Hepatoprotective effects of ethanolic extract of *Boerhaavia diffusa* against oxaliplatin induced hepatotoxicity in albino rats

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Abstract: The hepatoprotective effects of *Boerhaavia diffusa* was studied against the hepatotoxicity induced by oxaliplatin. Male Wistar rats were divided in three groups.Group N* control group (0.9% normal saline), Group NP0 oxaliplatin treated group and Group NP2 were prophylactically treated with *Boerhaavia diffusa* and then with oxaliplatin in order to assess the protective effects of *Boerhaavia diffusa* against the toxicity of oxaliplatin. The levels of liver enzymes ALT, AST and γ-GT were significantly reduced in the group prophylactically treated with *Boerhaavia diffusa* (NP2) compared with the group treated with oxaliplatin (NP0). *Boerhaavia diffusa* was effective in reducing risk of hypercholestremia associated with oxaliplatin. Histopathological examination of rat liver revealed that prophylactically treated group with *Boerhaavia diffusa* was effective in reducing oxidative stress induced steatohepatitis by oxaliplatin.

Keywords: *Boerhaavia diffusa*, hepatotoxicity, Oxaliplatin, wistar rats.

INTRODUCTION

Platinum derivative, Oxaliplatin possesses considerable activity in colorectal cancer (Carrato *et al.,* 2002). Irinotecan and oxaliplatin both have a significant effect in metastatic disease (Souglakos *et al.,* 2002; Schmoll, 2002). Oxaliplatin based chemotherapy regimen caused sinusoidal obstruction syndrome (SOS) which were associated with veno-occlusive lesions and sinusoidal fibrosis and rarely related with nodular regenerative hyperplasia (Alexandrino *et al.,* 2015). The sinusoidal lesions observed were morphologically comparable to those lesions seen in veno-occlusive disease (Eberhardt *et al.,* 2000). The sinusoidal wall integrity is disrupted when the strength of the sinusoidal dilatation and congestion become severe. Sinusoidal wall integrity was well explained via the erythrocytes extravasation in Disse's space and by help of ultra structural studies and immune histochemical, which express the lack of endothelial lining in the dilated areas (Rubbia-Brandt *et al.,* 2004).

The alcoholic extract of roots of *Boerhaavia diffusa* display a considerable protecting action against liver, indicated by a decrease in high levels of serum lysosomal enzymes specifically ALT and AST (Goyal *et al.,* 2010; Chandan *et al.,* 1991). The aqueous extract of roots can treat ascites and edema due to liver cirrhosis or chronic peritonitis. *Boerhaavia diffusa* have protective effects against hepatic injury caused by thioacetamide (Rawat *et al.,* 1997). It enhances bile flow signifying a strong choleretic activity and did not illustrate any signs of toxicity up to an oral dose of 2 g/kg in animals (Salman Khan *et al.,* 2013). The hydro alcoholic extracts of *Boerhaavia diffusa* show protective effects against acetaminophen stimulated hepatic damage by lowering the levels of ALT, AST, lactate dehydrogenase and total bilirubin and protect against lipid peroxidation induced by acetaminophen (Olaleye *et al.,* 2010; Govindarajan *et al.,* 2005). The extract of methanol and chloroform shows inhibition of propagation of human cervical cancer, HeLa cell lines. The inhibition of cell cycle in S-phase and apoptosis are important factor in antiproliferative property. The components which have anti proliferative activity are liriodendrin, boeravinones, lignans and syrigaresinol (Srivastava *et al.,* 2011). The chemo-preventive property of *Boerhaavia diffusa* was assessed in skin papillomagenesis induced by 7,12-dimethyl-benz (a) anthracene (DMBA) (Hiruma-Lima *et al.,* 2000).

The purpose of this study was to reduce the hepatotoxic effects of oxaliplatin and enhances the clinical efficacy of oxaliplatin by using the herbal drug which possesses hepatoprotective effects (*Boerhaavia diffusa*).

MATERIALS AND METHODS

Study design

The experimental study was designed in the Department of Pharmacology, Ziauddin University and conducted in DUHS (Dow University of Health Sciences), succeeding institutional and ethicalapproval. *Boerhaavia diffusa* was selected after 15 year literature survey to overcome the liver toxicity of oxaliplatin. Three groups each containing

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6 animals were designed N* is the group receiving normal saline 0.9 %, which is used as s control to assess the changes in the other two groups i.e. NP0 (receiving oxaliplatin) and NP2 (receiving prophylactic treatment with *Boerhaavia diffusa* and then oxaliplatin).Second phase of our study is to assess the changes in the enzymes levels of hepatic markers and lipid profile of each group following histological examination of liver tissue of each group.

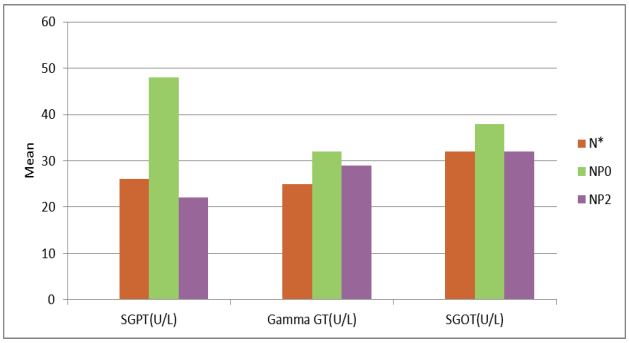

Fig. 1: Comparative hepatic profile.

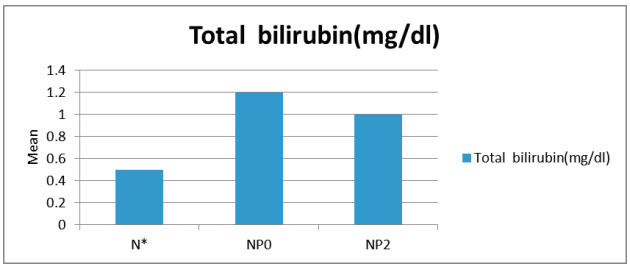

Fig. 2: Comparative Total bilirubin levels.

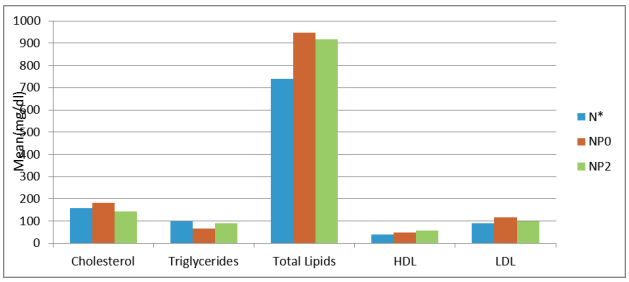

Fig. 3: Comparative lipid profile.

Animal protocol

Male albino Wistar rats (220-320g) were obtained from Dow University animal house which were chosen from inbred species and divided into three treatment groups of 6 animals in each group. Animals were housed in properly ventilated, spacious animal house of Dow University of health sciences. Relative humidity (65-75%) and temperature 23±2°C was maintained with alternating 12 hr light and 12 hr dark cycles. Animal Food and tap water was provided *adlibitum*.

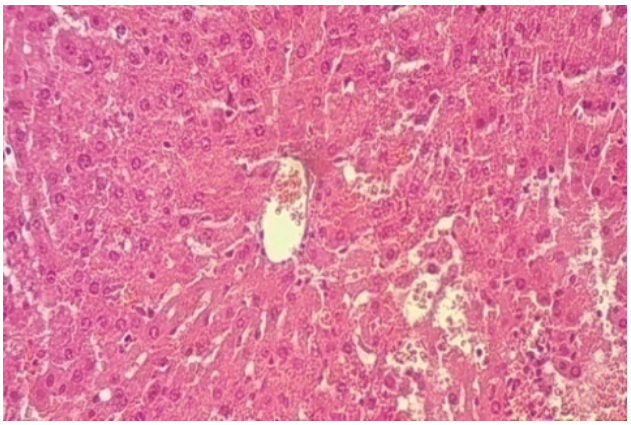

Fig. N* (H&E Stain) - 200x

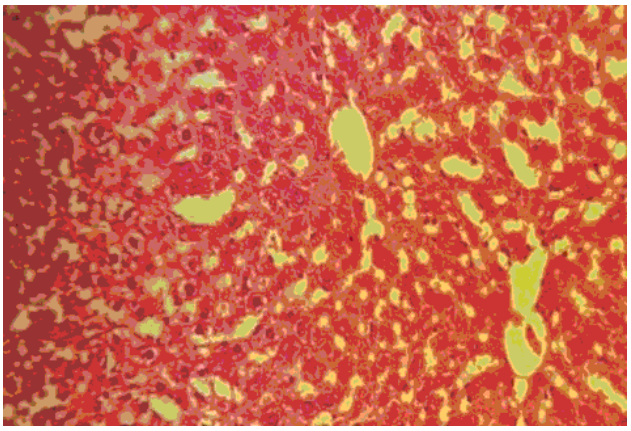

Fig. NP2 (H&E Stain) - 200x

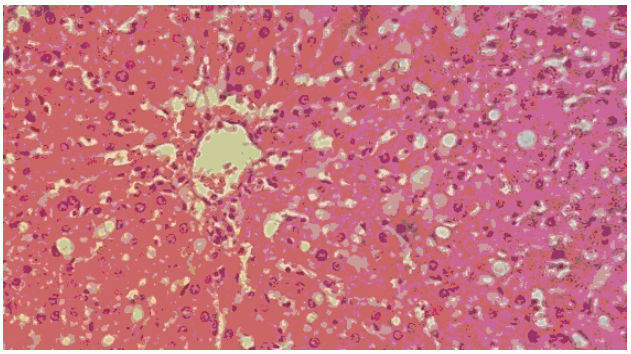

Fig. NP0 (H&E Stain) - 200x

Extract preparation

One thousand grams freshly collected roots of *Boerhaavia diffusa* were washed and air dried. Air dried leaves were finely grinded using a standard grinding machine. Powder drug was macerated in ethanol for 48 hours. 10 grams of powdered roots were extracted out successively with 100ml of ethanol. Extraction was carried out in standard soxhlet apparatus at 40-50°C. Rotary evaporator was used to concentrate the extract to dryness. The concentrated extract was stored in a refrigerator at 4°C. The extracts were prepared by using analytical grade ethanol.

Table 1: Comparative hepatic profile of NP2 (*Boerhaavia diffusa* group) with NP0 (oxaliplatin group)

Paired Samples Test - Hepatic profile									
Hepatic profile			Mean	Std. Deviation	Paired Differences		t-value	df	p-value
					Mean	Std. Deviation			
Alanine Amino Transferase (ALT) (U/L)	NP0 - NP2	NP0	48.000	0.632	26.000	1.414	45.033	5.000	0.000
		NP2	22.000	1.789					
		NP1	30.000	1.414					
Gamma Lutamyl Transferase (U/L)	NP0 - NP2	NP0	32.000	1.414	3.000	2.000	3.674	5.000	0.014
		NP2	29.000	2.280					
Aspartate Aminotransferase (AST) (U/L)	NP0 - NP2	NP0	37.830	0.753	6.000	2.000	7.348	5.000	0.001
		NP2	31.830	1.329					
Total Bilirubin (mg/dL)	NP0 - NP2	NP0	1.333	0.216	0.300	0.529	1.389	5.000	0.224
		NP2	1.033	0.344					

p-value< 0.05 (Significance at 5%), p-value < 0.01 (Significance at 1%), p-value < 0.001 (Significance at 0.1%)

Table 2: Comparative lipid profile of NP2 (*Boerhaavia diffusa* group) with NP0 (oxaliplatin group)

Paired Samples Test - Lipid profile									
			Mean	Std. Deviation	Paired Differences		t-value	df	p-value
					Mean	Std. Deviation			
Cholesterol (mg/dL)	NP0 - NP2	NP0	181.000	1.414	39.000	3.521	27.129	5	0.000
		NP2	142.000	2.280					
Triglycerides(mg/dL)	NP0 - NP2	NP0	65.830	5.076	-25.167	3.312	-18.615	5	0.000
		NP2	91.000	2.000					
TotalLipids (mg/dL)	NP0 - NP2	NP0	947.000	9.675	30.000	5.899	12.457	5	0.000
		NP2	917.000	3.950					
HDL (mg/dL)	NP0 - NP2	NP0	48.000	7.694	-8.000	10.714	-1.829	5	0.127
		NP2	56.000	7.071					
LDL (mg/dL)	NP0 - NP2	NP0	118.000	8.099	20.000	10.881	4.502	5	0.006
		NP2	98.000	2.828					

p-value < 0.05 (Significant), p-value < 0.01 (highly significant), p-value < 0.001 (very highly significant)

Dilutions

Boerhaavia diffusa extract concentrate was prepared in 5% DMSO solution (dimethyl sulfoxide with water for injection) for administration by oral route. Water for injection was used as a vehicle in administration of oxaliplatin by i.p. route.

Treatment schedule

The male wistar rats were randomized to experimental and control groups, and divided into 3 treatment groups comprising of 6 animals in each group.

Group N*

Animals in group N* served as the control group, where 2ml 0.9% normal saline was administered i.p.for 6 weeks.

Group NP0

Animals in group NP0 received oxaliplatin 0.8mg/kg body mass/day intraperitoneally for 1week (Najam *et al.,* 2014).

Group NP2

Animals in Group NP2 were administered prophylactically *Boerhaavia diffusa* orally 400mg/kg body mass/day for 6 weeks and then oxaliplatin 0.8mg/kg for 1 week.

Animals were housed for 56 days in animal house. Control time before and after dosing is 7days with sufficient rat chow. Scheduled sacrifice/cardiac puncture/organ excision was carried out on 7th day after last dose i.e. on day 56.

Biochemical assessment

Cardiac puncture was done to collect the blood in anticoagulant tubes, 3ml in Lavender top EDTA (Ethylene diamine tetraacetic acid) tube, 3ml in Light blue top Citrate tube and 3ml in Green top Heparanized tube. The blood cells are separated from the plasma by centrifugation for 10 minutes (1000-2000×g) in a refrigerated centrifuge. The centrifuge time iskept at 10 minutes to attain designated plasma for serological testing immediately after transferring the plasma into polypropylene tubes with Pasteur pipettes at a temperature of 2-8°C.

The following parameters were assessed by biochemical testing using standardized kits.

1. Hepatic profile (Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Gamma - glutamyl transpeptidase (GGT) and Total bilirubin.
2. Lipid profile (LDL, HDL, TGS, Total Cholesterol and Total lipids).

Histopathological examination

The livers were collected from all the groups, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast at 57-63ºC. Four micron thick sections were obtained, stained by H&E (Hematoxylin & Eosin) and Periodic Acid Schiff (PAS). Any structural or morphological changes in the stained cells of each tissue (organ) were evaluated under bright field microscope with the components, Model U-MDOB3, SN 6M 02703, Olympus corporation (device complies with par 15 of FCC rule) and Model LB X 41TF, T5 SN 7A25800, Olympus corporation. The slides were coded and examined along with a skilled pathologist unaware of the treatment (blinded assessment).

STATISTICAL ANALYSIS

Data was analyzed on SPSS version 19 with pairedsample test, p value <0.05 was considered significant, p value <0.01 highly significant and p value <0.001 was considered very highly significant.

RESULTS

Hepatic profile

As shown in table 1 and fig.1, the mean levels of Alanine aminotransferase were decreased very highly significantly ($p<0.001$) in the group treated prophylactically with *Boerhaavia diffusa* (NP2) compared with the standard group (NP0). Whereas the GGT and Aspartate aminotransferase levels were decreased significantly ($p<0.05$) compared with the standard group (NP0). Figure 2 and table 1 show Levels of total bilirubin were insignificantly affected ($p>0.05$) compared with the standard.

Lipid profile

Table 2 shows the difference in the lipid levels in treatment group (NP2) with the animals included in the standard group (NP0). As shown in table 2 and fig. 3, the levels of cholesterol and total lipid content were very highly significantly decreased ($p<0.001$) and LDL levels were highly significantly decreased ($p<0.01$) in the group prophylactically treated with *Boerhaavia diffusa* (NP2) compared with the standard (NP0). Triglycerides levels were very highly significantly increased ($p<0.001$) in the treated group NP2 compared with the standard (NP0). HDL levels were insignificantly affected ($p>0.05$) in the group treated prophylactically with *Boerhaavia diffusa* compared with the standard (NP0).

Liver histopathology

Fig. N*A shows that the group receiving normal saline 0.9%, section of liver reveals intact architecture. The hepatocytes are unremarkable with intact Portal tract and central vein. Fig. NP0A reveals that the group receiving oxaliplatin showing hepatocytes with intact architecture. There were hydropic changes in the hepatocytes, sub capsular area was observed, lobular inflammation, mild to moderate portal inflammation was observed. Fig. NP2A reveals that the group receiving prophylactically *Boerhaavia diffusa*, shows hepatocytes with intact architecture. There were no hydropic changes in the hepatocytes. No lobular inflammation however mild portal inflammation was present. The sinusoids and central veins were mildly dilated and congested.

DISCUSSION

Increased levels of Alanine aminotransferase, Gamma glutamyl transferase, Aspartate amino transferase and total bilirubin were observed in patients receiving Oxaliplatin (Gurzu *et al.,* 2013). Levels of Alanine aminotransferase, Aspartate aminotransferase and Gamma glutamyltransferase were decreased in the group prophylactically treated with *Boerhaavia diffusa* (NP2) compared with the standard (NP0) (fig.1). The Alanine aminotransferase (ALT) originates primarily from the hepatocytes and was more specific marker of liver disease (Pratt *et al.,* 2000).Whereas Aspartate aminotransferase (AST) is also abundant in heart, skeletal muscle tissue, kidneys and the brain and AST often shows increased activities due to extrahepatic reasons, including muscle diseases or strenuous exercise (Pratt *et al.,* 2000). This indicates that the drug *Boerhaavia diffusa* possesses hepatoprotective effects because the prophylactic administration of *Boerhaavia diffusa* decreases the levels of ALT and AST. Total bilirubin was not significantly reduced in the group prophylactically treated with *Boerhaavia diffusa* compared with the standard (NP0) (fig. 2). But *Boerhaavia diffusa* enhances bile flow signifying a strong choleretic activity (Salman Khan *et al.,* 2013). The increased bilirubin production may be due to the deficiencies in bilirubin metabolism or because of obstruction of the bile ducts (Jungst *et al.,* 2013).Therefore, jaundice may be present and the drug has no potential to decrease the jaundice associated with oxaliplatin. Elevated serum ALT and Gamma glutamyltransferase were independent markers of the activation of systemic inflammation and increased oxidative stress (Yamada *et al.,* 2006). This indicates that *Boerhaavia diffusa* was effective in reducing the oxidative stress induced inflammation caused by chemotherapy because it decreases the levels of Gamma glutamyltransferase and ALT. Oxaliplatin caused steatohepatitis which is due to oxidative stress produced by this drug. *Boerhaavia diffusa* may decreases the incidence of steatohepatitis induced by oxaliplatin,

because it possess antioxidant and anti-inflammatory activity which is also indicated by decreased levels of Gamma glutamyl transferase. Oxaliplatin induced hepatic damage associated with progressive inflammation is referred to as Chemotherapy associated steatohepatitis (CASH) (Schwingel *et al.,* 2014). Histological results show that prophylactic treatment with *Boerhaavia diffusa* (NP2) overcomes the hydropic changes and decreased dilation of sinusoids and central vein induced by Oxaliplatin compared with standard NP0 (fig. NP2A). Histological result shows hepatocytes with intact architecture. There were no hydropic changes in the hepatocytes. No lobular inflammation however mild portal inflammation was present (fig. NP2A) i.e. the drug is effective in reducing inflammation.

The pathogenesis of Non alcoholic fatty liver disease is based on a ‘‘two-hit hypothesis’’ (Donnelly *et al.,* 2005). The first hit involves triglyceride accumulation within hepatocytes, which results in simple steatosis (Marchesini *et al.,* 1999). The second hit was primary lipotoxicity caused by oxidative stress from increased lipid peroxidation, high reactive oxygen species production within hepatocytes, mitochondrial dysfunction and inflammation (Wobser *et al.,* 2009). The sinusoid and central vein were mostly dilated and congested and show mild to moderate lobular and portal tract inflammation (fig NP0A). This indicate that the Oxaliplatin induced hydropic changes was may be due to oxidative stress induced lipotoxicity, because the standard group (NP0) biochemical analysis show increased levels of cholesterol and Gamma glutamyltransferase (figs. 3 & 1). *Boerhaavia diffusa* may decrease steatohepatitis because it possesses lipid lowering and antioxidant activity. Chemotherapy with oxaliplatin causes obstruction of sinusoids which was associated with veno-occlusive lesions and sinusoidal fibrosis (De Leve *et al.,* 2002). Venoocculsive lesion occurred in sequence of steatohepatitis and the drug *Boerhaavia diffusa* decreased the inflammation induced by Oxaliplatin. This indicates the drug *Boerhaavia diffusa* decreases the incidence of sinusoidal obstruction syndrome indirectly by decreasing the oxidative stress induced inflammation associated steatohepatitis.

Hypercholestremia was observed in patients treated with oxaliplatin (Najam *et al.,* 2014). Triglycerides levels were found to be reduced in the group NP0 compared with the control. High triglycerides levels were the delayed toxic manifestation of Oxaliplatin in rats (Najam *et al.,* 2014). This study also reveal increased levels of cholesterol in the group receiving Oxaliplatin alone and decreased triglyceride levels compared with the control (N*). Levels of cholesterol and LDL were decreased in the group treated prophylactically with *Boerhaavia diffusa* compared with the standard (NP0) (fig. 3). Triglycerides levels were found to be increased in the treated group (NP2) compared with the standard but histological results did not indicate accumulation of fats in liver tissue. This indicates that the increased levels of triglyceride were in the normal range. Both male and female wistar rats showed increased lipid amount in the liver and increased triglycerides levels as compared to the other rat species (Willebrords *et al.,* 2016). *Boerhaavia diffusa* protects against lipid peroxidation induced by acetaminophen (Olaleye *et al.,* 2010). Levels of total lipid content and HDL were not affected compared with the standard (NP0). Histological examination reveals that the group receiving prophylactic treatment with *Boerhaavia diffusa* showed hepatocytes with intact architecture. There were no hydropic changes in the hepatocytes (fig. NP2A). This indicates that the *Boerhaavia diffusa* had the potential to overcome steatosis produced by oxaliplatin by decreasing the lipid peroxidation and oxidative stress.

CONCLUSION

Prophylactic treatment with *Boerhaavia diffusa* demonstrates hepatoprotective effects against the toxicity induced by Oxaliplatin. *Boerhaavia diffusa* decreases the levels of hepatic enzymes i.e. Alanine aminotransferase, Gamma glutamyltransferase and Aspartate aminotransferase. Prophylactic treatment with *Boerhaavia diffusa* was effective in reducing the hypercholesteremia associated with Oxaliplatin. Histological examination also indicates that drug overcomes the fatty changes andoxidative stress associated inflammation induced damages in the liver tissue. Prophylactic treatment with *Boerhaavia diffusa* is effective in reducing oxidative stress induced steatohepatitis by oxaliplatin.

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