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AMELIORATIVE EFFECTS OF GALLIC ACID AGAINST OXIDATIVE STRESS IN ERYTHROCYTES INDUCED BY BPA IN MALE ALBINO RATS.

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Bisphenol A (BPA) is a chemical material that using in polycarbonate plastic that is widely used in daily life items. Several studies showed the adverse effect on the health of erythrocytes in both human and animals, while gallic acid (GA) is a known antioxidant capable of counteracting free radicals. In this study included 4 groups 10 rats each. The first was the control. The second received BPA in drinking water was given *ad libitum* at dose 40mg/kg.b.w. The third and fourth groups was given BPA by the same dose and by the same route in addition to receive GA in the diet at doses of 50 and 200 mg/kg.b.w, respectively. In erythrocytic hemolysate, the antioxidants: glutathione (GSH), activities of both superoxide dismutase (SOD) and catalase (CAT) were estimated. Also, lipid peroxidation (LPO) and carbonyl protein (CrPr) were determined. The results showed that BPA significantly increased LPO and CrPr and decreased the activities of CAT, SOD and the GSH content. However, the treatments of the BPA-intoxicated rats with GA reversed the erythrocytic oxidative damage and curtailed the decrease in antioxidants. In conclusion, the GA with both doses could attenuate the oxidative damage in erythrocytes of rats-treated with BPA.

Key words: Bisphenol A, Gallic acid, Oxidative stress, Antioxidant enzymes.

INTRODUCTION

Bisphenol A (BPA) is an environmental chemical that has been widely used in the manufacture of polycarbonate plastics and epoxy resins for many years. BPA is 2,2-bis (4-hydroxy- phenyl)

propane), a xeno estrogen, is an important monomer of polycarbonate plastics and a constituent of epoxy and polystyrene resins **(Ranjit *et al*., 2010).** BPA is one of the most produced chemicals in the world and has been widely employed in the food industry. It is a monomer used in the production of a multitude of chemical products, including epoxy resins and polycarbonates. Furthermore, BPA has become a public health concern due to its extensive and continuous exposure through food and drinking water. BPA may be absorbed in the gastrointestinal tract after ingesting products packed in plastic containers. BPA is conjugated by glucuronic acid in bowel liver and excreted in urine as BPA-glucuronide **(Rahimi *et al*., 2015)**. The red blood cell has a variety of mechanisms to protect hemoglobin and membrane function from free radicals. While superoxide dismutase (SOD) and catalase enzymatically remove superoxide and H_2O_2, respectively, red cell reducing equivalents, mainly glutathione, NADH and NADPH may react directly with superoxide and H_2O_2. The activity of the hexose monophosphate shunt is important since it produces NADPH which is necessary for glutathione regeneration by glutathione reductase **(DiGuiseppi and Fridovich, 1984).**

Lipid peroxidation (LPO) of the erythrocytes causes membrane injury, osmotic fragility and destruction of the cell **(Harvey, 1997).** Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce MDA, LPO product **(Saxena *et al*., 2011)**. Increased LPO has been reported to cause an increase in osmotic fragility and a decrease in cell fluidity of erythrocytes **(Jain *et al*., 1989).** Pesticides toxicity directly or indirectly increase ROS

steady-state level **(Atamaniuk *et al*., 2013)**. Also, xenobiotics can generate reactive oxygen species (ROS) such as superoxide anion H_2O_2, hydroxyl radical (OH), and singlet oxygen, which can induce cell and tissue damage associated with different pathological processes **(Wilhelm-Filho *et al*., 2001)**. **Mathur and Cruz (2011)** have showed that BPA causes an imbalance in the prooxidant and antioxidant status of the testis and increased amount of ROS. Moreover, BPA induced hepatic oxidative stress via depletion of antioxidant enzymes concomitant with augmentation of LPO **(Abdel-Rahman *et al*., 2018)**. BPA (25-150 µg mL^{-1}) was found to cause an increase in the LPO in human RBC **(Sangai *et al*., 2018)**. The administration of BPA significantly decreased glutathione levels and increased malondialdehyde levels in rat erythrocytes and tissues **(Acaroz *et al*., 2019)**. Furthermore, **the authors** added that when erythrocyte suspensions were treated with different concentrations of BPA/H_2O_2, a dose-dependent increase in hemolysis occurred.

Effects of BPA on oxidative stress: (carbonyl protein: CrPr) in RBCs: CrPr are formed either by oxidation of certain amino acid residues or by reaction with LPO products **(Mateen *et al*., 2016)**. It has been found that bisphenols enhanced ROS (including $^{\bullet}OH$) formation **(Maćczak *et al*., 2015)**. **Almroth *et al*. (2005)** found that the formation of carbonyl derivatives is non-reversible, causing conformational changes, decreased catalytic activity in enzymes and ultimately resulting in breakdown of proteins by proteases due to increased susceptibility. Oxidation of proteins can introduce carbonyl groups at some amino acids residues such as lysine, arginine, proline, and threonine in the polypeptide chains **(Amici, 1989)**. The highly reactive hydroxyl radical (OH•), which is one of the ROS generated

in the process leading to oxidative stress, is considered to be responsible for the formation of carbonyl groups in proteins **(Oliver, 1987)**. All bisphenols examined caused methemoglobin formation with BPA inducing the strongest oxidative potential **(Maćczak *et al*., 2015)**. The author added that flow cytometry analysis showed that all bisphenols (excluding BPS) induced significant changes in erythrocytes size.

Moon *et al*. (2012) showed that BPA increased the hepatic levels of malondialdehyde (MDA), a naturally occurring product of LPO, while decreased expression of glutathione peroxidase (GPx). BPA can increase mitochondrial ROS, particularly superoxide, in some body tissues **(Leem *et al*., 2017)**. Also, in human RBCs, BPA (25-150 µg mL^{-1}) induced a decrease in the activities of CAT and SOD, and GPx **(Sangai *et al*., 2018).** BPA treatment reduced the CAT and superoxide SOD in tissues and erythrocytes **(Acaroz *et al*., 2019)**. It was shown that BPA and its metabolite hydroquinone (HQ) to varying extents caused oxidative damage in human erythrocytes: hemolysis, decreased GSH level, and methemoglobin formation **(Olcowik-Grabarek *et al*., 2018)**. Furthermore, It has been found that bisphenols depleted GSH level, increased LPO and changed the activities of SOD, CAT and GSH -Px in human erythrocytes **(Maćczak *et al*., 2015)**. It has been found that decreased NADPH levels were correlated with a loss of CAT activity in human erythrocytes **(Gaetani *et al*., 1989)** and that enhanced oxidant sensitivity of G6PDH-deficient cells is most likely due to the absence of NADPH rather than to a reduced GSH level **(Scott *et al*., 1991)**.

The concurrent addition of BPA (150 µg mL^{-1}) and quercetin (10-50 µg mL^{-1}) lead to significant amelioration in oxidative status in human erythrocytes **(Sangai *et al.*, 2018)**. Boron exhibited antioxidant and anti-inflammatory effects and regulated metabolic and histopathological alterations in male Wistar albino rats exposed to BPA **(Acaroz *et al.*, 2019).** It is found oxidative stress caused antineoplastic agent methotrexate -induced nephrotoxicity and gallic acid (GA) prevent the kidney from the nephrotoxicity due to its antioxidant and anti-inflammatory activities **(Ozmen *et al.*, 2017)**. Furthermore, Pre-treatment with GA suppressed LPO in erythrocytes in a dose-dependent manner against sodium fluoride-induced oxidative stress in rat erythrocytes **(Nabavi *et al.*, 2013)**. Moreover, In GA-treated diabetic rats, LPO in RBCs incubated with and without H_2O_2 was significantly lower compared with control **(Ramkumar *et al.*, 2014)**. Besides, GA decreased lead (Pb) induced oxidative damages by improving antioxidant defenses, thus GA may be promising in the treatment of Pb intoxications **(Reckziegel *et al.*, 2016).**

This study was an attempt to investigate the protective effects of oral supplementation of antioxidant GA against erythrocytic oxidative stress damage induced by BPA in male rats.

MATERIALS AND METHODS

2.1. Chemicals:

BPA were purchased from Alpha company, GA were purchased from Oxfored company, sodium dodecyl sulfate, epinephrine, potassium dibasic, potassium monobasic, trichloroacetic acid, EDTA, DTNB (5,5-dithiobis-2-mtrobenzoic acid), hydrogen

peroxide, salfosalsylic acid Fluka company. All other chemicals were of highest quality available

2.2. Experimental Animals

Forty male Wister albino rats weighting 180±30g were used. They were purchased from Assiut University joint Animal Breeding Unit and were kept there for complete care during the period of the experiment. The animals were kept in a controlled light room with normal photoperiod (dark light cycle 12:12) at a temperature of 23±2 ºC. Animals were allowed to food and water *ad libitum*.

2.3. Experimental design

Rats were randomly divided into four groups, 10 each. The first group was negative control with normal diet and tape water. The second group positive control, drinking water (DW) *ad libitum* containing BPA which (40mg/100ml D.W) which nearly equivalent (40mg/kg.b.w) according to previous finding **(Wahbby *et al*., 2017)**. The third and fourth groups was given BPA by the same of both dose and route in addition to receive GA in the diet at doses of 50 and 200 mg/kg.b.w, respectively.The GA dosage based on aprevious study of **Mansouri *et al*. (2012) and Karimi-Khouzani *et al*. (2018).** The period of the experiment extended for 30 days. All experimental protocols held on animals were done according to regulations set by the Institutional Animal Care and approved by Assiut University.

2.4. BPA grains preparation

BPA (40mg /100ml drinking water), 40 mg BPA grains was firstly dissolved in 1ml absolute ethanol to prepare 100ml drinking water contain 40mg BPA (1% ethanol). For one liter drinking water containing BPA was used 10 ml absolute ethanol alcohol to dissolve 400 mg BPA in 1 liter drinking water daily. **(Mahmoudi *et al*., 2015).**

2.5. GA preparation

GA 50.200 mg/60 gram food, to avoid the losing of GA doses in normal diet, GA powder firstly dissolve in so little drinking water and mix it with the food diet of rats daily.

2.6. Blood collection

At the end of experiment 30 days, the animals were deprived of food and the drinking water become devoid of BPA and GA for one day. Animals were anesthetized with ether, then killed for blood collection.

2.7. Erythrocytes lysate preparation

Immediately after collection, blood containing EDTA samples were centrifuged at 3000 rpm for 15 min at 4-8C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by resuspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate.

2.8. Assessment biochemical determinations

CAT and SOD activities were measured in the erythrocytes lysate by the methods of **Beers and Sizer. (1952) and Misra and Fridovich (1972),** respectively. The GSH level was determined in the erythrocytes lysate based on the method of **Ellman (1959).** The concentration of LPO was estimated in the erythrocytes lysate, malondialdehyde which can be measured by thiobarbituric acid reaction **(Ohkawa *et al.*, 1979).** The concentration of CrPr was estimated in the erythrocytes lysate by reaction with 2,4dinitrophehyl hydrazine (DNPH) according to **Stadtman and Levine (2000).**

The hemoglobin content (Hb) of the erythrocytes was determined in hemolysate by colorimetric method using commercial kits (Spectrum Diagnostics Company, Egypt) according to **Tietz (1990).**

Statistical analysis:

The data were expressed as mean ± SEM. The results were analyzed statistically using column statistics and one t-tests. These analyses were carried out using computer statistics Prism5.0 Package (Graph and Software, Inc, San Diego, USA). The minimum level of statistical significance was set at $P< 0.05$, 0.01 or 0.001.

RESULTS

3.1. Antioxidant enzymes

3.1.1. Catalase (CAT)

It was observed that the administration of BPA in rats led to significant inhibition ($P < 0.001$) in CAT activity by **-56.4%** in erythrocytes compared with control. This inhibition of CAT activity was counteracted and increased significantly ($P < 0.001$) after supplementation with GA (50 mg/kg.bwt) compared with BPA group, and become less than the normal value by only **-11.1%** compared with control. Followed by GA (200mg/kg.bwt) group, ($P < 0.001$) compared with BPA group, and also increased by **7%** compared with control, respectively in BPA-treated rats. **Table (1) and Fig (1:a).**

3.1.2. Superoxide dismutase (SOD)

Administration of BPA in rats led to significant inhibition ($P < 0.01$) in SOD activity by **-68.4%** in erythrocytes compared with control. This diminishment of SOD was improved and increased significantly ($P < 0.05$) after supplementation with GA (50mg/kg.bwt) compared with BPA group, and become less than the normal value by only **-21.7%** compared with control. Followed by GA (200mg/kg.bwt) group, ($P < 0.05$) compared with BPA group, by **-7.8**% compared with control, respectively in BPA-treated rats. **Table (1) and Fig (1:b).**

3.1.3. Glutathione (GSH)

In addition to these present results, the administration of BPA in rats led to significantly diminishment ($P < 0.001$) in GSH level by **-83.2%** compared with control. This diminishment in GSH level was counteracted and increased significantly ($P < 0.001$) after supplementation with GA (50mg/kg.bwt) compared with BPA group, and also GSH level increased significantly ($P < 0.001$) by **74.1%**

compared with control. Followed by GA (200mg/kg.bwt) group, ($P < 0.001$) compared with BPA group, by **104.0**% ($P < 0.001$) compared with control group, respectively in BPA-treated rats. **Table (1) and Fig (1:c).**

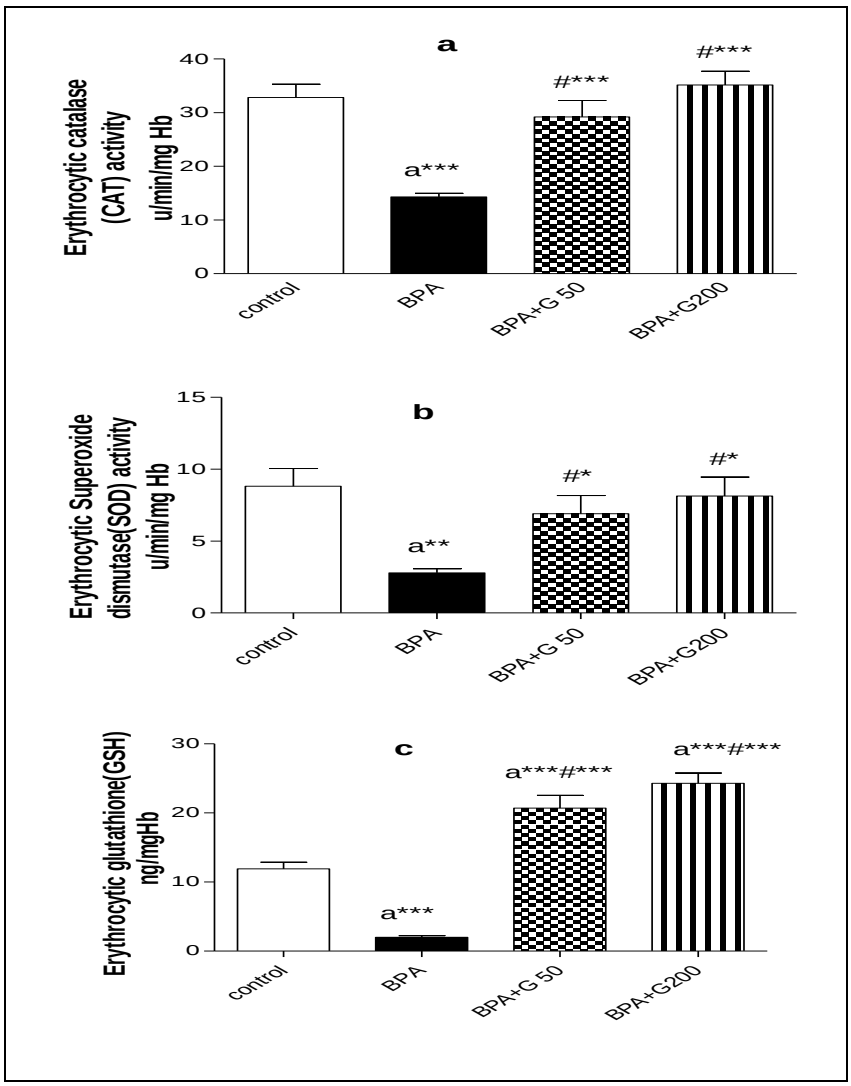

Fig.1: Ameliorative effects of GA (50mg and 200mg/kg.bwt) on some erythrocytic antioxidant enzymes activities, catalase (CAT) (a) and superoxide dismutase (SOD) (b) , in addition of glutathione level (GSH) (c) in rats-treated with BPA. Columns with different superscript are significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Table (1): Ameliorative effects of GA of two doses (50 and 200 mg/kg b.wt) on some antioxidant parameters in erythrocytic hemolysate of rats-treated with BPA:

Parameter	Control	BPA	% of change of control	G.A (50mg/kg.b.wt)+BPA	% of change of control	G.A (200mg/kg. b.wt)+BPA	% of change of control
CAT activity U/min/mgHb	32.84± 2.46	14.32± 0.65 ^{a***}	-56.4	29.21± 3.08 $^{\#***}$	-11.1	35.15 ± 2.57 $^{\#***}$	7.0
SOD activity U/min/mgHb	8.82± 1.22	2.79± 0.29 ^{a**}	-68.4	6.91± 1.26 $^{\#*}$	-21.7	8.13± 1.33 $^{\#*}$	-7.8
GSH level ng/mgHb	11.91± 0.98	2.00± 0.24 ^{a***}	-83.2	20.72± 1.85 $^{a***\#***}$	74.1	24.29± 1.50 $^{a***\#***}$	104.0

Data are present as means ± SE. Number of rats (n) = 10, (BPA) is bisphenol A, (GA) is gallic acid, catalase (CAT), super oxide dismutase (SOD) and glutathione (GSH), ^{a**}= Highly significant difference compared with negative control at $P < 0.01$, ^{a***}= Very highly significant difference compared with negative control at $P < 0.001$, $^{\#*}$ = Significant difference compared with positive control (BPA) at $P < 0.05$, $^{\#***}$ = Very highly significant difference compared with positive control (BPA) at $P < 0.001$.

2. Pro-oxidants parameters

2.1. LPO: as malondialdehide (MDA)

In addition to this inhibition of antioxidant enzymes in the present results, there was also observed significant rise in some pro-oxidant parameters. It was observed significant rise ($P < 0.001$) in LPO by **151.4**% compared with control, in response to BPA intoxication. This increase was improved and decreased significantly ($P < 0.01$) after treatment with GA (50mg/kg.bwt) compared with BPA group, and there were improvement in percentage of increase in LPO during treatment with GA (50mg/kg.bwt) to **78.9%** ($P < 0.05$) compared with control. Followed by GA (200mg/kg.bwt) group, ($P < 0.001$) compared with BPA group by **48.1%** compared with control, respectively in BPA-treated rats. **Table (2) and Fig (2:a)**.

2.1. CrPr

Moreover, it was observed significant elevation ($P < 0.001$) in CrPr by **189.1**% compared with control, in response to BPA intoxication. This increase was improved and decreased significantly ($P < 0.001$) after treatment with GA (50mg/kg.bwt) compared with BPA group, but still CrPr increasing significantly ($P < 0.001$) by **79.9**% compared with control. Followed by GA(200mg/kg.bwt) group which counteracted this increasing in CrPr significantly ($P < 0.001$) compared with BPA and GA (50mg/kg.bwt) group, also by **-35.8**% compared with control, respectively in BPA-treated rats. **Table (2) and Fig (1:b)**.

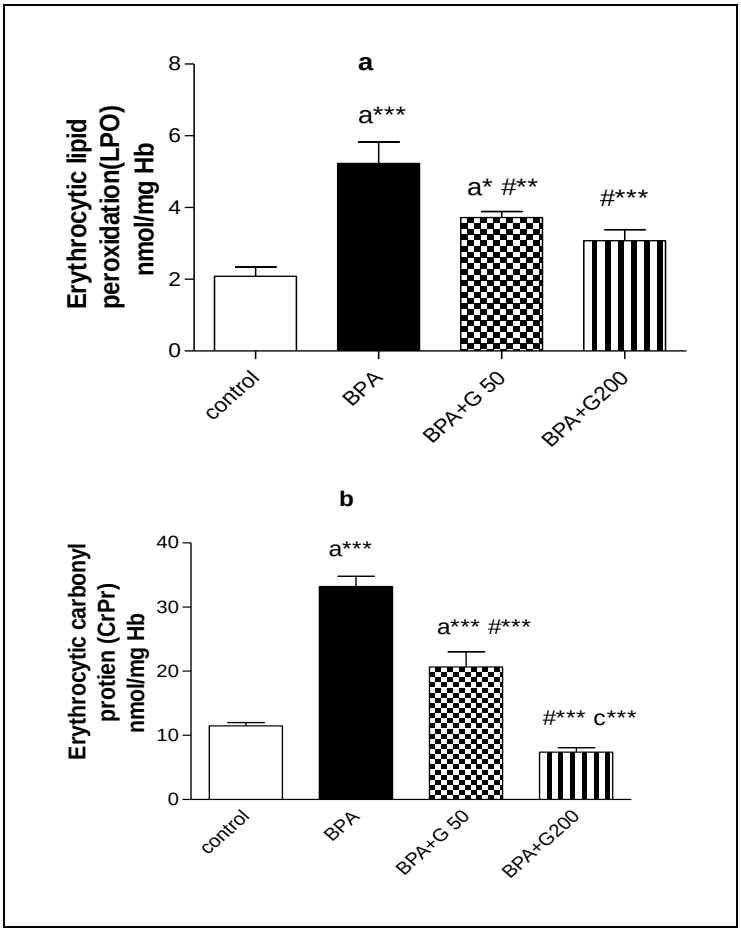

Fig.2: Ameliorative effects of GA (50mg and 200mg/kg.bwt) on some pro-oxidants parameters in erythrocytes, lipid peroxidation (LPO) level (a) and carbonyl protein (CrPr) level (b) in rats-treated with BPA. Columns with different superscript are significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Table (2): Ameliorative effects of GA of two doses (50 and 200) mg/kgb.wt on some pro-oxidants in erythrocytic hemolysate of rats-treated with BPA:

Parameter	Control	BPA	% of change of control	G.A (50mg/kg.b. wt)+BPA	% of change of control	G.A (200mg/kg.b. wt)+BPA	% of change of control
LPO nmol / mgHb	2.08± 0.26	5.23± 0.59 ^{a***}	151.4	3.72± 0.17^{a*} $^{\#**}$	78.9	3.08± 0.31 $^{\#***}$	48.1
CrPr nmol / mgHb	11.49± 0.50	33.22± 1.57 ^{a***}	189.1	20.67± 2.38 ^{a***} $^{\#***}$	79.9	7.38± 0.69 $^{\#***}$ ^{c***}	-35.8

Data are present as means ± SE. Number of rats (n) = 10, (BPA) is bisphenol A, (GA) is gallic acid, lipid peroxidation (LPO), carbonyl protein (CrPr), ^{a*} = significant difference compared with negative control at $P < 0.05$, ^{a***} = Very highly significant difference compared with negative control at $P < 0.001$, $^{\#**}$ = Highly significant difference compared with positive control BPA at $P < 0.01$, $^{\#***}$ = Very highly significant difference compared with positive control BPA at $P < 0.001$ and ^{c***} = Very highly significant difference compared with GA (50mg/kg b.wt+BPA) at $P < 0.001$.

DISCUSSION

CAT and SOD activites were significantly elevated, while GSH levels, glutathione-S-transferase and GPx activities were markedly reduced in the erythrocytes of CCl(4)-treated rats **(Makni *et al.*, 2012)**. Moreover, BPA induced hepatic oxidative stress via depletion of antioxidant enzymes concomitant with augmentation of LPO **(Abdel-Rahman *et al.*, 2018).** Furthermore, BPA treatment

reduced the CAT and SOD activities in tissues and erythrocytes **(Acaroz *et al*., 2019).** The present study showed that BPA led to pronounced decrease in CAT activity by -56.4%.BPA (25-150 µg mL^{-1}) induced a decrease in the activities of CAT and SOD in human RBC **(Sangai *et al*., 2018)**. BPA significantly decreased CAT in rat tissues and erythrocytes **(Acaroz *et al*., 2019).**This finding may be associated with little production of NADPH required for CAT activation. Supporting this finding, it has been demonstrated that NADPH protects CAT from inactivation **(Gaetani *et al*., 1989)**.The authors added that each of the four monomers of CAT contains an NADPH-binding site necessary for enzymatic activity. Also, it is found that decreased NADPH levels were correlated with a loss of CAT activity in human erythrocytes **(Gaetani *et al*., 1989).** The present study showed that BPA significantly decreased SOD activity by -68.4%. BPA (25-150 µg mL^{-1}) induced a decrease in the activities of CAT and SOD and GPX in human RBC **(Sangai *et al*., 2018).**

The expression of GPx decreased, after BPA treatment in mice **(Moon *et al*., 2012)**. BPA (25-150 µg mL^{-1}) induced a decrease in GPX in human RBC **(Sangai *et al*., 2018)**. BPA significantly decreased GSH levels in rat tissues and erythrocytes **(Acaroz *et al*., 2019). Rahman and MacNee (1999)**. GSH acts as a substrate in the detoxification of peroxides such as H_2O_2 and lipid peroxides in presence of GPx, this reaction generates oxidized GSH (GSSG), which is subsequently reduced by glutathione reductase. **Kiefmann *et al*.(2008**) stated that increased Hb autoxidation augments superoxide production in RBCs leading to RBCs release of H_2O_2 which diffuses into the lung, consequently it induced inflammation.

The present study showed that BPA significantly decreased GSH by -83.2%. this depletion may be due to the damaging effect of BPA on hexose monophosphate shunt to produce NADPH required for GSH regeneration. Also, the GSH depletion may led to fillness of RBCs with H_2O_2 which represent athreaten when exploded near any organ exerting probably inflammation as claimed by **Kiefmann *et al*. (2008).** The erythrocyte, due to its role in respiration and O_2 and CO_2 transporter, is under constant exposure of free radical effects **(Harvey, 1997)**. One source of oxidative stress for the RBC is plasma xanthine oxidase, an enzyme that produce superoxide anion **(Luchtemberg *et al*., 2008)**. It has been found that bisphenols enhanced reactive oxygen species(ROS) (including $^{\bullet}OH$) formation **(Maćczak*et al*., 2015)**. ROS- induced protein oxidation in tissues **(Dean *et al*., 1997).** In addition, **Khan *et al*. (2016)** showed that BPA led to a significant increase in mitochondrial superoxide generation was also observed.

Increased LPO has been reported to cause an increase in osmotic fragility and a decrease in cell fluidity of erythrocytes **(Jain*et al*., 1989)**. BPA (25-150 µg mL^{-1}) caused an increase in the LPO **(Sangai *et al*., 2018)**. In the present study, BPA significantly increased the level of LPO by 151.4%. Due to the ROS mediated oxidation of proteins, arthritic rats have also shown increased CrPr in the brain and liver **(Wendt *et al*., 2015)**. During oxidation, several amino acid residues (e.g., arginine, proline, histidine and lysine) irreversibly form carbonyl products which is protein oxidation **(Deavall *et al*. 2012).** Brodifacoum (coumarin derived rodenticide) administration, increased CrPr in cerebellar neurons **(Kalinin *et al*.,**

2017). Oxidative damage generated in erythrocytes in rat treated with CCl(4) as evidenced by increased thiobarbituric acid reactive substances, CrPr levels was accompanied with osmotic fragility **(Makni *et al*., 2012).** In the present study, BPA significantly increase the level of CrPr by 189.1%.

GA has been identified as an antioxidant component of the edible and medicinal plant Peltiphyllumpeltatum. **(Nabavi *et al*., 2013).** The authors showed that pre-treatment with GA suppressed LPO in erythrocytes in a dose-dependent manner against sodium fluoride-induced oxidative stress in rat erythrocytes. In GA-treated diabetic rats, LPO in RBCs incubated with and without H_2O_2 was significantly lower compared with control **(Ramkumar *et al*., 2014)**. Pre-treatment with GA or vitamin C significantly attenuated the deleterious effects against sodium fluoride-induced oxidative stress in rat erythrocytes **(Nabavi *et al*., 2013)**. The present study showed that in spite of BPA led to pronounced decrease in CAT and SOD activities by -56.4% and-68.4%, these percentages revealed relative improvements by either to -11.1 % and 7.0% or -21.7 % and -7.8 %, respectively after treatment with low and high GA doses in rats-treated with BPA. Also, the current work showed that in spite of BPA led to pronounced decrease in GSH by -83.2% , this percentage revealed a relative improvement to 74.1% after supplementation of GA_1 (low dose) supplement, while the percentage continuously exceeded to 104.0% after supplementation of GA_2 (high dose) supplement, respectively in rats-treated with BPA. Furthermore, this findings showed that GA may possess a power full antioxidant ability and may promote the synthesis of NADPH required for activity of both GSH and CAT as mentioned by **Gaetani *et al*. (1989).** In the

present study, in spite of BPA significantly increased the level of LPO by 151.4%, there was a relatively improvement in this percentage to 78.9% and 48.1 after treatment of low and high dose of GA, respectively in rats-treated with BPA. In the present study, in spite of BPA significantly increased the level of CrPr by 189.1%, there was a relatively improvement in this percentage to 79.9% and -35.8 % after treatment of low and high dose of GA, respectively in rats-treated with BPA. Consequently, GA could restore some protein damaged by BPA in erythrocytes. Also, the counteracting power of GA against LPO may be attributed to its free radical scavenger potency.

CONCLUSION

In conclusion, BPA significantly reduced erythrocytic antioxidants and increased LPO and CrPr in rat-treated by BPA, and this result may increase erythrocytic hemolysis as well as increased methemoglobin. In turn, supplementation of GA showed pronounced extent of improvement on the oxidative damage of erythrocytes in rat-treated by BPA.

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الملخص العربي

مع تزايد استخدام البلاستيك الضار على مستوى العالم في كل وسائل وأساليب الحياة تزداد المخاطر على صحة الانسان والإنتاج الحيواني والنباتي ولذا جاء الاهتمام الكبير عبر الأبحاث في التصدي لتلك المشكلة من خلال اظهار الاثار السلبية لاستخدام البلاستيك على وظائف أعضاء وأجهزة الجسم المختلفة وايضاً من خلال تقديم أفكار للتغذية للتقليل من هذه الاثار السلبية الناتجة عن استخدام المواد البلاستيكية.

البيسفينول (أ) يمثل المكون الأول والغالب في صناعة البلاستيك (البولي كربونات) التي يتم استخدامها في مختلف أساليب الحياة نظراً لرخص ثمنه. وبما انه يمثل خطراً كبيراً عندما يتم استخدامه خصوصاُ في تغليف الطعام

(الاكياس البلاستيكية- الاوعية البلاستيكية) وأوعية المياه المصنوعة من البلاستيك وغيره.

الجاليك هو واحد من اهم مضادات الاكسدة (البولي فينول) ولذلك تم استخدامه في هذه الدراسة كمضاد للأكسدة الناتجة عن استخدام البسفينول (أ).

وفي هذه الدراسة تم استخدام أربعين من ذكور الجرذان البيضاء متوسط اوزانها من ٣٠ ± ١٨٠ جرام، تم تصميم هذه الدراسة لتقييم فاعلية تأثير حمض الجاليك كمضاد للأكسدة الناتجة من استخدام البيسفينول (أ).تم تناول هذه المكونات عن طريق الفم اما في ماء الشرب مثل البيسفينول (أ) أو عن طريق الطعام مثل حمض الجاليك. تم تقسيم الجرذان إلى أربع مجموعات كل مجموعة عشرة جرذان.المجموعة الأولى ضابطة والمجموعة الثانية تناولت البيسفينول (٤٠ مللي جرام / ١٠٠ مللي ماء شرب)، المجموعة الثالثة استقبلت حمض الجاليك (٥٠ مللي جرام / ٦٠ جرام غذاء) بالإضافة الى البيسفينول (أ) والمجموعة الرابعة استقبلت حمض الجاليك (٢٠٠ مللي جرام /٦٠ جرام غذاء) بالإضافة الى البيسفينول (أ) .امتدت فترة التجربة ٣٠ يوماً وفي نهاية التجربة تم حرمان الحيوانات من الغذاء في اليوم الأخير من التجربة مع عدم أضافة البيسفينول الى مياه الشرب. تم تشريح الحيوانات في اليوم التالي وجمع الدم الذي تم تقسيمه الى جزئين ، الأول به مضاد للتجلد للدراسات الهيماتولوجية والباقي كان خالياً من مضاد التجلط لتحضير السيرم و الهيموليزات.

تم عمل القياسات التالية في الهيموليزات:

تم قياس المؤشرات الحيوية للإجهاد التأكسدي مثل :

الكاربونيل بروتين والاكسدة الفوقية للدهون كمؤشر للأضرار التأكسدية لخلايا الدم الحمراء.

ومستوى الجلوتاثيون ونشاط كلاً من الكاتالاز والسوبر أوكسيد ديسميوتيز كمؤشر لمضادات الاكسدة.

<u>وكانت نتائج هذه الدراسة كما يلي:</u>

١) الاثار السامة للبيسفينول (أ) على كريات الدم الحمراء.

(أ) أدت المعاملة بالبيسفينول الى انخفاض معنوي في مضادات الاكسدة ونشاط انزيمات الكاتالاز بنسبة **56.4%-** والسوبراوكسيد ديسميوتيز بنسبة - **68.4%**ومستوى الجلوتاثيون بنسبة **83.2%-** مقارنة بالكنترول.

(ب) أدت المعاملة بالبيسفينول (أ) الى زيادة معنوية في الاكسدة الفوقية للدهون بنسبة **151.4%** والكاربونيل بروتين بنسبة **189.1%**مقارنة بالكنترول.

٢)تأثير حمض الجاليك كمضاد للأكسدة على كربات الدم الحمراء

(أ) حدث تأثير محسن معنوي ضد سمية البيبسفينول (أ)على مضادات الاكسدة في المجموعتين التي تناولا حمض الجاليك كمادة معالجة. وكان افضل تحسن ظهر في المجموعة التي تتناول حمض الجاليك بجرعة عالية (المجموعة الرابعة) حيث تم زيادة نشاط كلاً من الكاتالاز والسوبر اوكسيد ديسميوتيز ومستوى الجلوتاثيون وكان مستوى نسب التغير **7%**، **7.8%-** ، **104%**على التوالي مقارنة بقيم الكنترول.

(ب) حدث تأثير محسن معنوي ضد سمية البيسفينول (أ) على دلالات الاكسدة في المجموعتين التي تتناول حمض الجاليك كمادة معالجة وكان افضل تحسن ظهر في المجموعة التي تتناول حمض الجاليك بجرعة اكبر (المجموعة الرابعة).حيث تم انخفاض مستوى الاكسدة الفوقية للدهون والكاربونيل بروتين بنسبة **48.1%**، **35.8%-** على التوالي مقارنة بقيم الكنترول.

وبناء عليه يمكن الاستنتاج من هذه الدراسة ان حمض الجاليك يمكن ان يستخدم كمكمل غذائي جيد ضد الآثار الضارة عن استخدام البيسفينول وتخفيف التلف التأكسدي في كرات الدم الحمراء.