# Effect of Moringa Oleifera Leaves on Hepatocytes Glycogen Content After Hepato-toxicity with Bisphenol-A in Albino Rat

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## Abstract

**Objective:** To reveal the effects of Moringa Oleifera leaves extract on depleted glycogen stores after hepatotoxicity with Bisphenol-A in albino rats by using PAS staining.

**Material and Methods:** This experiment was carried out at Post Graduate Medical Institute, 32 adult rats were procured and distributed into 4 groups A, B, C and D. The study duration was 6 weeks. Group A was control and was given corn oil only. Group B, received BPA, 50mg/kg/bw. Group C and D received BPA 50mg/kg along with MoLE 250mg/kg and 500mg/kg. Liver was removed, fixed and slides were prepared by using PAS stain. The number of positively PAS stained (magenta colour) liver cells/40 cells were counted from every group and their mean was compared among groups. The statistical analysis was carried out by applying SPSS 21.

**Result:** The mean value of PAS positive cells in group B was lowest (14.25±4.27). However, in groups A and D, mean was calculated as 27.6±4.7 and 24.25±5.39, respectively.

**Conclusion:** Administration of MoLE (250mg/kg & 500mg/kg) restored the depleted glycogen content in cells, which was due to the toxic effect of BPA. The number of cells rises with increasing the dose of MoLE.


**Keywords:** MoLE: Moringa Oleifera Leave extract, BPA: Bisphenol-A, PAS: Periodic Acid Schiff stain.

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## Introduction

The compound called Bisphenol A (BPA) is defined as a synthetic high production monomer utilized in polycarbonate plastics and the resins of epoxy in consumer products. This compound is used to bottles made of plastic, plastic food containers, warm receipts,[1] (Mourad and Khadrawy, 2012). Epoxy resins containing BPA are used to coat the inside of food and beverages metallic cans (Carwile et al., 2009).[2] Exposure to high temperature, the presence of acidic food or drinks in containers or cans may result in leaching of BPA into food. Thus the exposure of human beings is mainly through the use of contaminated food and drinks packed in containers with BPA. The liver assumes an essential part in the human body; it is responsible for blending glucose, plasma proteins, clotting factors, and urea; storing glycogen, fat,[3] and nutrients; regulating amino acids; and creating bile for metabolizing fat.[4]

Therapeutic plants have been investigated as a characteristic solution for liver diseases.[5] Moringa oleifera is an excellent source of phenolics and flavonoids comprise various pharmacological activities.[6] Different concentrates from restorative plants showing high hepatoprotective action in creature models.[7] Dynamic parts in restorative plants, like phenolic, and flavonoid compounds, display antioxidant and anti-inflammatory properties that assist with blocking lipid peroxidation

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and abatement the oxidative stress marker malondialdehyde (MDA) and hepatic enzymes,[8] Moringaoleifera (has a place with family Moringaceae) is a fast-growing tree with the tripinnate leaves structure as and is dispersed in African and Asian nations. In Pakistan, Moringa oleifera is known as "Suhannjana" and is adored as the "Miracle tree" for its restorative properties.[9] The leaf powder of M. oleifera is plentiful in calcium, iron, protein, starches, copper, and nutrients, iron, vitamin A, and L-ascorbic acid.[10] M. oleifera is demonstrated to be used in the treatment of various diseases, ascites, viral diseases (e.g., flu infection), bacterial contaminations, and various kinds of boil. Its leaves are eminent for their hepatoprotective, anti-inflammatory, antihypertensive, and antimicrobial exercises and lessen hyperglycemia and dyslipidemia.[11] Wealthy in antioxidants, for example, superoxide dismutase, catalase, phenols, flavonoids, and carotenoids, Moringa oleifera leaves display powerful hepatoprotective potential[12] and restores Glutathione. Treatment with Moringa oleifera leaves relieves liver harm and advance recovery, conceivably credited to its safeguarding effects on the plasmalemma and proteins.[13] Its hepatoprotective capacities have been widely considered with different hepatotoxicants, including antitubercular drugs and diclofenac sodium, yielding impressive outcomes.[14] This recommends the capability of Moringa oleifera as a characteristic remedial specialist for liver-related messes, inferable from its multi-layered pharmacological profile and significant protective effects against hepatotoxicity.[15]

## Material and Methods

This experiment was carried out at Post Graduate Medical Institute, 32 adult rats were procured and distributed into 4 groups A, B, C and D. The study duration was 6 weeks. After taking approval from Ethical Committee No 5999-6000/PGMI Dated 10-07-2014. Thirty two adult albino rats of either sex, weighing (170-200g) were procured Post Graduate Medical Insti-tute Males and females were kept in separate cages. They were kept at temperature of 28.0±2.0ºC under 12hr light/dark cycles and were given rat diet and water ad libitum. After seven days, using lottery method, rats were divided into four equal groups. A, B, C and D, each comprising of 8 rats. They were put in respective labe-lled cages. BPA was procured from Daejung –Korea. Moringa leaves were obtained from the gardenof Uni-versity of the Punjab, Lahore, Pakistan. The leaves were authenticated by Professor Abdul Nasir Khalid, Department of Botany, from that same University. 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. It was fixed with formalin, slides were made, labeled according to the rat number and group and stained with Periodic Acid Schiff Stain. The PAS stain demonstrates glycogen content and produce a bright pink or magenta colour. Two slides of each rat were observed from every group and number of positive PAS stained cells (magenta colour)/40 cells in each slide were counted .Their mean was taken and was compared among groups. The data was evaluated by applying SPSS 21. The quantitative data (PAS positive cells) 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.

## Results

The mean value of PAS positive cells for group A, B, C and D are given in Table 1. The group B had of the lowest mean of 14.25±4.27 while group A has highest mean value of 27.6±4.7. ANOVA revealed markedvariations among four groups with p-value <0.001. Photomicrograph of the liver from group A, The Post Huc-Tockey test revealed that the group B and C when compared to group A had p-values <0.001 and 0.280 respectively. Group C had high value as compared to group B and the difference was significant with p-value 0.009. (Table- 2).

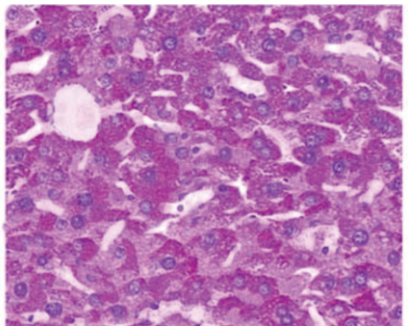

**Figure 1a:** *Magenta granules seen in cytoplasm of hepatocytes, indicates the presence of glycogen.*

**Table 1:** *Mean value of PAS positive cells in various animals groups.*

| Parameters | Groups | | | | P-value |
|---|---|---|---|---|---|
| | A Mean ±SD | B Mean ±SD | C Mean ±SD | D Mean ±SD | |
| Number of PAS positive cells/40 cells | 27.6±4.7 | 14.25±4.27 | 23.00±5.68 | 24.25±5.39 | <0.001 |

**Table 2:** *Mean value of PAS positive cells in various animals groups by applying Post HucTockey test.*

| Groups | Group Comparisons | Mean Difference Between groups | Std. Error | P-value |
|---|---|---|---|---|
| **Group A** | Group B | **(A-B)** 13.38 | 2.52 | <0.001 |
| | Group C | **(A-C)** 4.63 | 2.52 | 0.280 |
| | Group D | **(A-D)** 3.38 | 2.52 | 0.547 |
| **Group B** | Group C | **(B-C)** -8.75 | 2.52 | 0.009 |
| | Group D | **(B-D)** -10.00 | 2.52 | 0.002 |
| **Group C** | Group D | **(C-D)** -1.25 | 2.52 | 0.059 |

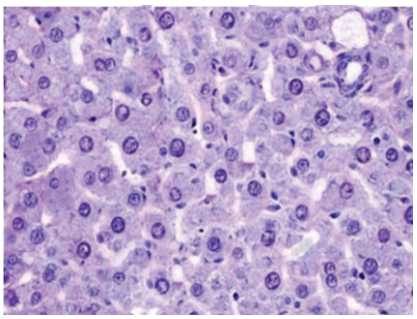

**Figure 1b:** *Subjecting the same preparation to PASD staining technique, which envisaged digestion of glycogen by Diastase. X400.*

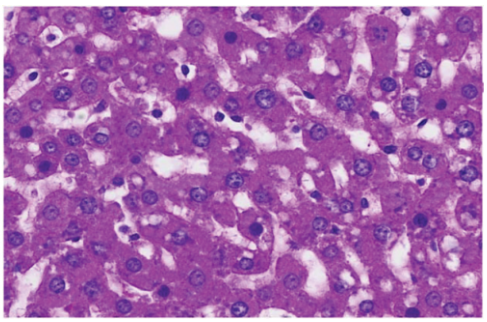

**Figure 2:** *Photomicrograph of liver from group B. PAS stain showing that hepatocytes cytoplasm was depleted of glycogen. PAS stain. X400.*

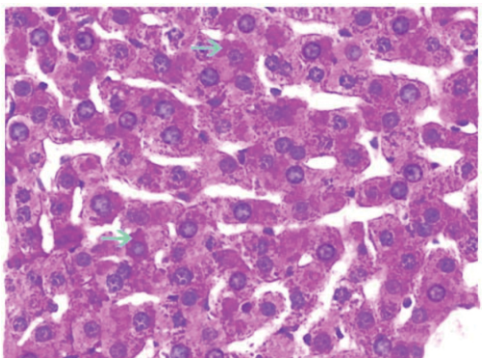

**Figure 3:** *Photomicrograph from group D. Magenta granules (green arrow) seen in hepatocytesindicated the presence of glycogen. PAS staining shows presence of glycogen. PAS stain. X400.*

## Discussion

BPA is a compound widely used in plastic manufacturing and for coating the inside of the cans, thus it is found in many plastic products, bottles, food and beverage cans. Liver is the major organ which is affected following an oral exposure to BPA. BPA disturbs the normal function of liver by generating ROS leading to oxidative stress. This oxidative stress lead to lipid peroxidation and causes damage to hepatocytes and normal liver architecture. Moringa oleiferaleaves have preventive and curative properties for many liver diseases. The leaves of Mo plant possess high nutritional value and are good source of antioxidants. In current study, we observed that the liver section from group A when stained by using the PAS technique, revea-led strong magenta colour in the cytoplasm of hepato-cytes, which indicated the presence of glycogen **(Fig.1a)** We calculated the number of these positively PAS stained (magenta colour) cells from every group and compared their mean among groups by using Anova **(Table.1)**. The variation in batches was remarkable (p-value <0.001). The group B had lower mean value which proved that the number of cells with prominent PAS staining were decreased, thus pointed towards depleted glycogen content in hepatocytes **(Fig-2)**. Mean value of PAS positive cells in various animals groups by applying Post HucTockey test is shown in **Table.2**. This result matches with a study done by Ahmed et al., 2015, in which PAS staining revealed a decrease in glycogen content after administration of BPA. Ahmed et al., 2015, Exposure to BPA reduces glycogen content by decreasing the glycogen synthesis and promoting glycolysis.[16] After administration of MoLE in group C and D the glycogen content was improved. A research by Ndong

et al., 2007[17] showed decreased glycogen stores in liver cells due to the effect of anemia, but this glycogen was increased after treatment with Mo leaves. Omotoso et al., 2015, reported that in lead treated group there was a decrease in hepatocytes glycogen deposits, which were improved with Mo leaves.[18] It is presumed that the flavonoids in Mo leaves are strong inhibitors of an enzyme glycogen phosphorylase, which can break down glycogen. It was proven in studies that in diabetic rats, administration of Mo extract improved glycogen synthesis as it enhances glycogen synthase activity.[19] Another study manifested improvement in muscle and hepatic glycogen levels after administration of Mo leaves to the rats.[20]

In current experiment, BPA at dose of 50mg/kg caused decreased glycogen content These harmful effects were presumably due to oxidative stress and impaired enzymes activity Moreover this damage was ameliorated by co-administration of MoLE, as proved on the basis of histological and biochemical grounds. MoLEsignicantly improved the glycogen content in hepatocytes and these effects were more evident in group D, in which 500mg of moringa extract was given, as compared to group "C" which had 250mg of moringa extract. The healing effects of these leaves were more as we increased the dose. It was evident that the protective effects of Mo leaves were dose dependent; the liver damage restored more towards normal with increasing the dose of MoLE. This was accredited to the improved SOD, GSH and catalase activities after administration of leaves extract.[21]

## Conclusion

Moringa oleifera leaves have protective effect on liver. It restored the depleted glycogen content in rats after administration of BPA. The effect of Moringa rises as dose of Moringa is increased. The powder of the leaves can also be used as a nutrient product to improve the diet. These results highlight that MoLE could be regarded as a source of natural antioxidants, thus its use in different medicinal fields should be encouraged.

**Conflict of Interest:** *None*

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## Authors Contribution

**AS:** Conceptualization of Project
**NH:** Data Collection
**AW:** Literature Search
**NN:** Statistical Analysis
**FI:** Drafting, Revision
**AH:** Writing of Manuscript