ORIGINAL ARTICLE

EFFECT OF MORINGA OLEIFERA LEAVES ON BISPHENOL-A INDUCED HISTOLOGICAL CHANGES OF HEPATOCYTES IN ALBINO RATS

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ABSTRACT

Objective: To reveal the effects of Moringa Oleifera leaves extract on Bisphenol-A generated variations (Diameter of hepatocytes and vacuolization) in liver of rats.

Methods: It was experimental study, conducted at Post Graduate Medical Institute. This study was performed on 32 adult rats, which were then randomly separated into 4 groups A, B, C and D. The experiment lasted for 42 days. Group A was control received corn oil only. Group B, received BPA only 50mg/kg/bw. Group C and D received BPA 50mg/kg along with *MoLE* 250mg/kg and 500mg/kg. Liver was removed and fixed in 10% formalin. To observe the effect of BPA and *MoLE*, slides were prepared for histological examination. The diameter of hepatocytes and vacuolization was observed. The evaluation of results was done by using SPSS 21**.**

Results: In group B, vacuolization was seen in 87.5% of animals and significant increase in mean diameter (19.7±1.3 µm) of hepatocytes was seen. However in Groups C, 50% of animals showed vacuolization and mean hepatocyte diameter was 17.0±1.1µm. In Group D, 25% of animals showed vacuolization and mean hepatocyte diameter decreased to14.6±1 after administration of *MoLE.*

Conclusion: Administration of *MoLE* (250mg/kg & 500mg/kg) exerts a protective effect against damaging effects of BPA (50mg/kg) on hepatocytes in Albino rats. This hepatoprotective role becomes greater with increasing the dose of *MoLE*.

Key words: BPA: Bisphenol-A, *MoLE*: Moringa Oleifera Leave extract.

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INTRODUCTION

Bisphenol-A (BPA) is a compound that has been utilized globally for formation of plastics products and epoxy resins[1]. It is found in disposable plastic ware, children toys, baby bottles, dental fillings and paints[2] and to coat the inside of food and beverages metallic cans[3].

Human are at risk due to the use of food and drinks, packed or stored in containers made with BPA. When temperature rises, BPA molecules undergo hydrolysis and leaching of BPA into surrounding takes place. Liver is the organ which is mainly affected following an oral exposure to BPA, disturbing its oxidant/antioxidant status by generating Reactive oxygen species (ROS)[4,5]. ROS are considered as harmful for cell proteins, nucleic acids and lipids and cause defective enzyme function[6].

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A study on rats revealed that BPA in doses of 48mg/kg and 60mg/kg produced changes in liver histology like hepatocytes vacuolization, dilatation and congestion of vessels (portal vein, central vein and sinusoids) and Kupffer cells proliferation[7.]

Moringa *oleifera (Mo)* belongs to genus *Moringacea* and is commonly known by names Drumstick-tree or Horse radish-tree[8]. In Pakistan *Mo* is known by the name 'Suhannjana'. This is also considered as "Miracle tree" and its leaves hold hepatoprotective, anti-inflammatory, antihypertensive and antimicrobial activities[9]. The leaves of this miraculous plant are rich in antioxidants (SOD and Catalase) phenols, flavonoids and caretenoids[10], which imparts to its hepatoprotective potential Therapy with *Mo* leaves reduces the liver damage and promotes regeneration[11], this may be associated with the preventive and preserving potential of *Moringa oleifera* on plasmalemma and increased protein synthesis. These leaves possess combination of calcium, iron, protein, carbohydrates, copper and vitamins are helpful in

lowering blood pressure and glucose levels and exhibit anti-ulcer and anti-inflammatory effect.

The hepatoprotective abilities of *Mo* have been studied with various hepatotoxicant such as antitubercular drugs and diclofenac sodium[12] and outcomes were impressive.

METHODS

Thirty two adult albino rats of either sex, weighing (170-200g) were acquired from National Institute of Health, Islamabad. The rats were handled in accordance with the guidelines for care of experimental animals, as promoted by the Canadian Council of Animal Care. Males and females were kept in separate cages. They were kept at temperature of 28.0±2.0ºC under 12 hr light/dark cycles and were given rat diet and water *ad libitum.*

After seven days of acclimatization, using lottery method, rats were separated into 4 equal groups. These groups were named as A, B, C and D and each group comprised of 8 rats. Animals were placed in their respective labelled cages. BPA was procured from Daejung –Korea. Moringa leaves were obtained from Botanical garden, University of the Punjab, Lahore, Pakistan. Leaves were authenticated by Professor Abdul Nasir Khalid, Department of Botany, University of the Punjab. A voucher specimen of no LAH35146 was kept in herbarium for future reference.

BPA and *MoLE* were dissolved in corn oil. Dose was freshly prepared on daily basis and was given through oral gavage. Animals were sacrificed at end of the 7th week and liver was dissected out. Liver was examined for any gross abnormality. It was weighed and then fixed with formalin. Sections were taken from liver. Tissue processing was done. Slides were made after embedding and sectioning, labeled according to the rat number and group and stained with H&E.

Parameters

- Hepatocyte diameter (Quantitative)
- Hepatocyte vacuolization (Qualitative)

Statistical analysis

The quantitative data (hepatocyte diameter) was presented in the form of Mean ± S.D (standard deviation) and were assessed by using micrometer. The qualitative data (hepatocyte vacuolization) was presented in the form of frequency and percentages. Anova and Fischer exact test were applied to Quantitative and Qualitiative parameters respectively.

RESULTS

After seven weeks of experiment all rats had normal weight gain and remained active. Liver tissue was examined under microscope for hepatocytes diameter and vacuolization.

When diameters of hepatocytes were measured, it was noted that the group A had mean value of 12.0±0.6 µm with group B had highest of 19.7±1.3µm. The group C and D had mean diameters of 17.0±1.1µm and 14.6±1.7µm respectively. The diameter of hepatocyte increased in group B animals and ANOVA showed statistically significant difference among groups with p-value <0.001.

Table 1: Mean diameter of hepatocytes (µm) in various animals groups.

Parameters	Groups				P-value
	A Mean ±SD	B Mean ±SD	C Mean ±SD	D Mean ±SD	
Hepatocyte diameter	12.0±0.6	19.7±1.3	17.0±1.1	14.6±1.7.	<0.001

*p<0.05 is considered statistically significant.

When comparison was made group wise by applying Post Hoc Tuckey test, it was observed that the group A had significantly smaller diameter as compare to group B, C and D with p-values <0.001, <0.001 and 0.001 respectively.

Table 2: Comparison of difference of mean hepatocyte diameter among groups by applying Post Hoc Tukey test.

Group (I)	Group (J)	Mean Difference (I-J)	Std. Error	P-value
Group A	Group B	-7.64*	0.62	<0.001
	Group C	-5.01*	0.62	<0.001
	Group D	-2.57*	0.62	0.001
Group B	Group C	2.63*	0.62	0.001
	Group D	5.07*	0.62	<0.001
Group C	Group D	2.43*	0.62	0.003

Table 3 shows that in group A (control) vacuolization was absent. It was present in 7(87.5%) of group B animals. In group C and D it was present in 4(50.0%) and 2(25.0%) of animals .

Table 3: Illustrates association of hepatocyte vacuolization in groups.

Groups	Hepatocyte vacuolization					
	Present		Absent		Total	
	N	%	n	%	n	%
Group A	0	0.0	8	100.0	8	100.0
Group B	7	87.5	1	12.5	8	100.0
Group C	4	50.0	4	50.0	8	100.0
Group D	2	25.0	6	75.0	8	100.0

Fisher's Exact test = 13.912 p-value = 0.002

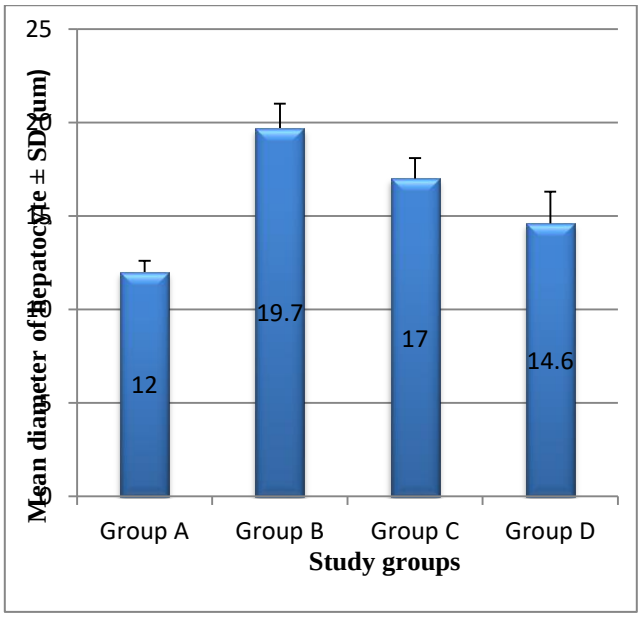
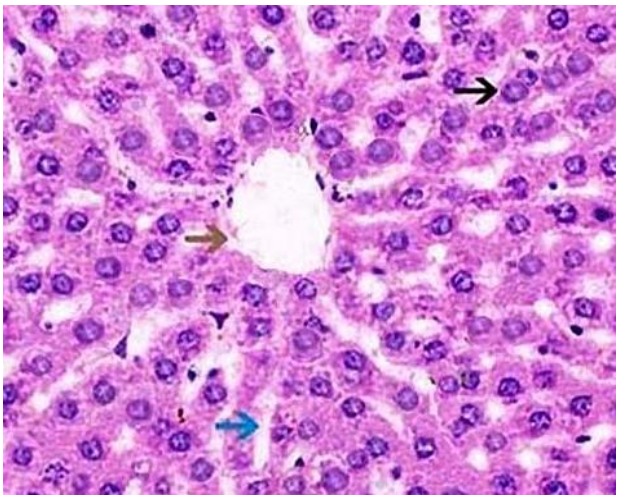


Fig.1: Bar chart showing comparison of mean diameter of hepatocytes (µm) among groups.



Photomicrograph 1: Liver section taken from group A. Hepatocytes (black arrow) were seen to have vesicular nucleus (black arrow). Sinusoids were also shown (blue arrow). Central vein (brown arrow). No vacuolization is seen. H&E. X 400.

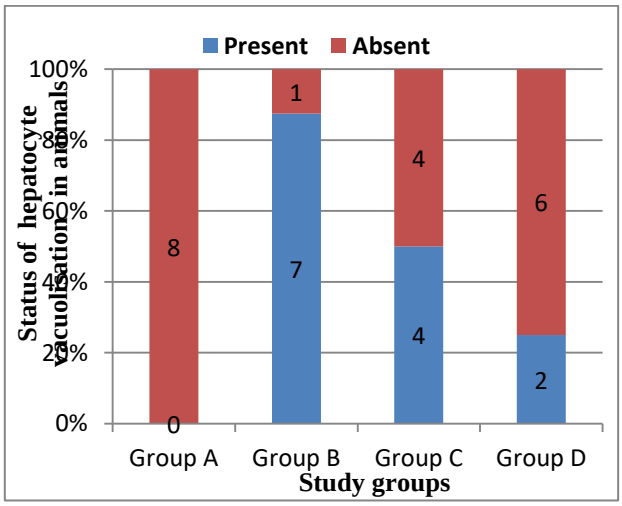
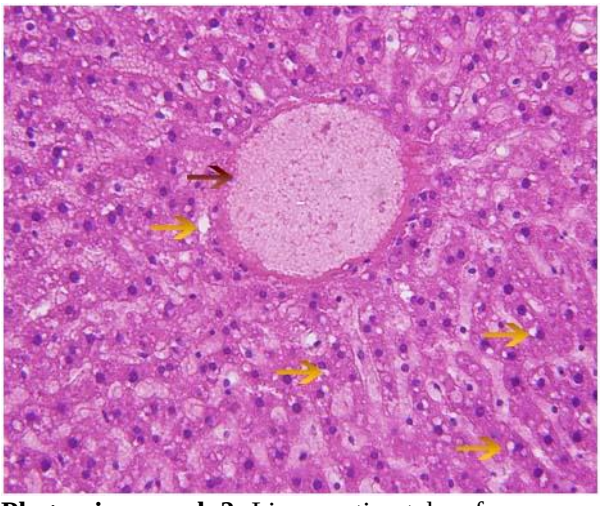
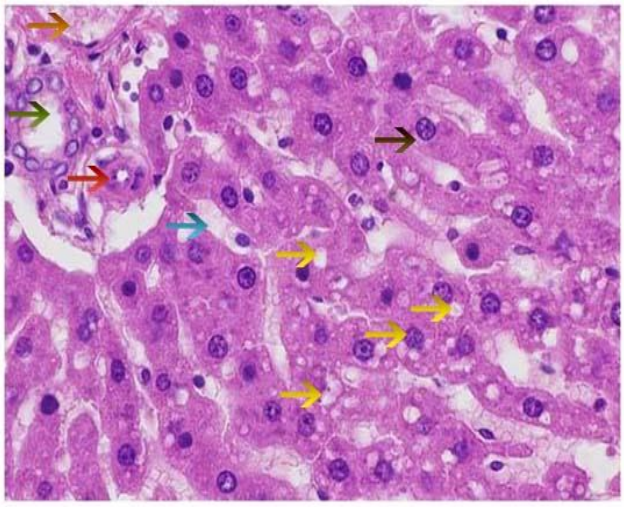


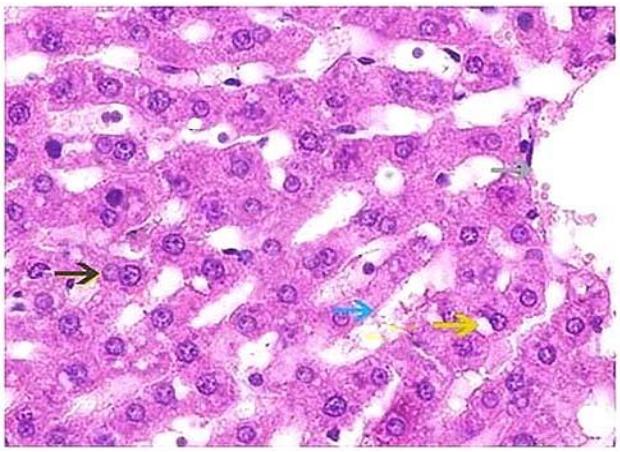
Fig. 2: Bar chart presenting status of hepatocyte vacuolization in animals groups.



Photomicrograph 2: Liver section taken from group B. Vacuolization of hepatocytes (yellow arrow). Showing central vein (brown arrow), H&E staining., X200.



Photomicrograph 3: Liver section from group B showing portal vein (dark yellow arrow), bile duct (green arrow) and hepatic artery (red arrow). Hepatocytes (black arrow). Sinusoidal spaces (blue arrow) were running between cords of hepatocytes. Vacuolization of hepatocytes (yellow arrow). H&E stain. X400.



Photomicrograph 4: Liver section from group C. Central vein is seen (grey arrow). Few hepatocytes with vacuoles (dark yellow arrow) were visible. Nucleus (black arrow) with clear nuclear membrane and prominent nucleoli were seen, indicating regeneration of hepatocytes. H&E stain. X400.

DISCUSSION

Liver is the major organ which is affected following an oral exposure to BPA. BPA disturbs the normal function of liver through generation of ROS. *Moringa oleifera* leaves have preventive and curative properties for many liver diseases. The leaves of *Mo* plant possess high nutritional value and are good source of antioxidants.

A major requirement to assess the potential protective agent for hepatic injury is to observe it effect on liver histology, so this study was based on histological observations.

Current study was an effort to observe the results of Ethanolic extract of *Mo* leaves on liver changes brought about the use of Bisphenol-A. BPA causes localized depletion of glutathione, catalase, SOD and GSH that would result in oxidation stress. This oxidative stress lead to mitochondrial dysfunction, lipid peroxidation and formation of ROS, which are important causes for the damage to hepatocytes and liver architecture[13].

The leaves of *Mo* are good source of vitamins, flavonoids, phenols and carotenoids which contribute to their antioxidant potential[8]. BPA leads to the formation of ROS which are thus scavenged by the antioxidants in *Mo* leaves. It could be postulated that the hepatoprotective effect of *moringa* was due to its property to prevent oxidative degradation of lipids thus protecting the cell membrane[14]. Studies have reported the effect of *Moringa Oleifera* leaves after administration of hepatotoxicants, like acetaminophen, and antitubercular drugs and results were encouraging. The animals showed recovery from damage and elevated liver enzymes returned to normal[8].

Due to mitochondrial dysfunction after exposure to BPA, depletion of ATP occurs which results in failure of the sodium pump with influx of sodium and water. This leads to cell swelling and vacuolization. In group B, cytoplasm of hepatocytes were filled with vacuoles of different sizes and presumably showed vacuolar degeneration and cell swelling in response to toxin (Pic: 2&3) and lead to increase in size of cells in group B .

Our findings were supported by a study was conducted, which revealed vacuolar degeneration in hepatocyte after BPA administration at dose of 50mg/kg[15]. These findings were comparable with observation of a study[16], in which BPA at dose of 50mg/kg, resulted in vacuolated, swollen hepatocytes (Pic: 2&3). This vacuolization was milder in group C and D, after administration of *MoLE* (Table.3; Pic:4). It was presumably due to the antioxidant potential and cell membrane stabilizing effect of *Moringa*[13]. This study revealed that in BPA treated group, the size of hepatocyte was remarkably increased when comparability was checked with other groups (p-value <0.001) (Table. 2; Fig.1). In group C and D, there was decrease in the size of hepatocytes (Table; 1&2).

This generalized increase in size of hepatocytes in all the experimental groups confirms BPA toxicity. After administration of *MoLE* for few weeks, the size of

hepatocytes in group D were restored close to the control group.

The present results were strengthened by research works, in which administration of lead[17] and carbon tetracholride[18] caused hepatocytes vacuolization, vascular congestion and cellular infilterate and were improved upon treatment with *Moringa* extract.

CONCLUSION:

MoLE shows a protective effect on hepatocytes when administered with BPA. Thus to counteract the liver damage by BPA, an effective hepatoprotective action can be provided by natural valuable compounds of *Mo* leaves. *Moringa* leaves act as a source of antioxidant agent and can be used to combat different diseases. Moreover, this study can be carried out on other organs of the rat like brain, testis, ovary and kidney

ETHICAL APPROVAL:

The study was approved from Ethical Review Committee of Postgraduate Medical Institute, Lahore, Pakistan.

AUTHORS' CONTRIBUTION:

AZ: Study design

SN: Proof reading

NS, MA: Drafting

FQ: References search

AH: Analysis and interpretation of data

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