A STUDY OF GINGER EXTRACT ROLE ON THE TOXIC EFFECTS OF BISPHENOL-A ON THE LIVER IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Bisphenol -A (BPA) is a monomer used in the manufacture of polycarbonate plastics. BPA is used in diverse forms of plastic products in the food and electronic industries. BPA has been shown to leach out of products, and high levels of the monomer have been identified in human and animal samples. Many pharmacological effects were reported on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumor, anti-rhinoviral and anti-hepatotoxicity.

Aim of the work: The current study was designed to investigate the effects of ginger extract on biochemical histomorphological and immunohistochemical changes induced in liver of albino rats by bisphenol A.

Materials and methods: Sixity healthy adult male albino rats of initial body weight 150-200 gm were included. Rats were randomly and equally divided into 4groups, group (I): subdivide into: group Ia negative control group, group (Ib): olive oil treated group, group (Ic):saline treated group, group (II): ginger group, group (III): bisphenol A group, group (IV) bisphenol A and ginger group. Treatment will continue for 8 weeks (three times a week). After the end of 8 weeks, the rats from each group will be sacrificed for biochemical, histopathological and immuno histochemical studies in liver.

Results: There was no significant difference between the negative, the positive control and ginger groups as regard all biochemical, histopathological and immune histochemical parameters.

Bisphenol- A significantly decreased hepatic glutathione peroxidase (GPx) activities but significantly increased serum aspartate transaminase (AST), alanine transaminase (ALT) and hepatic malondialdehyde (MDA) levels together with deterioration of the hepatic histoarchitecture after eight weeks. Moreover, it was found that exogenous administration of ginger extract resulted in a significant improvement of all the above-mentioned parameters.

Conclusion: Ginger extract improved the hepatic toxicity induced by BPA that could be explained by the anti-oxidant effects of ginger extract.

Key words: Bisphenol-A, ginger extract, oxidative stress.

INTRODUCTION

Bisphenol- A is widely used in plastic products such as water dispensers, clear plastic bottles and food can linings. Human exposure to BPA is extensive **(Calafat et al., 2005).**

Kabuto et al. (2003), Vandenberg et al. (2007) and Lang et al. (2008) reported that some animal studies suggest that exposure to BPA induced lipid peroxidation and tissue oxidative stress.

Bindhumol et al. (2003) found that bisphenol-A induce oxidative stress in liver, kidney, testes and epididymal sperms of animals by generating free radicals and altering the endogenous antioxidants.

Lopez-Torres et al. (2002) and Reddy et al. (2008) reported that oxidative stress and antioxidant capacity of the body modulated by nutritional**,** environmental and physiological.

From times unknown, ginger or *Zingiber officinale* has become a subject of interest because of its beneficial effects on human health. Ginger has been found to possess antioxidant effect that can control the generation of free radicals **(Ahmad et al., 2006)**.

AIM OF THE WORK

The current study was designed to investigate the effects of ginger extract on biochemical histo- morphological and immune-histochemical changes induced in liver of albino rats by bisphenol A.

MATERIAL AND METHODS

MATERIAL

Bisphenol -A

Bisphenol- A (CAS No. 80-05-7; purity of 97%) and 4-Tert-octylpenol (CAS No. 140-66-9; purity of 97%) were purchased from Sigma–Aldrich Company, Germany. Chemicals were dissolved in olive oil.

Ginger

Ginger extracts were obtained from Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO, Cairo, Egypt) in tablet form. Each tablet contained 400 mg of ginger extract. The tablet was crushed and dissolved in 4 ml

saline; hence, each ml contained 100 mg ginger.

Animals

This study was conducted on 60 healthy adult male albino rats weighing 150-200 gm, were obtained from the animal house of Faculty of Medicine- Zagazig University. All animals were subjected to 14 days period of passive preliminaries in order to be adapted the new environment, to ascertain their physical wellbeing and to exclude any diseased animals. The rats received balanced food rich in all stuffs necessary to maintain their health before and during drug administration. It consisted of bread, barley and milk. Water was offered in separate clean containers. All investigations were conducted in accordance with the guiding principles for the care and use of research animals and were approved by the Institutional Research Board.

Treatment protocol

Rats were randomly divided into four groups, group (I): control group were subdivided into three group as follows: group (I a) (negative control group), will be lifted without intervention to measure the basic parameters, group (I b) (positive control group), each rat will be treated daily orally with olive oil (vehicle of bisphenol) once daily via gastric intubations, group (I c) (positive control group) rat will be treated daily orally with saline (vehicle of ginger) group (II): ginger group: ginger extract (200 mg/kg body weight) **(Maralla, 2013)**. Group III (bisphenol-A group), each rat will be treated orally with bisphenol-A (50 mg/kg/day) **(vomSaal et al., 2007).** LD50 in rats is 3250 mg/kg orally **(Chapin et al., 2008)** and group IV: (bisphenol-A and ginger extract), each rat will be treated orally with bisphenol-A (50 mg/kg/ day) and ginger extract (200 mg/kg body weight).

Treatment will be three times a week for 8 weeks**.** After the end of the study, rats from each group will be sacrificed.

Sample collection:

Venous blood samples were collected from the retro-orbital plexus of the animals by capillary glass tubes using light ether anesthesia according to procedure described by **Nemzek et al. (2001).** 3 ml of blood were collected from each rat in clean centrifuge tube and incubated at 37°C until blood clotted and then centrifuged to separate the serum that is used to measure Serum liver enzymes; aspartate transaminase, alanine transaminase.

After collecting blood samples, laparotomy was conducted after the animals were sacrificed by cervical dislocation under mild ether anesthesia.

Biochemical Analysis

1) Serum Aspartate aminotransferase (AST) by Dimession-ES (clinical chemistry auto-analyzer**):** According to **Saris, (1978)**

2) Serum Alanine aminotransferase (ALT) Dimension-ES (clinical chemistry auto-analyzer): According to **Bergmeyer et al., (1978).**

3) Hepatic antioxidant system evaluation:

Tissues were perfused in 0.9 % NaCl containing 0.16 mg / ml heparin. Tissues was washed and minced in ice-cold 0.25 M sucrose, then homogenized, diluted and centrifuged at 4000 rpm and 4°C for two minutes. The supernatant was used to measure

- Assay of glutathione peroxidase (GP_X) activity: according to the method described by **Paglia and Valentine (1967).**
- Assay of MDA level: according to the method described by **Jain et al. (1989).**
- All are measured by using spectrophotometer.

Histopathological examination:

Livers from all groups were removed and fixed in 10% formalin solution and followed by dehydration in a descending series of ethyl alcohol, were cleared in xylene and embedded in paraffin. Paraffin sections of testes were cut at 5 μm on a rotary microtome, mounted on slides and stained with heamatoxylin eosin (H&E) **(Horobin and Bancroft, 1998)** and examined under a light microscope.

Immunohistochemical examination:

A rabbit monoclonal antibody of IgG type was carried out for localization of caspase-3 (apoptosis marker) in paraffin sections. (The kits were delivered from Lab Vision Laboratories; Cat. #:1475-1) according to **Joyner and Wall, (2008)**

Statistical Analysis:

Data were analyzed by Statistical Package of Social Science (SPSS), software version 22.0 **(SPSS Inc., 2013).**

RESULTS

1) Biochemical studies:

*** As regard the control groups; group Ia (-ve control), group Ib (olive oil treated group), group Ic (saline treated group) and group II (ginger extract):**

There were a non significant difference ($p > 0.05$) between groups all over the period of the study by ANOVA as regard ALT, AST, GPx and MDA in hepatic tissues **(table 1).**

So the negative control group was chosen to be compared with the results of the treated groups;

group IV (bisphenol-A) and group V (bisphenol-A + ginger extract)**.**

*** As regard the treated groups; group IV (bisphenol-A) and group V (bisphenol-A + ginger extract):**

There were significant increase in AST, ALT in bisphenol-A group when compared with negative control group by ANOVA **(table 2)**, while, there was significant decrease in those parameters in bisphenol-A +ginger extract group when compared with bisphenol-A groups by ANOVA **(table 2).**

Also, there was significant decrease in hepatic GPx with significant increase in hepatic MDA in bisphenol-A group when compared with those of negative control group. while there was significant increase in hepatic GPx with significant decrease in hepatic MDA in bisphenol-A+ ginger extract group when compared with bisphenol-A groups **(table 3).**

2) Histopathological results:

Light microscopic examination of H&E stained sections from the livers of the control groups revealed hepatocytes arranged in plates radiating from the central vein and separated by blood sinusoids hepatocytes are polygonal in shape, with central rounded vesicular nuclei and acidophilic cytoplasm **(figure1).**

While caspase immune-staining of the control groups showed positive reaction in few hepatocyte **(figure2).**

In bisphenol-A group, dilated portal vein and bile duct proliferation were observed in H&E examination **(figure 3).** Also, marked positive reaction for activated caspase 3 in many hepatocytes in bisphenol -A group were noticed **(figure 4).**These changes become less in group taken ginger extract with bisphenol –A either detected by H&E examination **(figure 5)** or caspase immune-staining **(figure 6).**

Table 1: Statistical comparison among the negative control, olive oil treated group, ginger extract treated group as regard AST (IU/L), ALT (IU/L), GPX and MDA in liver tissue along the period of the study by ANOVA.

Group / Parameter	Negative control Mean ± SD	Olive oil treated group Mean ± SD	Saline treated group Mean ± SD	Ginger extract treated group Mean ± SD	F	P
AST	14.13±3.47	14.11±3.40	14.11±3.41	14.10±3.15	0.000	>0.05
ALT	25.3±3.40	25.1±3.43	25.4±3.40	24.9±3.15	0.044	>0.05
GPX in liver tissue	28±4.62	28.2±4.66	28.1±4.62	27.9±4.01	0.008	>0.05
MDA in liver tissue	98.35±8.99	97.35±8.11	98.34±8.90	99.01±8.02	0.065	>0.05

Number of sacrificed rats for each group was 10 rats.

ANOVA: Analysis of variance

SD : Standard Deviation.

p>0.05 : non-significant

AST: Aspartate transaminase

ALT: Alanine transaminase

GPX: Glutathione peroxidase

MDA: Malondialdehyde

Table 2: Statistical comparison among the negative control, Bisphenol-A, Bisphenol-A + ginger extract groups as regard AST (IU/L), ALT (IU/L) along the period of the study by ANOVA.

Group Parameter	Negative control group(I) Mean ± SD	Bisphenol-A group(III) Mean ± SD	Bisphenol-A + ginger extract group(IV) Mean ± SD	F	P
AST	14.13±3.47	95.29±3.47 [a]	25.12±3.45 [b]	1,616.199	<0.0001***
ALT	25.3±3.40	105.77±1.74 [a]	40.4±3.51 [b]	2,039.689	<0.0001***

Number of sacrificed rats for each group was 10 rats
ANOVA: Analysis of variance
SD: Standard Deviation
a = significant versus group I
b = significant versus group IV
P <0.0001*** highly significant
AST: Aspartate transaminase
ALT: Alanine transaminase

Table 3: Statistical comparison among the negative control, Bisphenol-A, Bisphenol-A + ginger extract groups as regard GPx (ng/ml) and MDA (mmol/l) levels in hepatic tissues along the period of the study by ANOVA.

Group Parameter	Tissue	Negative control group(I) Mean ± SD	Bisphenol-A group (III) Mean ± SD	Vit C+ Bisphenol-A group (IV) Mean ± SD	F	P
GPx (ng/ml)						
	Liver	28±4.62	10.62±1.6 [a]	24.70±5.01 [b]	52.159	<0.0001***
MDA (mmol/l)						
	Liver	98.35±8.99	402.34±4.69 [a]	105.93±4.8 [b]	7,163.980	<0.0001***

Number of sacrificed rats for each group was 10 rats
ANOVA: Analysis of variance
SD: Standard Deviation
a = significant versus group I
b = significant versus group IV
P <0.0001*** highly significant
GPX: Glutathione peroxidase
MDA: Malondialdehyde

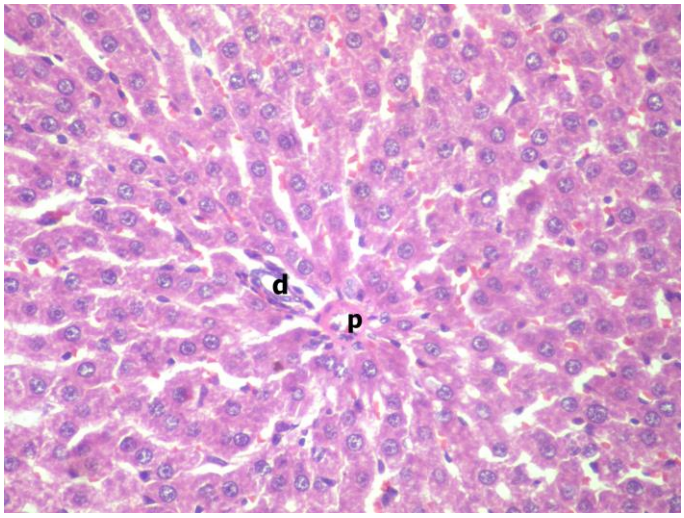

Figure 1: A photomicrograph of a section from the liver of a control group showing the portal area containing the portal vein (P) and bile duct (d).
(H&E X 400).

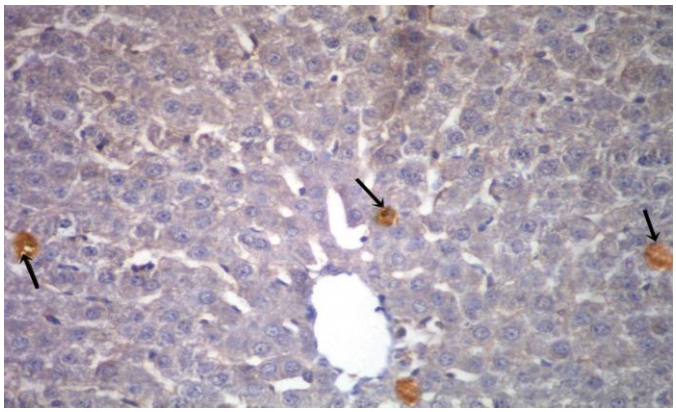

Figure 2: photomicrograph of a section from the liver of a control group showing positive reaction for activated caspase 3 in few hepatocyte (arrow) (Caspase-3 immunostaining X 400).

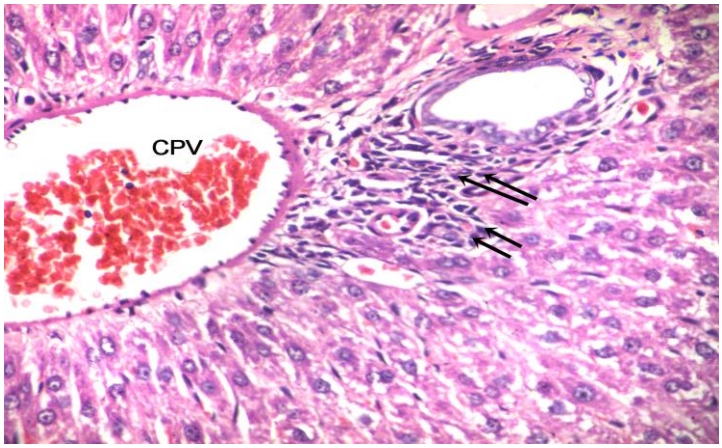

Figure 3: A photomicrograph of a section from liver of bisphenol A group showing dilated congested portal vein (CPV), bile duct proliferation (arrows).
(H&E X 400).

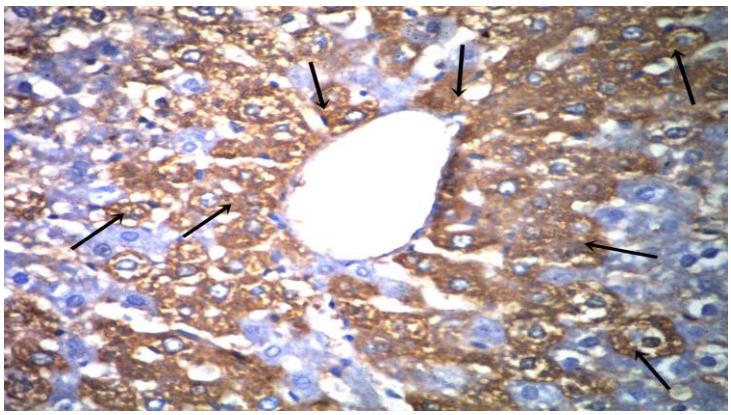

Figure 4: A photomicrograph of a section from liver of bisphenol A group showing positive reaction for activated caspase-3 in many hepatocytes (arrows). (Caspase-3 immunostaining X 400).

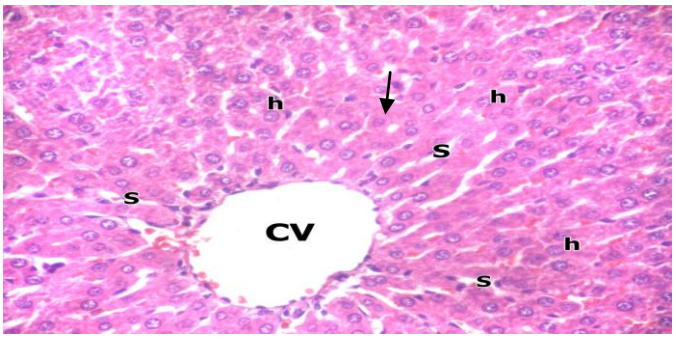

Figure 5: A photomicrograph of a section from liver of bisphenol + ginger extract group showing most hepatocytes of the pericentral zone showing non congested central vein (CV) with minimally stained nuclei (h) and clear sinusoids (s).(H&E X 400)

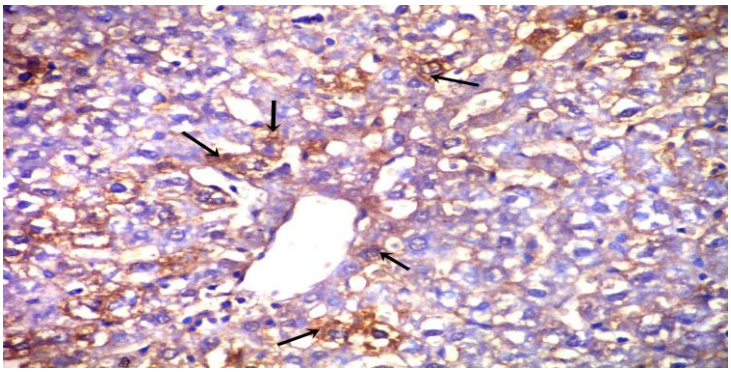

Figure 6: A photomicrograph of a section from liver of bisphenol A + ginger extract group showing positive reacted cells to caspase-3 (minimal staining) (arrows) (Caspase-3 immunostaining X 400).

DISCUSSION

Vandenberg et al. (2007) explained that bisphenol- A is one of the highest volume chemicals produced worldwide**.** This compound is a building block of polycarbonate plastics and epoxy resins that are used to line food and beverage containers.

Calafat et al. (2005) reported that this compound is also found in an enormous number of other products that we come into contact with daily, and therefore it has been detected in the majority of individuals examined **.**

Vandenberg et al. (2007) studied that BPA can leach with food and drink.

Possible target organs of toxicity identified in repeat-dose animal studies with oral dosing included liver, kidney, and reproductive systems **(Yamasaki et al., 2002 and European-Union, 2003).**

Muthuvel et al. (2006) reported that ginger extract is an important dietary antioxidant which significantly decreases the adverse effects of reactive oxygen species implicated in chronic diseases.

Therefore, this study was designed to explore the probable effects of ginger extract in modulating the effects of BPA on hepatic tissue.

The schedule of the present study included four groups: **group Ia (negative control group):** will be lifted without intervention to measure the basic parameters**, group Ib (positive control group):** each rat will be treated daily orally with olive oil (vehicle of bisphenol) once daily via gastric intubations**, group Ic (positive control group):** each rat will be treated daily orally with saline (vehicle of ginger) once daily via gastric intubations **group II (ginger extract group):** each rat will be treated orally with ginger extract (200 mg/kg/day) dissolved in saline, **group III (bisphenol-A group):** Each rat will be treated orally with bisphenol-A (50 mg/kg/day) orally and **group IV (bisphenol-A and ginger extract):** each rat will be treated orally with bisphenol-A (50 mg/kg/ day) and ginger extract (200 mg /kg/ day). Treatment will continue for 8 weeks (three times a week). After the end of the eight weeks, ten rats from each group will be sacrificed for biochemical, histopathologial and immune histochemisy studies.

1-Control Groups (negative and positive control groups) and group II (ginger extract group):

Rats of these groups showed no abnormal findings as regards biochemical studies. There was no significant difference between these groups as regard all these parameters.

Also, there were no abnormal histopathological changes in the liver specimens of the adult male albino rats of these groups all over the periods of the study.

2-Treated groups:

Bisphenol-A treatment had induced a significant increase in the mean values of serum AST and ALT when compared with the control groups.

While bisphenol-A + ginger extract treatment group had induced a significant decrease in the mean values of serum AST and ALT when compared with bisphenol-A groups.

The disturbance of liver AST & ALT of the rats treated with bisphenol-A could be explained by **Jaeschke et al. (2002)** who stated that leakage of the enzymes were produced within hepatocytes and small amounts constantly leak through the cell membrane which gave the normal serum enzymes level of these enzymes. Liver damage caused by liver cell injury (hepatocellular toxicity) made the membranes more permeable. So, greater amounts of enzymes leaked out with subsequent elevation of serum enzymes above normal level.

A significant increase in ALT and AST activities in rats treated with BPA for six and ten weeks (**Mourad and khadrawy, 2012)**.

On the other hand, the present study resulted in significant decrease in GPx and significant increase in MDA in hepatic tissue of bisphenol-A group when compared with control groups.

While there were also significant increase in hepatic GPx and significant decrease in hepatic MDA in bisphenol-A + ginger extract groups when compared with those in bisphenol-A group.

Bindhumol et al. (2003) stated that the activities of antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase decreased while the levels of hydrogen peroxide and lipid peroxidation increased significantly in mitochondrial and microsome-rich fractions of liver when compared with the corresponding group of control animals.

Pigeolet et al. (1990) reported that the reduction in activities of antioxidant enzymes showed the failure of primary antioxidant system to act against free radicals. Antioxidants which are located throughout the cell can provide protection against ROS toxicity. ROS play an important role in the defence mechanisms against pathological conditions but excessive generation of free oxygen radicals may damage tissues **.**

An enhanced ROS generation by polymorphonuclear neutrophils (PMNs) at the site of inflammation causes endothelial dysfunction and tissue injury. The vascular endothelium plays an important role in passage of macromolecules and inflammatory cells from the blood to tissue. Under the inflammatory conditions, oxidative stress produced by PMNs leads to the opening of inter-endothelial junctions and promotes the migration of inflammatory cells across the endothelial barrier. The migrated inflammatory cells not only help in the clearance of pathogens and foreign particles but also lead to tissue injury **(Mittal et al., 2014).**

Mitochondria are a major source of intracellular reactive oxygen species (ROS) and are particularly vulnerable to oxidative stress. Oxidative damage to mitochondria has been shown to impair mitochondrial function and lead to cell death via apoptosis and necrosis. Because dysfunctional mitochondria will produce more ROS, a feed-forward loop is set up whereby ROS-mediated oxidative damage to mitochondria favors more ROS generation, resulting in a vicious cycle **(Szeto, 2006).**

Bai and Odin (2003) explained that Oxidative stress can lead to damage of the mitochondrial inner membrane, resulting in mitochondrial permeability transition pore (MPTP) formation and subsequent release of cytochrome c and apoptosis inducing factor from the mitochondria. In the cytosol, cytochrome C complexes with apoptotic protease activating factor (Apaf-1) to activate procaspase 9, which in turn activates downstream effector caspases (3, 6 and 7) **.**

Nakagawa and Tayama (2000) reported that bisphenol-A has been shown to reduce mitochondrial function in hepatocytes.

Moon et al. (2012) reported that a low dose of BPA induces mitochondrial dysfunction in the liver, and this is associated with an increase in oxidative stress and inflammation and this go parallel with our study.

Measurement of MDA levels in the tissue is a marker of lipid peroxidation which is among the chief mechanism of cell damage. Bisphenol-A is lipophilic in nature due to which it can easily penetrate/interact with the lipid membrane of the hepatocytes **(Doerge and Fisher, 2010)**.

The results of the present study were in agreement with the study of **Suthar and Verma** (**2014)** which concluded that treatment of BPA for 30 days cause increase in lipid peroxidation as well as alterations in the anti-oxidative system ultimately causing oxidative stress in experimental animals.

A study of **Sangai et al. (2014)** also revealed decrease of oxidative damage in liver and kidney of mice after exposure to BPA by potent antioxidants such as quercetin. They also showed that exposure to BPA caused significant reduction in the activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase as well as in the levels of glutathione and total ascorbic acid contents; however, significant increase was found in the levels of malondialdehyde.

Therefore, we hypothesized that ginger extract could act as an antioxidant against BPA.

Results of light microscopic examination and immunohistochemical staining of stained liver sections obtained in the present study have supported the above mentioned biochemical results.

Light-microscope examination of H&E stained liver sections of BPA treated group after 8 weeks showed congested portal vein, bile duct proliferation and cellular infilteration, which was minimally observed in the group taken ginger extract with bisphenol.

Venous congestion was a permanent feature in the treated liver sections in this study which accordingly might interfere with the hepatic arterial blood supply. This might lead to the development of ischemia and subsequent necrosis as mentioned by **(Majno and Joris, 1995).**

Our results were matched with **Helal et al. (2013)** who noticed that administration of BPA for 30 days revealed The nuclei of hepatocytes are mostly large with prominent one or more nuclei and with **Hussein and Eid (2013)** who revealed that oral administration of BPA for 21 days gave light abnormal pathological change compared with the control, dilatation and congestion of the central vein, portal vein and hepatic sinusoids.

Also, it was matched with results of **Korkmaz et al. (2010)** in which treatment of rats with bisphenol for 8 weeks resulted in congestion and necrotic areas. These observations may be explained by BPA and induced peroxidation of membrane lipids in the liver cells.

Hussein and Eid (2013) noticed that bisphenol-A caused cell infiltration was observed in focal manner surrounding the dilated bile duct.

Immune-histochemical reaction to caspase 3 revealed positive reaction within cytoplasm of most hepatocytes in BPA group after 8 weeks which became less in group taken BPA + ginger extract for 8 weeks.

Caspases, intracellular cysteine proteases that cleave various substrates including structural proteins such as caspase–3, are the key mediators of apoptosis. Caspase-3, as a main final common executor of apoptosis, is responsible for the cleavage of the key cellular proteins, leading to typical morphological changes observed in cells undergoing apoptosis **(Budihardjo et al., 1999; Saikumar et al., 1999 and Fischer et al., 2003).**

Asahi et al. (2010) found that BPA induced endoplasmic reticulum (ER) stress-associated apoptosis in hepatocytes. The ER stress was due to ROS production and was independent of estrogen receptors.

Iida et al. (2003) had shown that apoptosis induction by BPA was associated with caspase activation.

The overproduction of ROS may be an inducible factor of apoptosis. Previous studies reported that BPA exposure produced ROS by inhibiting antioxidant enzymes **(Chitra et al. 2003 and Kabuto et al. 2003).**

Our results were matched with **Ahmad et al. (2006)** in which they found that ginger extract may have antioxidant effect by replacing SOD activities and reducing the level of superoxide radicals in liver cancer induced rats. This is similar to the findings of **Park et al. (1998)**, in which the bioactive component in ginger reduced the production of ROS such as superoxide anions.

Chang et al. (1994) found the bioactive component of ginger, namely gingerol, possessed antioxidative effect by inhibiting peroxidation of phospholipids induced by xanthine oxidase activity.

Ahmad et al. (2006) concluded that ginger extract may have bioactive components with antioxidant activity in scavenging free radicals such as superoxide anions and H2O2 as well as decreasing the MDA level for the reduction of lipid peroxidation.

CONCLUSION

From this study we concluded that bisphenol-A induced oxidative stress and apoptosis in hepatic tissue which decreased with administration of ginger extract which acts as an antioxidant agent.

RECOMMENDATION

1-More attention should be paied to health education about sources of exposure to Bisphenol-A in the environment and we should try to minimize them.

2-The imposition of strict laws to prevent the use of bisphenol-A in many products.

3-The use of plastic products free of bisphenol-A or replace the container glass or other materials free of bisphenol-A to save the foods and beverages.

4- Further studies should be done on the toxic effects of Bisphenol-A and the use of antioxidants in the prevention of toxic effects.

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دراسة دور مستخرج الزنجبيل على التأثيرات السامة لمادة البيسفينول-أ على الكبد فى ذكور الجرذان البيضاء البالغة

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الملخص العربي

المقدمة: البيسفينول - أ هو مونومر يستخدم في صناعة البلاستيك البولي ويستخدم في أشكال متنوعة من المنتجات البلاستيكية في المواد الغذائية والصناعات الإلكترونية. وقد وجد أنه عن طريق الأكسدة يسبب آثاراً سامة فى الكبد والكلى والخصيتين. وقد تم تأكيد العديد من الآثار الدوائية للزنجبيل ومكوناته الطازجة والمجففة. ومن بينها مضادات الأكسدة ومكافحة الأورام ومكافحة الفيروسات ومكافحة التسمم الكبدي.
هدف البحث: تم إجراء هذا البحث لدراسة دور مستخرج الزنجبيل على التأثيرات السامة لمادة البيسفينول-أ على الكبد فى ذكور الجرذان البيضاء البالغة.
خطة البحث: تم عمل هذا البحث على عدد 60 من ذكور الجرذان البيضاء البالغة . تم تقسيمها إلى اربع مجموعات كالآتى: المجموعة الأولى وقد تم تقسيمها كالاتى (مجموعة ضابطة سالبة): تم إعطاؤها الوجبة العادية والماء بدون أي علاج لقياس المعايير الأساسية و (مجموعة ضابطة موجبة): تم إعطاؤها زيت زيتون كمذيب لمادة البيسفينول عن طريق الفم مرة واحدة يومياً و (مجموعة ضابطة موجبة): تم إعطاؤها محلول الملح كمذيب لمادة الزنجبيل عن طريق الفم مرة واحد. المجموعة الثانية (مجموعة مستخرج الزنجبيل): تم إعطاؤها مستخرج الزنجبيل عن طريق الفم (200 مجم\كجم) مرة واحدة يومياً. المجموعةالثالثة (مجموعة البيسفينول-أ): تم إعطاؤها البيسفينول-أ عن طريق الفم (50 مجم\كجم) مرة واحدة يومياً. المجموعةالرابعة (مجموعة البيسفينول-أ + مستخرج الزنجبيل): تم إعطاؤها البيسفينول-أ (50مجم\كجم) + مستخرج الزنجبيل (200 كجم\كجم) عن طريق الفم مرة واحدة يوميا. استمرت الدراسة ثمانية أسابيع تم بعدها أخذ عينات الدم ثم ذبح جرذان كل مجموعة وعمل الآتي: قياس إنزيمات ترانسفيراز الألانين وترانسفيراز الأسبرتات، تحديد مستوى مضادات الأكسدة\ بيروكسيداز الجلوتاثيون فى نسيج الكبد، تحديد مستوى المالونديالدهايد فى نسيج الكبد وأخذ عينات من الكبد لفحصها بالميكروسكوب الضوئى ودراستها هستوكيميائياً.
ثم تم تجميع النتائج وتحليلها بطرق إحصائية مناسبة ووضعها في جداول وصور وتم مناقشتها.
النتائج: من هذه الدراسة تم استنتاج ما يلي: المجموعة التي أخذت البيسفينول-أ قد اختلت بها وظائف الكبد فى صورة ارتفاع ذي دلالة إحصائية فى إنزيمات الكبد بالمقارنة مع المجموعة الضابطة السالبة مصحوباً بخلل فى التركيب الهستولوجي والهستوكيميائي ناتج عن زيادة فى الشوارد الحرة فى صورة انخفاض ذي دلالة إحصائية فى دلالات الأكسدة (بيروكسيداز الجلوتاثيون) وزيادة ذات دلالة إحصائية فى نواتج الأكسدة (المالونديالدهايد). وقد قل هذا التأثير على الكبد فى المجموعة التي أخذت كل من البيسفينول-أ ومستخرج الزنجبيل.
الخلاصة: التعرض لمادة البيسفينول-أ عن طريق الفم بجرعة 50 مجم\كجم له تأثير سام على الكبد. هذا التسمم الكبدي ناتج عن زيادة الشوارد الحرة والموت المبرمج للخلايا. و قد قل التأثير السام للبيسفينول-أ على الكبد عند إعطاء مستخرج الزنجبيل (200مجم\كجم) مع البيسفينول-أ.
التوصيات: الاهتمام أكثر بالتثقيف الصحي لمعرفة مصادر التعرض للبيسفينول-أ فى البيئة ومحاولة الإقلال منها. فرض قوانين صارمة لمنع استخدام البيسفينول-أ فى المنتجات. استخدام المنتجات البلاستيكية الخالية من البيسفينول-أ أو استبدالها بالحاويات الزجاجية أو غيرها من المواد الخالية من البيسفينول-أ لحفظ الأطعمة والمشروبات. ينصح بإجراء المزيد من الدراسات على التأثيرات السامة للبيسفينول-أ واستخدام مضادات الأكسدة فى الوقاية من آثارها السامة.