

EFFECT OF VITAMIN C AND THYMOQUINONE ON EXPERIMENTALLY BISPHENOL A INDUCED HEPATO - RENAL TOXICITY IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Bisphenol-A (BPA) is the building block of polycarbonate plastics, a hard plastic used to make numerous consumer products, including most baby bottles and water bottles. **Objective:** Assessing the adverse effect of oral administration of BPA on liver and kidney of adult male albino rats and evaluation of the role of vitamin C and thymoquinone (TQ) in alleviating the possible detrimental effects of BPA. **Material and Methods:** Fifty six adult male albino rats of local strain were housed in 14 suitable metal cages (20 × 32 × 20 cm for every 4 rats). They were divided into seven equal groups: The 1st group was served as a control group, the 2nd group was treated with bisphenol-A, the 3rd group was normal and treated with vitamin C alone, the 4th group was normal and treated with thymoquinone alone, the 5th group was administered by bisphenol-A and treated with vitamin C, the 6th group was administered by bisphenol-A and treated with thymoquinone and the 7th group was administered by bisphenol-A and treated with both vitamin C and thymoquinone. All animals were treated for four weeks. **Results:** Administration of bisphenol-A (50mg/Kg body weight orally) to rats resulted in an increase in alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatine, and hepatic and renal MDA level, and decreased reduced glutathione (GSH) and superoxide dismutase (SOD) contents of the liver and kidney as compared to control group. In contrast, the administration of vitamin C (500 mg/ kg body weight) and/ or thymoquinone (10 mg /kg body weight) to bisphenol-A treated rats attenuated the toxicity of bisphenol. This alleviation was more pronounced in the bisphenol-A group treated with both vitamin C and thymoquinone. **Conclusion:** BPA has detrimental effect on liver and kidney of adult male albino rats. It supported the possibility that the synergistic effect of thymoquinone and vitamin C played a notable role in protecting the liver and kidney against BPA-induced toxicity and oxidative stress in male rats.

Key words: Bisphenol-A, vitamin C, thymoquinone.

INTRODUCTION

BPA is an additive that has been used for more than 40 years to harden plastics, keep bacteria from growing in foods and prevent cans from rusting (Maćczak et al., 2017). BPA is the building block for polycarbonate, a hard strong plastic used to make compact disks, eye glass lenses, drinking glasses, water bottles, epoxy resins and baby bottles (Crain et al.,

2007). Human exposure to BPA occurs through multiple routes. However, oral exposure is considered the major route of exposure, while air, dust, and water are other possible sources of exposure (Di Donato et al., 2017).

BPA may be absorbed in the gastrointestinal tract after ingesting products packed in plastic containers. Like intestinal phenols, BPA is conjugated by

glucuronic acid in intestine and liver, and excreted in urine within 24 as BPA-glucuronide . BPA is only biologically active in its unconjugated form (**Wang et al., 2017**).

Reactive oxygen species (ROS) are cytotoxic agents causing oxidative damage by attacking cell membrane and DNA (**Ameziane et al., 2017**). Antioxidants are scavengers that prevent cell and tissue damage that could lead to cellular damage and disease (**Cooper et al., 2011**). Antioxidant agents have been used to prevent tissue damage in various clinical settings and experimental models, and could help in preventing lipid peroxidation and hydrogen peroxide levels, resulting in reduced kidney and hepatic injury (**Gong et al., 2013**).

Vitamin C is a potent natural antioxidant capable of reducing oxidative stress, scavenging free radicals and reestablishing anti oxidative systems (**Masaki, 2010**). Even in small amounts, vitamin C can protect indispensable molecules in the body such as proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species (ROS) that are generated during normal metabolism (**Sarita et al., 2011**). Thymoquinone (TQ) was shown to inhibit tissue inflammation and oxidative stress (**Muhammad et al., 2017**). The protecting effects of vitamin C and thymoquinone in the kidney and liver are reported to be due to their antioxidant activity (**Ayman et al., 2014**).

The present study aimed to evaluate the effect of vitamin C and thymoquinone on BPA-induced hepato– renal toxicity in adult male albino rats.

MATERIAL AND METHODS

Chemicals: Bisphenol-A, vitamin C and thymoquinone were purchased from Sigma –Aldrich Chemical CO.(USA). All other chemicals were of analytical grade and were obtained from commercial kits purchased from (Biodiagnostics Co. (Dokky, Giza, Egypt). Half gram of Bisphenol-A powder were dissolved in 50 cm sterile water, and 1 cm of solution contained 10 mg of Bisphenol-A. Half gram of vitamin C powder was dissolved in 5 cm sterile water, and 1 cm of solution contained 100 mg of vitamin C. Half gram of thymoquinone powder was dissolved in 50 cm sterile water and 1 cm of solution contained 10 mg of thymoquinone.

Animals: Fifty six adult male albino rats of local strain weighing 180 to 200 grams. They were chosen as an animal model for this study. They were brought from animal house, Faculty of Medicine, Assiute University, Assiute, Egypt. They were housed in metal cages (20 ×32× 20 cm for every 4 rats) at room temperature, with the natural light/dark cycle in animal laboratory of Pharmacology Department, Al-Azhar University of Medicine (Assiute). They were maintained on dry chow pellets and water ad libitum throughout the experimental period. All the experiments were performed during the same time of day, between 9 a.m. and 12 p.m. to avoid variations due to diurnal rhythms (**Shimizu et al., 2015**). They were kept for two weeks under this condition to adapt the laboratory conditions before the start of the experiment.

Methods: After 2 weeks of acclimatization, male albino rats were randomly divided into 7 equal groups as follows:

Group I was served as a control group.

Group II was administered freshly prepared BPA 50 mg /Kg body weight (5cm for each 100 g body weight of rat) in water once daily for four weeks orally via orogastric gavage (**Da Chen et al., 2016**).

Group III was administered vitamin C at a dose of 500 mg /kg body weight (1cm for each 50 g body weight of rat) once daily for four weeks orally via oro gastric gavage (**Adikwu and Deo, 2013**).

Group IV was administered thymoquinone (TQ) 10 mg /kg body weight (1cm for each 100 g from weight of rat) once daily for four weeks orally via oro gastric gavage (**Aycan et al., 2015**).

Group V received the same previous doses of BPA and vitamin C at the same time for four weeks orally via oro gastric gavage.

Group VI received the same previous doses of BPA and TQ at the same time for four weeks orally via oro gastric gavage.

Group VII received the same previous doses of BPA, vitamin C and TQ at the same time for four weeks orally via oro gastric gavage.

Collection of blood samples for laboratory assessment: After 24 hours of receiving the last dose, blood samples were obtained from the retroorbital venous plexus of the eye. Blood was collected into non heparinized tubes. Serum was separated from blood by centrifugation at 5000 rpm for 10 minutes and was stored at -20 C until used for estimation of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and urea and creatinine levels.

Tissue sampling and preparation of liver and kidney homogenate:

Immediately after blood samples were collected, under ether anesthesia, the abdomens of the rats were immediately opened after reaching the stage of surgical anesthesia as evident by loss of withdrawal reflex. Then the liver and kidney of different groups were excised, weighed, perfused with normal saline to remove blood, blotted between filter papers and used for the preparation of tissue homogenate. About 0.5 gm of each organ was homogenized in 4.5 ml of phosphate –buffered saline (pH 7). The crude tissue homogenate was then centrifuged at 8,000 rpm for 30 minutes. The clear supernatant from liver and kidney homogenate were stored at 4°C and were used for the assay of reduced glutathione (GSH) (**Beutler et al., 1963**), superoxide dismutase (SOD) (**Nishikimi et al., 1972**), and malondialdehyde (MDA) (**Ohkawa et al., 1978**) by colorimetric method.

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean \pm standard error (S.E). Statistical analysis of the difference between groups was performed by using One –way analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means. A value of $P < 0.05$ was used as the limit for statistical significance.

RESULTS

The liver functions (ALT, AST and ALP) in BPA treated group(II) resulted in significant elevation compared to control group (I). Also, liver functions (ALT, AST and ALP) in BPA treated group(II)

resulted in significant elevation as compared to group treated with either vitamin C(III) or thymoquinone (IV) each alone. Meanwhile, administration of either vitamin C(III) or thymoquinone (IV) each alone revealed non-significant changes in liver functions (ALT, AST and ALP) as compared to a normal control group (I). Also, administration of thymoquinone alone revealed non-significant changes in liver functions (ALT, AST and ALP) as compared to group treated with vitamin C (III) alone. The BPA group treated with either vitamin C (V) or thymoquinone (VI) each alone afforded significant decrease in liver

functions (ALT, AST and ALP) as compared to bisphenol-A group (II). Administration of a mixture of antioxidant elements (vitamin C + thymoquinone) into bisphenol A group(VII) achieved a significant reduction in liver functions (ALT, AST and ALP) as compared to the Bisphenol-A group (II), it was obvious that bisphenol -A group treated with a mixture of antioxidant element(vitamin C + thymoquinone) (VII) induced more decrement in liver functions levels than Bisphenol-A group treated with either vitamin C (V) or thymoquinone (VI) (Table 1).

Table (1): Changes in ALT, AST and ALP in response to Bisphenol-A, vitamin C and thymoquinone administration in different groups (Mean± SE).

Liver Functions	AST(U/L)	ALT(U/L)	ALP(U/L)
Group I (Control)	45±0.63	63.11±0.53	245.10±0.27
Group II(BPA)	71.52±0.50*	136.20±0.36*	357.34±0.28 *
Group III (Vit C)	44.50±0.64#	65.21±0.53#	243.50±0.27#
Group IV (TQ)	43.40±0.64#	64.11±0.53#	244.70±0.26#
Group V(BPA + Vit C)	63.45±0.53* #	80.33±0.47* #	292.34±0.25* #
Group VI(BPA + TQ)	62.31±0.54* #	82.21±0.47* #	290.22±0.25* #
Group VII (BPA+VitC+TQ)	54.31±0.56* # ±	70.21±0.51* # ±	280.42±0.24* #±

- Significant result as compared to group V, VI ±

- Significant result as compared to group II #

- Significant result as compared to group I *

Urea and creatine in BPA-treated group (II) showed significant elevation compared to control group (I). Also, urea and creatine in BPA treated group group (II) resulted in significant elevation as compared to group treated with either vitamin C group (III) or thymoquinone (IV) each alone. Meanwhile, administra-

tion of either vitamin C (III) or thymoquinone (IV) each alone revealed non-significant changes in kidney functions (urea and creatinine) as compared to control group (I). Also, administration of thymoquinone (IV) alone revealed non-significant changes in kidney functions (urea and creatinine) as

compared to group treated with vitamin C(III) alone. The BPA group treated with either vitamin C (V) or thymoquinone(VI) each alone afforded significant decrease in kidney functions (urea and creatine) as compared to the BPA group(II). It was obvious that BPA group treated with a mixture of antioxidant elements (vitamin C + thymoquinone) (VII) induced more

decrement in kidney functions levels than BPA group treated with either vitamin C(V) or thymoquinone(VI) each alone. Administration of a mixture of antioxidant elements (vitamin C + thymoquinone) in BPA group (VII) succeeded in restoring kidney functions (urea and creatine) to normal values as compared to the untreated BPA group (II) (Table 2).

Table (2): Changes in urea and creatine in response to Bisphenol-A, vitamin C and thymoquinone administration in different groups (Mean± SE).

Groups	Kidney Functions	Urea (mg/dl)	Creatine (mg/dl)
Group I (Control)		21.03 ±0.93	0.75±1.55
Group II(BPA)		46.35±0.62*	1.91±3.80*
Group III (Vit C)		22.35±0.84#	0.74±5.17#
Group IV (TQ)		23.55±0.83#	0.73±5.17#
Group V(BPA + Vit C)		25.78±0.84*#	0.98±4.55*#
Group VI(BPA + TQ)		27.68±0.83*#	1.02±4.40*#
Group VII (BPA+ Vit C+TQ)		24.34±0.86*# ±	0.77±4.80*# ±

- Significant result as compared to group V, VI ±
- Significant result as compared to group II #
- Significant result as compared to group I *

The level of liver GSH and SOD in the BPA group (II) significantly decreased as compared to control group(I). Also, level of liver GSH and SOD in BPA treated group(II) resulted in significant decrease as compared to group treated with either vitamin C (III) or thymoquinone (IV) each alone. Meanwhile, administration of either vitamin C (III) or thymoquinone (IV) each alone revealed non-significant changes in liver GSH and SOD level as compared to control group (I). Also, administration of thymoquinone (IV) alone induced non-significant changes in liver GSH and SOD level as compared

to group treated with vitamin C (III) alone. The treatment of BPA group with either vitamin C (V) or thymoquinone (VI) each antioxidant alone elicited a significant increase in liver GSH and SOD level as compared to BPA group (II). It was obvious that BPA group treated with a mixture of both vitamin C and thymoquinone (VII) induced more increment in liver GSH and SOD level than BPA group treated with either vitamin C (V) or thymoquinone (VI) each alone. The treatment of BPA group with both vitamin C and thymoquinone (VII) significantly increased level of GSH and

SOD in liver in comparison with the untreated BPA group(II) (Table 3).

The level of liver MDA in the BPA-treated rats (II) was significantly higher than that of control group (I). Also, level of liver MDA in BPA treated group (II) resulted in significant elevation as compared to group treated with either vitamin C (III) or thymoquinone (IV) each alone. Meanwhile, administration of either vitamin C (III) or thymoquinone (IV) each alone induced non-significant changes in liver MDA level as compared to the normal control group (I). Also, administration of thymoquinone (IV) alone revealed non-significant changes in liver MDA as compared to group treated

with vitamin C (III) alone. The treatment of BPA group with either vitamin C (V) or thymoquinone (VI) each antioxidant alone elicited a significant decrease in liver MDA level as compared to BPA group (II). It was obvious that BPA group treated with both vitamin C and thymoquinone (VII) induced more decrement in liver MDA level than BPA group treated with either vitamin C (V) or thymoquinone (VI) each antioxidant alone. The treatment of BPA group with both vitamin C and thymoquinone(VII) significantly inhibited the increase of liver MDA in comparison with BPA group (II).

Table (3): Changes in liver GSH, SOD and MDA activities in response to Bisphenol-A, vitamin C and thymoquinone administration in different groups (Mean± SE).

Oxidative markers in liver	GSH (U/ gm tissue)	SOD (U/ gm tissue)	MDA (U/ gm tissue)
Group I (Control)	34.50±0.72	70.11±0.51	250.10±0.27
Group II(BPA)	20.52±0.94*	40.20±0.67*	370.34±0.22*
Group III (Vit C)	32.50±0.77#	69.21±0.51#	248.50±0.27#
Group IV (TQ)	30.40±0.74#	68.11±0.51#	247.70±0.27#
Group V(BPA + Vit C)	28.45±0.80*#	64.33±0.53* #	280.34±0.23*#
Group VI(BPA + TQ)	29.31±0.78*#	65.21±0.53* #	281.22±0.23*#
Group VII (BPA+VitC+TQ)	31.31±0.76* # ±	67.21±0.52* # ±	249.42±0.27*# ±

- Significant result as compared to group V, VI ±

- Significant result as compared to group II #

- Significant result as compared to group I *

The levels of kidney GSH and SOD in the BPA group (II) were significantly decreased as compared to control group (I). Also, levels of kidney GSH and SOD in BPA treated group (II) resulted in significant decrease as compared to group

treated with either vitamin C (III) or thymoquinone (IV) each alone. Meanwhile, administration of either vitamin C (III) or thymoquinone (IV) each alone revealed non-significant changes in kidney GSH and SOD levels as compared

to the normal control group (I). Also, administration of thymoquinone (IV) alone induced non-significant changes in kidney GSH and SOD as compared to group treated with vitamin C(III) alone. The treatment of BPA group with either vitamin C (V) or thymoquinone (VI) each antioxidant alone elicited a significant increase in kidney GSH and SOD levels as compared to BPA group(II). It was obvious that BPA group treated with both vitamin C + thymoquinone(VII) induced more increment in GSH and SOD levels than BPA group treated with either vitamin C (V) or thymoquinone (VI) each antioxidant alone. The treatment of BPA group with both vitamin C and thymoquinone (VII) significantly increased level of GSH and SOD in kidney in comparison with the BPA group (II).

The levels of kidney MDA in BPA-treated rats (II) was significantly higher than that of control group (I). Also, levels of kidney MDA in BPA treated group(II) resulted in significant elevation as compared to group treated with either

vitamin C (III) or thymoquinone (IV) each alone. Meanwhile, administration of either vitamin C (III) or thymoquinone (IV) each alone induced non-significant changes in kidney MDA level as compared to the normal control group (I). Also, administration of thymoquinone (IV) alone revealed non-significant changes in kidney MDA as compared to group treated with vitamin C (III) alone. The treatment of BPA group with either vitamin C (V) or thymoquinone (VI) each alone elicited a significant decrease in kidney MDA level as compared to BPA group (II). It was obvious that BPA group treated with both vitamin C+ thymo-quinone (VII) induced more decrement in kidney MDA level than BPA group treated with either vitamin C (V) or thymoquinone (VI) each antioxidant alone. The treatment of BPA group with a mixture of antioxidant elements (vitamin C+ thymoquinone) (VII) significantly inhibited the increase of kidney MDA in comparison with the BPA group (II).

Table (4): Changes in kidney GSH, SOD and MDA activities in response to Bisphenol-A, vitamin C and thymoquinone administration in different groups (Mean ± SE).

Oxidative markers in kidney	GSH (U/ gm tissue)	SOD (U/ gm tissue)	MDA (U/ gm tissue)
Group I (Control)	60.50±0.55	22.11±0.92	0.5 ±6.00
Group II(BPA)	40.52±0.67*	10.20±1.33*	1.2 ±4.00*
Group III (Vit C)	61.50±0.54#	23.21±0.88#	0.3 ±6.00#
Group IV (TQ)	62.40±0.55#	24.11±0.87#	0.4±4.00#
Group V(BPA + Vit C)	50.45±0.60*#	15.33±1.10*#	0.7 ±4.80*#
Group VI(BPA + TQ)	52.31±0.58*#	17.21±1.03*#	0. 8±5.10*#
Group VII (BPA+VitC+TQ)	55.31±0.57*# ±	20.21±0.95*# ±	0.6±6.00*# ±

- Significant result as compared to group V, VI ±
- Significant result as compared to group II #
- Significant result as compared to group I *

DISCUSSION

This study set out to assess the impact of vitamin C and thymoquinone on bisphenol A - induced hepato-renal toxicity in adult male albino rats. The current study demonstrated that BPA caused elevation of kidney and liver enzymes (damage markers). Also, this study has revealed disturbances in oxidative/ anti oxidative status in rat liver and kidney with concomitant impairment in its proper functioning as a result of BPA administration. Supplementation with vitamin C and thymoquinone protected liver and kidney against BPA induced hepato-renal toxicity and improved its antioxidant status. In the present study, it has been observed that administration of a mixture of antioxidant elements (vitamin C + thymoquinone) in bisphenol A group succeeded in restoring liver and kidney functions to normal values.

Liver function tests are routinely used as diagnostic markers for hepatotoxicity. Aminotransferases (ALT and AST) and alkaline phosphatase (ALP) are the major markers in monitoring the functional status of liver. The ALT activity is an important index to measure the degree of cell membrane damage while AST is an indicator of mitochondrial damage. The activity of ALP is often employed to assess the integrity of plasma membrane (Shi et al., 2015).

BPA administration significantly increased the serum indices of liver function as indicated by elevation in the activity of ALT, AST and ALP as compared to the control group. The high levels of ALT, AST and ALP are attributed to damage in liver.

Moon et al., (2012) mentioned that BPA can induce hepatic damage and mitochondrial dysfunction by increasing oxidative stress in the liver. Similarly, elevated levels of serum indices as a result of liver damage in rats due to BPA toxicity have been previously reported by Korkmaz et al. (2010). Ronn et al. (2013) also reported an increase in AST activity in male rats treated with BPA 200 mg/kg/day and increased ALP, γ -glutamyl transpeptidase activity in male rats treated with 600 mg/kg/day.

The mechanisms involved in Bisphenol-induced hepato-renal damage were investigated and our results showed that BPA induced oxidative stress, evident by decreased GSH level and SOD activity with increased lipid peroxidation product, MDA, which was in consistent with previous studies of Zeinab et al.(2012) and Ozra et al.(2015). These results are consistent with the previous findings released by some research group who had found an association between Bisphenol A toxicity and the increased oxidative stress in rats (El-Megharbel et al., 2015). In the current study, it was noted that the elevation in liver enzymes was decreased significantly after treatment of Bisphenol rats with either vitamin C or thymoquinone each alone or their combination as compared to Bisphenol group. Similar results were obtained by Wesam (2014) who reported that treatment with thymoquinone at a dose of 10 mg/kg BW significantly suppressed the bisphenol-induced elevation in these hepatic biomarkers.

The efficacy of thymoquinone in protecting hepatic enzyme leakage may be related to its ability to preserve the

structural and functional integrity of the liver against the adverse effects of BPA as well as repair of hepatic tissue damage caused by BPA. These results come in accordance with studies which indicated the hepatoprotective effect of thymoquinone in models of liver injury (**Hamid et al.(2014)**). This result was in agreement with **Korkmaz et al.(2010)** who reported that vitamin C co-administration along with BPA protect against liver damage in male rats. **Milad et al. (2015)** also reported that the administration of vitamin C or vitamin C combined with chitosan restored MDA levels, which reveals that vitamin C preserves the integrity of cellular membrane and the normal physiological functions of hepatocyte.

Hepatoprotective property of vitamin C is attributed to its antioxidant property. Vitamin C which is a major water-soluble antioxidant is believed to decrease lipid peroxidation either directly or indirectly by regenerating vitamin E. Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. (**Adikwu and Deo, 2013**).

Furthermore, BPA administration elevated levels of serum renal injury markers such as urea and creatine as compared to control group. These results are in agreement with **Hassan et al. (2012)** and **Ahmed et al. (2015)** who reported that Bisphenol has a nephrotoxic effect and result in affecting the glomerular filtration. Also, these results were in agreement with the results of **Sangai et al., (2012)** and **Yldiz et al., (2013)** who reported that the bisphenol-treated rats showed severe degenerative

changes in the renal corpuscles and the renal tubules. **Jyothi et al. (2009)** observed significantly increased values in the concentration of serum creatinine when exposed to bisphenol in rats. The most likely explanation for these finding is by **ELadak et al.(2015)** who reported that Bisphenol A has a nephrotoxic effect due to accumulation of BPA toxic metabolites and inability of the kidney to eliminate them and reported that bisphenol A induces mitochondrial dysfunction and rough endoplasmic damage which in turn is important for protein pathway.

In the present study ,it was noted that the elevation in kidney function markers was decreased significantly after treatment of Bisphenol rats with either vitamin C or thymoquinone each alone or their combination as compared to Bisphenol group. The result of the present work was in agreement with **Mohamed and Emad, (2015)** where they noticed the protective effects of Nigella sativa oil (NSO) and/or ascorbic acid (AA), against oxytetracycline(OTC)-induced nephrotoxicity in rabbits. The activities of the enzymes involved in glutathione pathways were also disrupted in the bisphenol treated group (tab 3, 4), indicating the involvement of oxidative stress in hepato-renal damage.

Our results showed that administration of Bisphenol caused a significant increase in hepatic lipid peroxidation level (MDA), depletion of GSH contents and decreased SOD activity (Table 3) as compared to control group. These findings come in agreement with the results obtained by **Raluca et al. (2014)** who found that Bisphenol had increased the hepatic

oxidative stress and mitochondrial dysfunction leading to structural changes of rat liver and alteration of hepatic reduced glutathione (GSH) contents, glutathione peroxidase (GPx) and glutathione reductase (GR) activities. The result of the present work was in agreement with **Hassan et al.,(2012)** who noticed that Bisphenol induced hepatotoxicity through oxidative stress.

This study demonstrated that administration of Bisphenol caused a significant increase in renal lipid peroxidation level (MDA), depletion of GSH contents and decreased SOD activity (Table 4) as compared to that of rats of control group. This result was in disagreement with **(Mourad and Khadrawy (2012))** who reported that there were non-significant changes in oxidative stress parameters in the kidney and liver due to BPA treatment. All these effects are involved in Bisphenol -induced hepato-renal oxidative damage and toxicity, as a result of excessive generation of free radicals, which have been reported to affect various biological molecules, including lipids and induce lipid peroxidation.

Oxidative stress, mitochondrial damage and intracellular glutathione depletion are the most important factors contributing to the prediction of hepato-renal toxicity **(Ahmad and Basma2016)**. Reactive oxygen species (ROS) are instantly produced in the body due to exposure to a wide range of exogenous chemicals, drugs and xenobiotics.

In the current study, treatment with vitamin C or thymoquinone each alone or their combination played a role in ameliorating bisphenol-induced hepato-renal toxicity. Their free radical scaveng-

ing abilities seem to mediate such a protective effect, indicated by the reduction of MDA as well as the elevation of GSH and SOD levels in hepatic and renal tissue. But, this alleviation is more pronounced in the bisphenol group treated with both of the antioxidants. Also the improvement was more pronounced when both vitamin C and thymoquinone were used together in bisphenol group.

Thus, the synergistic effect of vitamin C and thymoquinone is most powerful in reducing the hepato-renal toxicity induced by BPA and improving the liver and kidney antioxidant status. This result was in agreement with **Saleem et al., (2012)** who noticed nephro-protective effect of vitamin C and Nigella sativa oil on gentamicin associated nephrotoxicity in rabbits.

The protective effect of vitamin C and thymoquinone against BPA-induced oxidative stress in our rat model could be either direct by inhibiting lipid peroxidation and scavenging free radicals, or indirect through the enhancement of SOD and GSH activities; the enzymatic free radical scavengers in the cells. Therefore, vitamin C and thymoquinone could be used in combination to prevent and treat hepatic and renal diseases, especially those induced by oxidative damage.

CONCLUSION

Based on the data of the current study, it may be concluded that BPA has detrimental effect on liver and kidney of male rats. Also it supports the possibility that the synergistic effect of thymoquinone and vitamin C played a notable

role in protecting the liver and kidney against BPA-induced toxicity and oxidative stress in male rats. The strong antioxidant activity could partially be the mechanism underlying the protective effects of thymoquinone and vitamin C. The versatility and potency of thymoquinone and vitamin C make it potential candidates for therapeutic and preventive drugs for hepato-renal toxicity resulting from toxicants, including BPA. To mimic the study's result, drink tap water or rely on BPA-free stainless steel water bottles. To be safe, avoid all canned foods and replace with non-canned variations.

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تأثير فيتامين سي والثيموكينون علي سمية الكبد والكلبي المستحدث عمليا بالبيسفينول أ في ذكور الجرذان البيضاء البالغة

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خلفية البحث: البيسفينول أ هو أساس العديد من المنتجات البلاستيكية حيث يستخدم في تصنيع العديد من المنتجات البلاستيكية كمعظم زجاجات المياه و زجاجات رضاعة الأطفال

الهدف من البحث: دراسة تأثير فيتامين سي والثيموكينون علي سمية الكبد والكلبي المستحدث بالبيسفينول أ في ذكور الجرذان البيضاء البالغة

طريقة البحث: أجريت هذه الدراسة علي ستة وخمسين من الجرذان البيضاء البالغة من سلالة محلية وقد قسمت الجرذان إلى سبع مجموعات متساوية : المجموعة الأولى هي الضابطة والمجموعة الثانية جرعت عن طريق الفم مادة البيسفينول أ يومياً لمدة ٢٨ يوماً متتالية ، والمجموعة الثالثة جرعت عن طريق الفم فيتامين سي يومياً لمدة ٢٨ يوماً متتالية، والمجموعة الرابعة جرعت الثيموكينون لمدة ٢٨ يوماً متتالية. والمجموعة الخامسة جرعت عن طريق الفم مادة البيسفينول أ مع إعطاء فيتامين سي يومياً لمدة ٢٨ يوماً متتالية ، والسادسة جرعت عن طريق الفم مادة البيسفينول أ مع إعطاء الثيموكينون يومياً لمدة ٢٨ يوماً متتالية ، والسابعة جرعت عن طريق الفم مادة البيسفينول أ مع إعطاء فيتامين سي والثيموكينون يومياً لمدة ٢٨ يوماً متتالية. وقد استمرت التجربة ٢٨ يوماً .

نتائج البحث : أسفرت النتائج إلى أن المجموعة المعاملة بمادة البيسفينول أ فقط (٥٠ ملجم /كجم بالفم) أظهرت إرتفاعاً في إنزيم ألانين ترانس أميناز، وإنزيم اسبارتات ترانس أميناز، وإنزيم الفوسفاتاز القلوية ، واليوريا ، والكرياتينين ، وزيادة في تركيز مالونداي الدهيد بينما كان هناك انخفاضاً في معدل مضادات الأكسدة (سوبر أكسيد ديسميوتاز والجلوتاثيون المختزل) بنسجي الكبد والكلبي. وفي المقابل أدت المعالجة بفيتامين سي (٥٠٠ ملجم /كجم بالفم) مع / أو الثيموكينون (١٠ ملجم /كجم بالفم) للمجموعة المعاملة بمادة البيسفينول أ إلى الإقلال من السمية المستحثة بالبيسفينول أ في معظم القياسات السابقة ولقد كان التقليل من سمية البيسفينول أ أكثر وضوحاً في المجموعة المعالجة بفيتامين سي والثيموكينون معا.

الاستنتاج : البيسفينول أ له تأثير ضار على الكبد والكلبي في ذكور الجرذان البيضاء البالغة . ولقد لعب التأثير التآزري لإستخدام فيتامين سي والثيموكينون معا دوراً بارزاً في حماية الكبد والكلبي ضد التسمم الناجم عن البيسفينول أ أو الأكسدة في ذكور الجرذان.