract of *Boerhaavia diffusa* could reduce the carcinogenic effect of DEN. On the basis of the present study it is concluded that *Boerhaavia diffusa* is a potent oxidant/ hepatoprotective agent.

ACKNOWLEDGEMENT

Authors are thankful to the management and Director of S.B.S.P.G.I., Balawala, Dehradun, Uttarakhand, India for providing all the facilities to carry out this study. Authors are also thankful to Dr. R. Somvanshi, Principal Scientist, Division of Pathology, IVRI, for carrying out the histopathological studies of the samples.

REFERENCES

1. Boyle P and Levin B. (eds.) World Cancer report 2008, Geneva : IARC : 2008.
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics. CA Cancer J. Clin. 2017;67(1):7-30.
3. Jemal A, Rebecca Siegel, Elizabeth Ward, Taylor Murray, Jiaquan Xu and Michael J Thun. Cancer Statistics. CA Cancer J. Clin. 2007;57:43-66.
4. Druckrey H, Preusmann R, Ivankovic S, Schmahl D. Organotrope carcinogen Wirkungen bei 65 verschiedenen N-nitroso-Verbindungen an BD-Ratten [organotropic carcinogenic effects of 65 various N-nitroso compounds on BD rats]. Z. Krebsforsch. 1969; 69:103-201.
5. Peto R, Gray R, Brantom P and Grasso P. Effects on 4080 rats of chronic ingestion of N-nitroso diethylamine or N-nitroso dimethylamine : a detailed dose response study. Cancer Res. 1991;51: 6415-6451.
6. Verna L, Whysner J and Williams G M. N-nitroso diethylamine mechanistic data a risk assessment bioactivation. DNA adduct formation, mutagenicity and tumor initiation. Pharmacol. Ther. 1996;71:57-81.
7. Kang J S, Wanibuchi, H, Morimura K, Gonzalez F J and Fukushima S. Role of CYP-ZEI in diethyl nitrosamine induced hepato-carcinogenesis in-vivo. Cancer Res. 2007; 67:11141-11146.

8. R Tolba, T Kraus, C Liedtke, M Schwarz and R Weilskrichen. Diethyl nitrosamine (DEN) induced carcinogenic liver injury in mice. *Laboratory Animals*. 2015;Vol.49(S1): 59-69.
9. Shweta Majgaine and DL Verma. Antibacterial Activity of *Boerhaavia diffusa* L. (Punarnava) On certain Bacteria. *IOSR Journal Of Pharmacy*, (2017) ; Volume 7, Issue 1 Version.2:Jan 2017;01-13.
10. Mishra H P. and Fridouovich I. Role of superoxide and ion in the auto oxidation of epinephrine and simple assay for SOD. *J. Biol. Chem.*1972;247: 370.
11. Beers R F and Seezers I W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. *J. Biol. Chem.*1952;195: 133-140.
12. Lowery O H, Farr A L and Randall R J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*1951;193:265.
13. Esterbauer H and Cheeseman K H. Determination of aldehyde lipid peroxidation product. Malonaldehyde and hydroxynoneal. *Methods in Enzymology*.1993;186:407.
14. Ramjee G, Berjak P, Adhikari M, Dutton M F. Aflatoxins and Kwashiorkor in Durban. South Africa. *Ann. Trop. Paediatr.* 1992; Vol. 12:241-248.
15. Bedi P S, Singh Harpreet, Johri T S and Agarwal R K. Effects of aflatoxin on growth and feed consumption in Guinea fowl. *XX World Poultry Congress*. 1996;Proc.Vol.(3): 665-667.
16. Zeliha Selamoglu Talas, Ismet Yilmaz, Ilknur Ozdemir, Burhan Ates, Yetkin Gok and Bekir Cetinkaya. Role of synthesized organoselenium compounds on protection of rat erythrocytes from DMBA induced oxidative stress. *Biol. Trace Elem. Res.*2009;128: 167-175.
17. Sadeghi Heibatollah, Nikbakht Mohammad Reza, Ghaitasi Izadpanah and Sabzali Sohailla. Hepatoprotective effect of *Cichorium intybus* on CCl₄ induced liver damage in rats. (2008). *African J. of Biochem. Res.*2008; Vol. 2 (6):141-144.
18. Guptha BHMRK, Kadali RMSLDV, Kumar VM, Revanasiddappa BC. Anti hyperlipidemic activity of *Chloroxylon swietenia* in triton WR 1339 induced hyper lipidemia. *Intl. J. Basic Clin. Pharmacol.* 2018;Vol.(7):518-523.
19. Williamson EM, Okpako DT, and Evans FJ. Selection, preparation and pharmacological evaluation of plant material.1996 John Wiley Publication, England. P. 13.
20. Pandey Maneesha and Kumar Saurabh Kapil. Antioxidant and protective effects of *Ganoderma lucidum* against CCl₄ induced toxicity in rats and evaluation of antimicrobial efficacy. M. Sc. Thesis (Biochemistry),2007. SBSPGI, Balawala, Dehradun, India.

21. M Halim Eshrat. Effect of *Coccinia indica* (L.) and *Abroma augusta* (L.) on glycemia, lipid profile and on indicators of end organ damage in streptozotocin induced diabetic rats. Ind. J. of Clinical Biochemistry 2003;18(2):54-63.

AJPTR is

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: editor@ajptr.com

