Effect of Moringa Oleifera Leaves Extract on Bisphenol-A Induced Changes in Venous Drainage of the Liver of Albino Rats

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Abstract

Objectives: To observe the effects of Ethanolic extract of Moringa Oleifera leaves on Bisphenol –A (BPA) induced changes in diameter of central vein and venous drainage (central vein, portal and sinusoidal) in liver of albinorats.

Methods: This was an experimental study conducted at the animal house of Anatomy Department, PGMI, Lahore. Thirty two albino rats of both sex, weighing 170-200 gms were divided equally into 4 groups as A (control), B, C and D (other groups were experimental). Group A, received corn oil only. Group B, received BPA only 50mg/kg/body weight (wt)., Group C and D received BPA 50mg/kg along with Moringa Oleifera leaves extract (MoLE) 250mg kg/body wt and 500mg kg/body wt respectively. BPA and MoLE was given in single dose through oral route. At the end of seven weeks, rats were dissected, liver was removed and slides were made by using H&E stain. The effect of MoLE on BPA induced venous changes was observed under light microscope. The statistical analysis of results was done by using SPSS 21.

Results: BPA increased the diameter of central vein and produced vascular congestion in group B, which was then reduced by administration of MoLE in group C and D. The healing effect of MoLE was augumented as dose was increased.

Conclusions: Moringa Oleifera leaves prevented the vascular congestion and dilatation induced by BPA in liver of rats . The preventive effect improved as dose was increased.

Key Words: BPA: Bisphenol-A; MoLE: Moringa Oleifera leaves extract.

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Introduction

Liver plays a major role in the regulation of many physiological processes in our bodies, which include metabolic and secretory functions. It is involved in detoxification of a variety of drugs, chemicals and xenobiotics.[1]

Bisphenol-A (BPA) is a synthetic chemical that has been widely used in synthesis of plastics and epoxy resins,[2] so people of every age are inevitably exposed to BPA in daily life.[3] It is commonly used in manufacturing of baby bottles, food storage boxes, dental sealants[4] and to coat the inside of metallic cans.[5] Leaching of BPA into surrounding occurs due to presence of acidic food or beverages in cans or plastic containers. Heating of cans for food sterilization and repeated washings of plastic products increases the rate of leaching of this chemical into environment.

The major exposure is through BPA leaking into food and beverages.[6] It has been detected in over 90% of all analyzed human urine samples, showing its wide human exposure.[7] BPA has harmful effects on body organs like kidney, testes, prostate but liver is the main organ which is affected following an oral exposure. This causes production of Reactive Oxygen Species (ROS) and disturbs the activities of normal antioxidant enzymes such as catalase, glutathione peroxidase (Gpx), superoxide dismutase (SOD) and glutathione (GSH) in liver.[8] Many agents such as alcohol, viruses, fatty acids, drugs and immune response, can lead to intracellular stress through lysosomal and mitochondrial

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dysfunction eventually leading to cell injury or death. Moringa oleifera (Mo) belongs to genus Moringacea and is commonly known by names Drumstick-tree or Horse radish-tree.[10] In Pakistan Mo is known by the name 'Suhannjana'. Several traditional systems of medicine in south Asia , use leaves of this marvelous tree in their medicinal recepies.[11] The leaves possess minerals like iron, calcium, vitamins, essential amino acids and many antioxidant compounds such as phenols and flavonoids.[12] These compounds scavenge free radicals, boost up the levels of other antioxidants and builds up the hepatoprotective activity in leaves.[13] Other traditional uses of the leaves are in healing skin infections, anxiety, asthma, wounds, fever, diarrhea, and sore throats. The Moringa Oleifera possess different pharmacological functions including anti-inflammatory, anti-ulcer, anti-cancer and anti-diabetic activity.[14] After an injury to the liver caused by some agent, the administration of Moringa leaves reduces the damaging effects and promotes formation of healthy tissue in liver.[15] This preventive effect of Mo have been studied with several hepatotoxicants such as drugs and chemicals and results were admirable. In a study, co-administration of MoLE with Diclofenac Sodium decreased the vascular congestion, promoted regeneration and decreased the levels of enzymes ALT and AST. The leaves are used as dietary supplements in Asia and are called mother's best friend as they increase the milk production during lactation. A study revealed that Moringa oleifera leaves exhibit higher antioxidant potential when compared with the plant's seed and root, as leaves possess high levels of flavonoids and phenolic acids.[16]

Methods

This was an experimental study conducted at the animal house of PGMI, Lahore. Thirty two albino rats of both sex, weighing 170-200 gms were procured from National Institute of Health, Islamabad. The male and female rats were housed 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 acclimatization for one week, 32 rats were divided through simple random sampling into 4 equal groups A, B, C and D (n=8) and weighed. Group A was control (received corn oil only) and rest of the groups were experimental. Dose of BPA and MoLE was prepared daily in corn oil and was given separately in single dose through oral route. Group B, received BPA only 50mg/kg/bw. Group C and D received BPA 50mg/kg along with MoLE 250mg/kg and 500mg/kg respectivley. BPA was acquired from Daejung–Korea and Moringa leaves were obtained from Botanical garden, University of the Punjab, Lahore, Pakistan. Ethanolic extract of Moringa leaves was prepared in PCSIR Laboratories Complex, Lahore.

Animals were sacrificed at end of the 7th week. All instruments needed for dissection were sterilized before dissection. Vertical and Transverse incision were made through skin and muscles. Liver was dissected out and washed with cold normal saline, blotted, and weighed with the help of an electronic weighing scale. Its macroscopic examination was carried out to note any gross abnormality. It was fixed with 10 % formalin. Tissue processing was done and paraffin embedded blocks were made. Tissue slides were stained with H&E and were observed under microscope. (Magnification: 10X and 20X)

Parameters

Quantitative:

- Diameter of central vein

Qualitative:

- Central vein congestion
- Portal triad congestion
- Sinusoidal congestion

Statistical Analysis

The data was analyzed by using SPSS 21. The quantitative data (central vein diameter) was measured by using micrometer and was presented in the form of Mean± standard deviation (S.D). ANOVA was applied to determine the statistical differences among groups. For comparison among groups, Post Hoc Tuckey was applied. A p-value of <0.05 was considered as statistically significant. For vascular congestion, five different fields were observed and the data was coded as: Absent = 0 and Present = 1. The qualitative data was presented in the form of frequency and percentages and was determined by applying Fischer's exact test.

Results

After 7 weeks of experiment, all animals remained active, had normal weight gain and no morbidity was observed. In control group "A", the central vein, portal vein and sinusoids did not show any change (Fig-1). However congestion was observed in these vessels in experimental groups "B" (Fig-2,3) which was then reduced in group "C" and "D" after administration of MoLE.

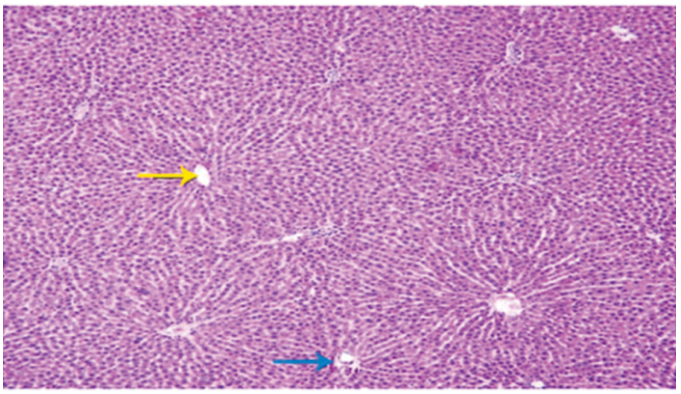

Fig.1: *Photomicrograph from Group A, Showing Normal Hepatic Lobules, Central Vein (yellow) and Portal Vein (blue). H&E Stain (10X)*

The diameter of central vein was compared in different groups by applying ANOVA. The group B had largest diameter while the group C and D had respectively smaller diameter than B. There was a significant difference among groups with p-value <0.001.

For comparison among groups, Post Hoc Tuckey was applied, it was observed that the group A had smaller diameter as compared to group B, C and D with p-values <0.001, 0.050 and 0.338 respectively.

Table 1: *Mean Diameter of Central Vein (µm) in Animals Groups*

Parameters	Groups				P-value (of comparison among groups)
	A Mean±SD	B Mean±SD	C Mean±SD	A Mean±SD	
Central vein diameter (µm)	101.8±13.5	212.1±39.5	155.4±49.3	33.4±44.6	<0.001

*p<0.05 is considered statistically significant.

The central vein congestion was present in 7(87.5%), 3(37.5%) and 2(25.0%) of animals in group B, C and D respectively. Fischer's exact test showed significant difference among groups with p-value 0.003 **(Fig: 1)**.

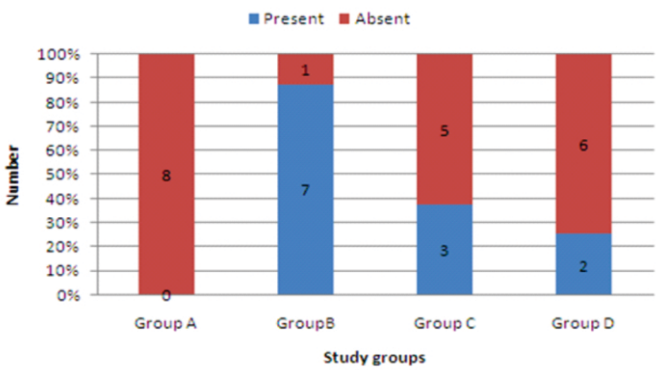

Fig-1: *Bar Chart Showing Status of Central Vein Congestion in Animals*

Portal vein congestion was present in all 8 animals of group B, 4(50.0%) and 3(37.5%) animals of group C and D respectively. Fischer's exact test showed significant difference among groups with p-value 0.004 **(Fig:2)**

Table 2: *Comparison of difference of Mean Central Vein 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	-110.38*	19.6	<0.001
	Group C	-53.63*	19.6	0.050
	Group D	-31.63	19.6	0.388
Group B	Group C	56.75*	19.6	0.035
	Group D	78.75*	19.6	0.002
Group C	Group D	22.00	19.6	0.680

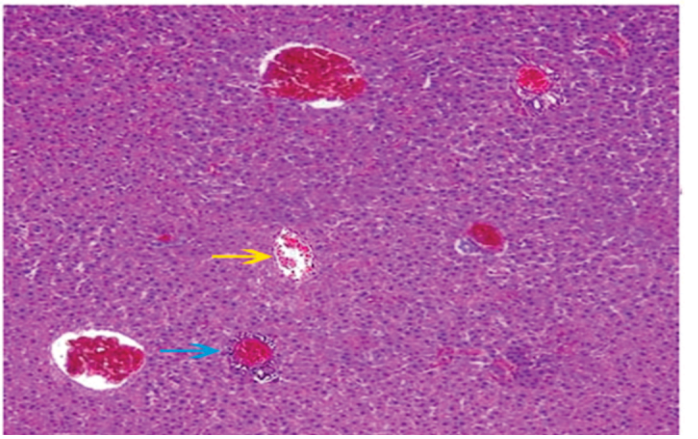

Fig-3: *Photomicrograph of Liver from Group B, Showing Congested Central Vein (Yellow Arrow) and portal vein (Blue arrow). H&E Stain (10X)*

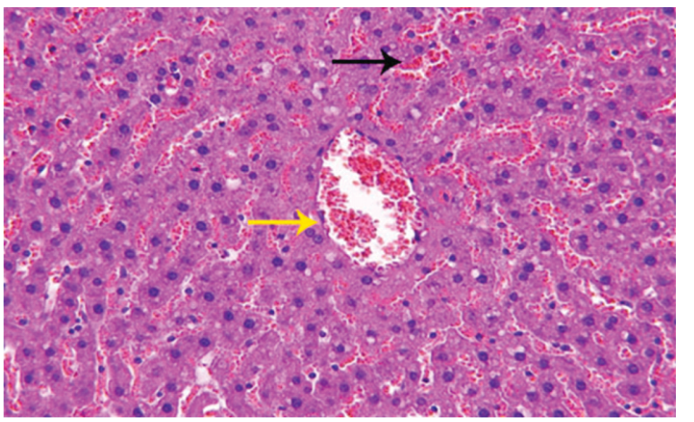

Fig-4: *Photomicrograph from Group B, Showing Congested Vessels. Central Vein (Yellow Arrow) and Sinusoids (Black Arrow). H&E Stain (20X)*

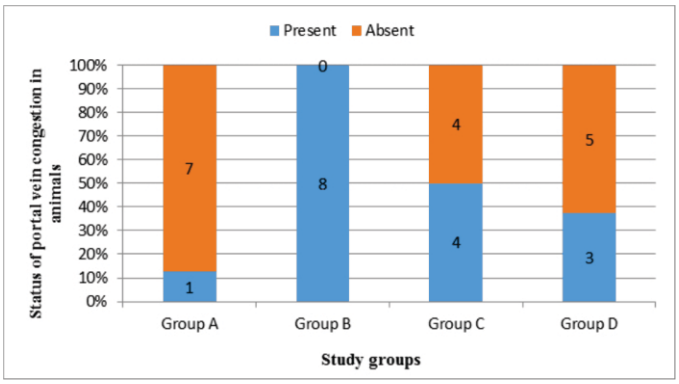

Fig-4: *Bar Chart Showing Status of Portal Vein Congestion in Animals of Each Group*

Discussion

BPA leads to the production of oxidative stress (ROS) in liver 8. ROS damages cell proteins, nucleic acids and lipids and impairs mitochondrial function, leading to cell damage.[9]

Moringa oleifera plant has high polyphenols and flavonoids content, which boosts up antioxidant status[15] and protects the cell membrane by augmenting protein synthesis and preventing lipid peroxidation.[17] The role of Moringa oleifera against oxidative stress in the heart of diabetic rats was also studied and a notable rise in levels of plasma insulin, superoxide dismutase (SOD) and catalase (CAT) and decrease in inflammatory changes were observed.[18] In another study after administration of Methotrexate, MO leaves reduced the inflammatory process and congestion in kidney and liver.[19]

Current study revealed congestion and dilatation of central vein (Pic 2&3). The diameter of central vein in group B was largest and in group D, it was close to Control group. However current study manifested a statistically significant decrease in mean diameters of central vein of group C and D after administration of MoLE **(Table-1)**.

Another study revealed that administration of BPA at dose of 50mg/kg resulted in congestion and dilatation of central vein[2] (**Fig.1**). Congestion in portal vein and sinusoids was also observed in group B (Fig-2,3; Fig 2). This could be attributed to reduced outflow of blood from a tissue. Congestion can be systemic, as observed in heart failure, or local, as in venous obstruction of an inflammed organ.[20] The oxidative stress produced due to BPA lead to failure of sodium pumps with water influx inside the cell which ultimately resulted in cellular swelling and injury. After an oral exposure to BPA, cytokines like IL-1beta and IL-6 are being released from the macrophages (Kupffer cells) of liver which further worsens the cell injury. These cellular changes may cause impaired venous drainage leading to congestion, as seen in group B. However in group C and D, this finding was reduced. The protective effect of Moringa leaves have been observed after administration of various hepatotoxicants like Carbon Tetrachloride and a decrease in inflammatory cell aggregates and congestion of blood vessel was reported.[13] Many studies have revelaed that the plant Moringa oleifera possess pharmacological activities and can be used for treatment of various diseases.[21]

Conclusion

Moringa oleifera leaves have protective effect on liver. It prevents the vascular congestion and dilatation induced by BPA. The healing effect improves as dose of Moringa is increased. These leaves can also be used as dietary supplement to improve the nutritional status.

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Authors Contributions

A.Z: Conceptualization of Project
A.B: Data collection
R.J: Literature Search
A.W: Statistical Analysis
Z.I: Drafting, Revision
R.S: Writing Manuscript