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RESEARCH ARTICLE

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Oxidative stress in liver and brain of male growing rats supplemented benzene sulfonic acid

ABSTRACT:

The synthetic organic azo dye sunset yellow is a widely used in food products, such as drinks, yoghurts, ice cream, and sweets. The solutions of this coloured dye are not stable in sunlight or in dark, particularly when common food acids (e.g. citric acid and ascorbic acid) are present. The degraded products from sunset yellow are sulphanilic acid and the sodium salt of 2-naphthalene-sulphonic acid-5-amino-6- hydroxyl. These products further undergo deamination upon prolonged sunlight irradiation to produce benzene sulfonic acid sodium salt (BSA-Na) and ammonia as final products. The purpose of this study was to assess the toxicity BSA-Na on the liver and brain of male growing Sprague-Dawley rats. Oxidative stress and related gene transcription of the antioxidant enzymes have been assessed in male growing rats supplemented the different concentration of BSA-Na (34.8, 69.4, and 153.6 µg/kg. B.W.) through the experimental period. The results showed that these concentrations of BSA-Na caused significant increase in the activity of the antioxidant enzymes, total superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), xanthine oxidase (XO) and lipid peroxidation level in liver and brain of growing male rats as compared with their control. A significant retardation in growth rate was observed in rat supplemented BSA-Na. In addition, these concentrations of BSA-Na significantly caused increase in Cu/Zn super oxide dismutase (Cu/Zn SOD) gene transcription. The data clearly demonstrate that BSA- Na can result in oxidative stress in both liver and brain of the growing rat.

KEY WORDS:

Oxidative stress, sunset yellow, benzene sulfonic acid sodium salt, liver, brain, Cu/Zn SOD.

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INTRODUCTION:

The use of synthetic food colours as an individual dye or in the form of mixtures has increased extremely as they enhance the attraction of foodstuff during the earlier several years. The artificial food additives include many food colorants and the most widespread one is sunset yellow FCF. The sunset yellow FCF (SY) present in marketable beverages are degraded by the action of sunlight, even when contained in their special sealed bottles, and the product is sulphanilic acid and the sodium salt of 2-naphthalene-sulhonic acid-5-amino-6-hydroxyl. These further undergo deamination upon prolonged sunlight irradiation to produce benzene sulfonic acid sodium salts and ammonia as final products (Osman *et al*., 2004). The bioassay of SY results with mammals was shown to be mutagenic and even carcinogenic. The side effects of sunset yellow have been investigated in many studies and it has been found that their toxic effect varies widely, depending on the consumed dose. The toxicity, carcinogenicity and oxidative stress of sunset yellow in the mammalian system were intensively studied (Sies, 1991; Seesuriyachan *et al*., 2007; Feng *et al*., 2012; Sayed *et al*., 2012; Farghali *et al.*, 2014; Sirmali *et al*., 2015). Such knowledge about the effect of SY is not available about its derivative, benzene

sulfonic acid sodium salts (BSA-Na), except a few (Attia *et al*., 2005; AbdElhalem and Hegazi, 2016).

Oxidative stress, and related gene transcription of the antioxidant enzymes have been assessed in male growing rats supplemented the different concentration of BSA-Na. These reactive oxygen species (ROS) radicals are generally eliminated by antioxidant enzymes. Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidant mechanisms to minimize the cellular damage that results from interactions between cellular constituents and ROS (Rahal *et al*., 2014).The enzymatic antioxidant mechanism involves many of enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx), as well as enzymes involved in recycling oxidized glutathione, such as glutathione reductase (GR) (Fouad *et al*., 2013). Superoxide dismutases (SODs) constitute the first line of defence against superoxide radicals (Xing *et al*., 2013). SODs are a group of metal-enzymes that catalyse the conversion of reactive superoxide anions into H_2O_2, which is an essential ROS (Kim *et al*., 2015). SODs are classified into three groups, based on the metal co-factor used by the enzyme, Fe SOD, Mn SOD, and Cu/Zn SOD (Kayihan *et al*., 2012). The three groups of super oxide dismutase are represented by different genes. The gene Sod1 encodes the cytoplasmic Cu/Zn SOD enzyme (Vaiserman *et al*., 2015). Cu/Zn SOD functions as a homodimer and is found both in the cytoplasm and the outer mitochondria space (Sturtz *et al*., 2001). The gene Sod2 encodes the Mn SOD enzyme.

Manganese SOD functions as a tetramer and is localized to the inner mitochondrial space (Vaiserman *et al*., 2015). The gene Sod3 encodes the extracellular form of Cu/Zn SOD, sometimes called ecSOD (Oberley *et al*., 2009). The objectives of this study were to test the effects of benzene sulphonic acid sodium salt BSA-Na on oxidative stress, body weight and antioxidant enzyme related gene of male growing Sprague-Dawley rats.

MATERIAL AND METHODS:

Animals and experimental design:

The experiment was performed on sixty young male Sprague-Dawley albino rats weighting 40 ± 10 g of one-week age. The rats were obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Helwan, Egypt.

The rats were housed in plastic mesh cages for one week before the experimental work that stayed for 8weeks. The institutional animal care and use facility of the Zoology Department, Faculty of science, Tanta University-Egypt, approved the experimental design. Animals were fed on standard protein diet and were carefully observed every day. Their body weights, food consumption, and water intakes were registered precisely every week to follow up any signs of toxicity or abnormality during the experiment.

Experimental groups:

The commercial beverages Sunset Yellow is not stable neither upon exposure to natural conditions of summer temperature nor to sunlight (Ibrahim *et al.,* 2008; Mahfouz and Moussa, 2015). It's known that the beverage contains sunset yellow as the colouring dye in concentration 1.9×10^{-4} M. There are many degradation products. including benzene sulfonic acid sodium salt (BSA-Na). The concentrations of BSA- Na in one beverage bottle were about 3.0525×10^{-5} M, 5.515×10^{-5} M and 15.87×10^{-5} M after exposure to sunlight for 5, 9 and 31 days, respectively (Attia *et al*., 2005; AbdElhalem and Hegazi, 2016). Even though the toxicity of these products is not explicitly given in the up-to-date literature, yet it is not licensed as food additives and some aromatic amines are reported in the literature to possess health hazards as being cancer suspect materials. In this study, some trials were adopted to throw a light on to oxidative state and molecular aspects on rats treated with BSA-Na (a degraded product of sunset yellow).

The dose of supplemented benzene sulphonic acid sodium salts **(**BSA-Na) administered to growing rats was calculated to study the effect of ingestion one or two bottles/day from these irradiated bottles (333 ml). The amount administered daily to the growing male Sprague-Dawley rats dissolved in 1 mL distilled water by intra gastrointestinal gavage (i.g.). The experiment work stayed 8 weeks and divided into 4 groups as follow:

Group 1 control: rats of this group are normal healthy.

Group 2: rats of this group orally received BSA-Na (34.8 µg/kg B.W.)

Group 3: rats of this group orally received BSA-Na (69.4 µg/kg B.W.).

Group 4: rats of this group orally received BSA-Na (153.6 µg/kg B.W.).

Tissue sampling:

At the termination of the experiment, the liver and brain were quickly excised, washed by ice-cold isotonic NaCl saline and processed for investigations.

Right lobe of liver and right half of brain were weighted and stored at - 80°C

and were processed for molecular analysis by quantitative real-time polymerase chain reaction (qRT-PCR) for detection of Cu/Zn SOD genes expressions.

Left lobe of liver and left half of brain were placed in ice, avoiding squeezing the tissue, washed in ice-cold isotonic NaCl saline, blotted dry with filter paper and weighed. The tissues were homogenized in ice-cold phosphate buffer containing triton X - 100 (50 mM, pH 7.4) 10% (w/v) using Omni international homogenizer (USA) at 22.000 rpm for 20 s each at 10 s intervals. The supernatant was freeze-thawed thrice to completely disrupt mitochondria. Then, the supernatant was centrifuged at 6000 xg in cooling centrifuged at 4°C for 15 min and the yielded supernatant which contains the cytosolic and mitochondrial enzymes was used for immediate enzyme assays. The UV/VIS spectrophotometer (JENWAY 6505, UK) used for the measurements of enzyme activities and oxidative stress parameters at 25°C.

Antioxidant enzyme activities and biochemical markers assays:

The activity of total superoxide dismutase (SODs, EC 1.15.1.1) catalase (CAT, EC 1.11.1.6), glutathione S-transferase (GST, EC 2.5.1.18), glutathione reductase (GR, EC 1.6.4.2), xanthine oxidase (XO, EC 1.17.3.2), and malondialdehyde (MDA, as an important lipid peroxidation indicator) were assayed according methods modified by Hegazi *et al*. (2010).

Gene expression analysis using Quantitative Real-Time PCR (qRT-PCR):

The m-RNA expression of antioxidant enzymes related gene Cu/Zn SOD was studied using qRT-PCR technique. The housekeeping gene glyceraldehyde-3- phosphate dehydrogenase (GAPDH) was used as a reference gene, endogenous control to normalize the expression level of the different genes analysed in the liver tissue.

RNA isolation and c-DNA preparation:

RNA was extracted from liver sections treated or not with BSA-Na using a total RNA isolation kit (analytikjena-Germany) according to the manufacturer's Single-stranded complementary DNA (c-DNA) was obtained from 1 µg of purified RNA using the Sensiscript Reverse Transcriptase (QIAGEN, Germany) Synthesis Kit, according to the manufacturer's directions using random hexamers. All the reaction tubes were loaded into a thermal cycler (Applied Biosystems, USA). The thermal cycler was set to the following conditions. The Thermal Cycler Reaction Conditions:

Step	Hexamer Incubation	Reverse Transcription	Reverse Transcription Inactivation
	HOLD	HOLD	HOLD
Temperature	25°C	37°C	95°C
Time	10 min	60 min	5 min
Volume		25 µL	

After thermal cycling, all cDNA samples were stored at - 20ºC. Primers' sequences are listed in table 1. All primer pairs were synthesized by Jena Bioscience GmbH (Jena, Germany).

Table 1. Primer Sequences of Rat Liver-Related Genes and Endogenous Reference Gene Name sequence GAPDH

Gene name	Sequence
Reference gene	F: 5'-AGTTCAACGGCACAGTCAAG-3' R: 5'-TACTCAGCACCAGCATCACC-3'
CU/Zn SOD	F: 5'- GCAGAAGGCAAGCGGTGAAC -3' R: 5'- TAGCAGGACAGCAGATGAGT -3'

GAPDH and CU/Zn SOD rat primer sequences for qRT-PCR were brought from the National Centre for Biotechnology Information (NCBI) Gene bank (Pubmed) and aligned against each of gene sequences to check their potential amplification products. The CT cycle (Threshold cycle) was used to determine the expression level in control cells and cells treated with BSA-Na. The gene expression level was then calculated as described by Yuan *et al*. (2016) using Applied Biosystems Step One™ Instrument software.

Chemicals and drugs:

The sodium salt of benzene sulphonic acid powder and all the chemicals used in this study was obtained from Sigma chemical co. (St. Louis, MO, USA).

Statistical analysis:

The data were statistically analysed using one-way Analysis of Variance (ANOVA) followed by Dunnett test using a computer program (GraphPad InState3 Software, Inc.). For all comparisons, P-values less than 95% ($P < 0.05$) were statistically significant.

RESULTS:

Results Growth rate:

There were significant decreases in growth rate of all groups versus control (Fig. 1).

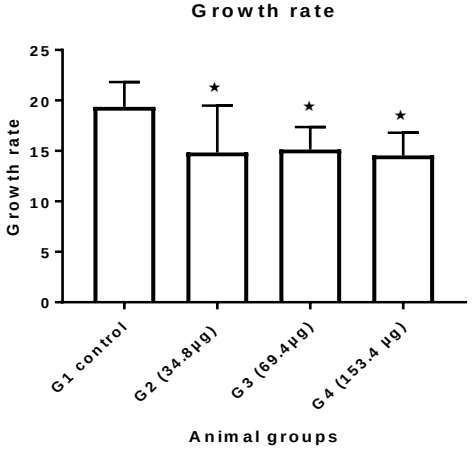


Fig. 1. Growth rate of male rats administered BSA-Na *: Significant vs. G1 (control) at $P < 0.05$.

Cu/Zn SOD gene expression enzymes activity:

The expression level of Cu/Zn SOD was up-regulated by 6.143, 13.83, and 28.59-fold in group 2, group 3, and group 4, respectively as compared with the control (Fig. 2). The mRNA expression data in group 4 was further significantly up- regulated as compared with the expression data of group 3. The mRNA expression data in group 2 was not significant.

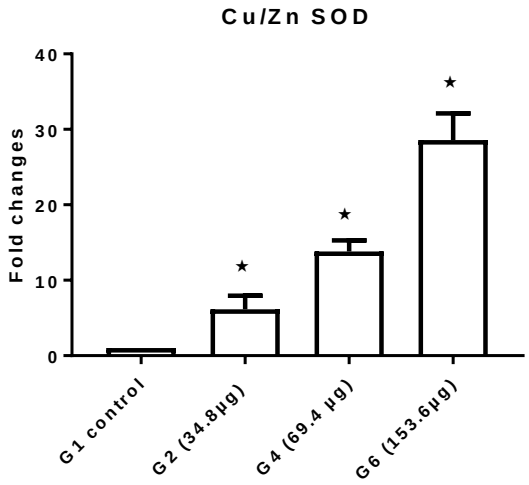


Fig. 2. qRT-PCR Data Analysis for liver of BSA-Na administered growing male rats Cu/Zn SOD Gene Relative to GAPDH Housekeeping Gene. *: Significant vs. G1 (control) at P < 0.05.

Lipid peroxidation:

MDA level in the liver and brain of growing male rats was significantly increased (P ≤ 0.05) in all rat groups except group 2 in comparison with their respective control (Fig. 3).

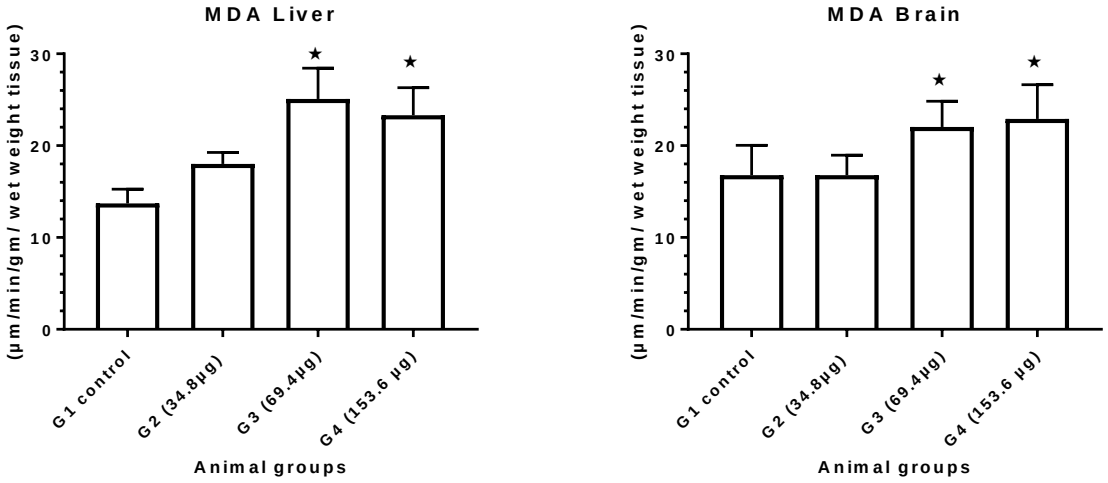


Fig. 3. MDA biomarker level in liver and brain of male rats administered BSA-Na *: Significant vs. G1 (control) at P < 0.05.

Enzymes activity:

The activity of the ROS producing enzyme XO in liver and brain of growing male rats were significantly increased (P ≤ 0.05) in all rat groups in comparison with their respective control (Fig. 4).

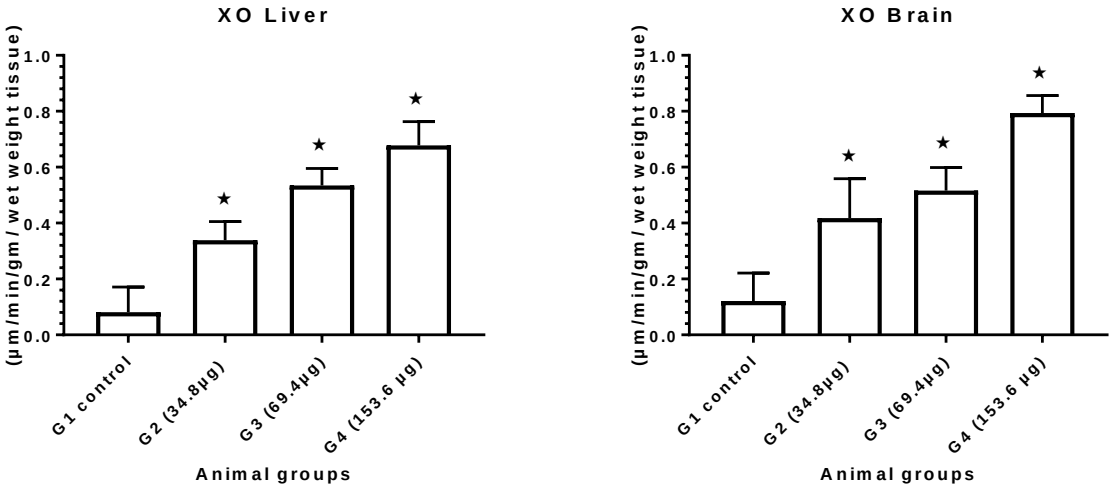
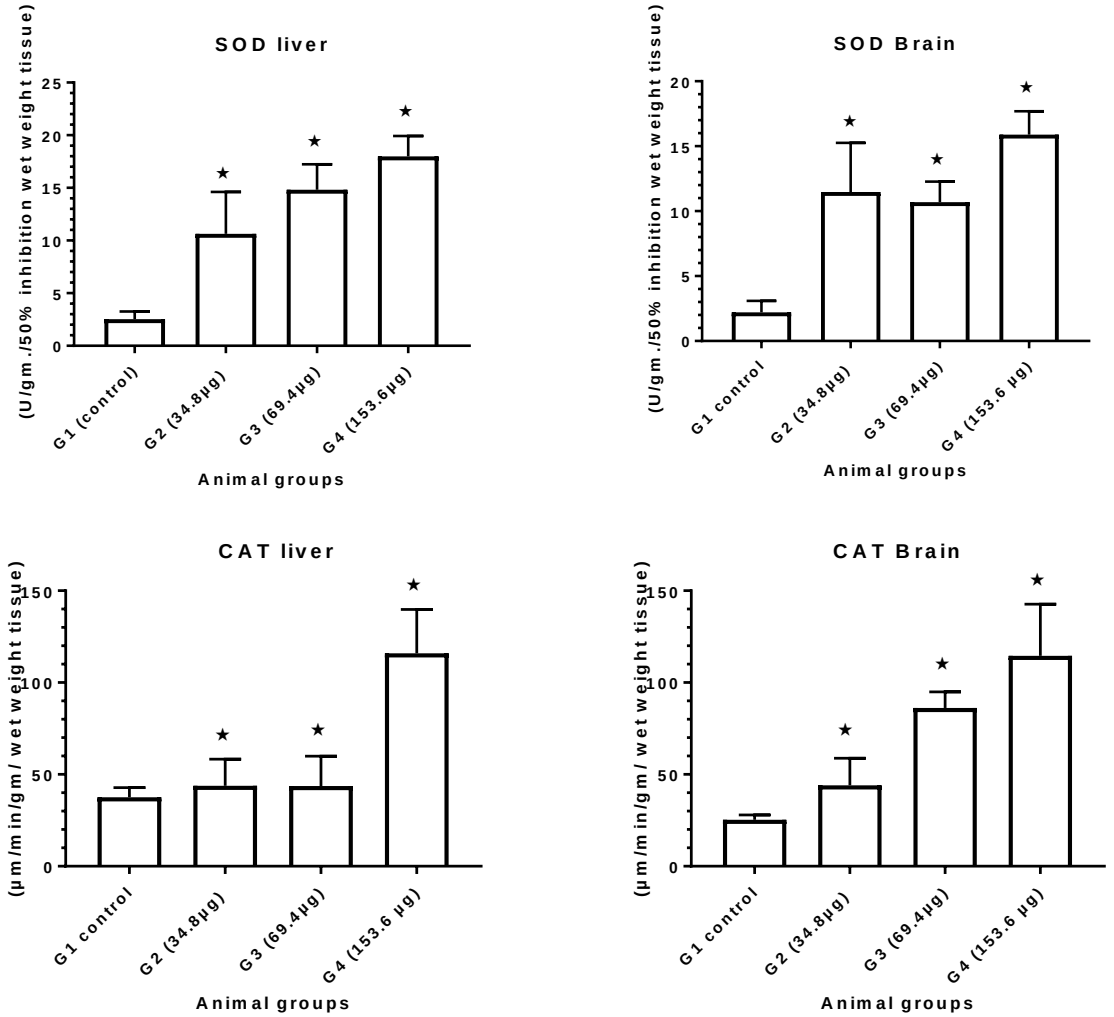


Fig. 4. XO enzyme activity in liver and brain of male rats administered BSA-Na *: Significant vs. G1 (control) at P < 0.05.

The activity of the antioxidant enzymes SOD, CAT, GST and GR in liver and brain of growing male rats were significantly increased (P ≤ 0.05) in all rat groups in comparison with their respective control (Fig. 5).



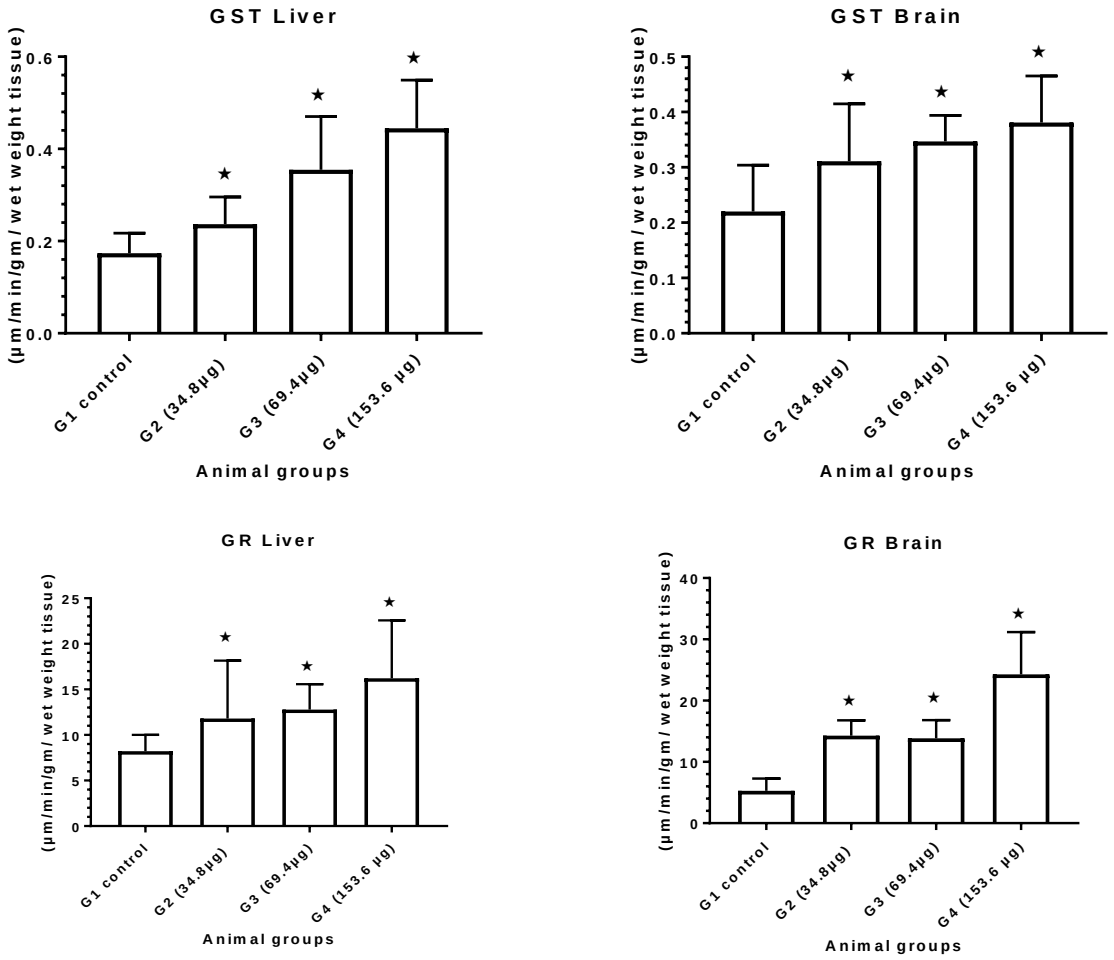


Fig. 5. SOD, CAT, GST, and GR enzymes activity in liver and brain of male rats administered BSA-Na *: Significant vs. G1 (control) at P < 0.05.

DISCUSSION:

The results revealed a significant decrease in growth rate of all rat groups. The present decrease in growth rate may be related to a reduction of food utilization and/or increased catabolic processes in the body. Unfortunately, the biological effects of BSA-Na on animal physiological indices had not been previously explored and the present investigation, to the best of our knowledge, is the first trial in this regard. However, there are many reports on weight loss due to other food additives (Helal, 2001; Attia *et al*., 2005; Amin *et al*., 2010; Fouad *et al*., 2013). Meanwhile, some studies reported a slight increase in body weight in food colour additives SY administered to experimental animals (Hasan, 2010; AbdElhalem and Hegazi, 2016).

The level of MDA in the liver and brain increased in rats treated with BSA-Na and the increase was positively parallel to the dose levels. This increase in agreement with previous studies (Amin *et al*., 2010; Fouad *et al*., 2013; AbdElhalem and Hegazi, 2016). Lipid peroxidation is the degradation of lipids that occurs because of oxidative damage and is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as MDA. MDA may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer (Colak, 2008). Also, MDA level was increased as a product of lipid peroxidation occurred by the Reactive oxygen species (ROS) action on lipids of the cellular membrane. ROS play an important role in pathological changes in the liver. Biological membranes are particularly prone to the ROS effect, the peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and disruption of membrane integrity and function, which is implicated in serious pathological changes (Amin *et al*., 2010).

The brain tissue is predominantly susceptible to membrane lipid peroxidation that interrupts essential functions of the brain. Neuronal cells are particularly sensitive to oxidative insults and therefor ROS are involved in many neurodegenerative processes (Bala *et al*., 2006). The iron from brain cells can be easily released on oxidative stress. If the

free iron content of the system were raised by cell injury, an ascorbate–iron salt mixture would be expected to promote lipid peroxidation and hydroxyl radical formation. This primary radical damage can result in cytotoxic oedema, which can have secondary effect by causing vascular compression (Carocho and Ferreira, 2013). Insistent oxidative stress may cause the permanent loss of nervous system cells of brain or spinal cord injury leading to deteriorating disorders. The damage in nerve endings may lead to disturbances in neurotransmitter transport, causing an alteration in CNS functions (Ostrowska *et al*., 2004). Thus, antioxidant defence is critically important in nervous tissue protection. The present findings may emphasize the role of BSA-Na on nervous tissues hazardous.

The activity of the enzyme XO was significantly increased in rats treated with BSA-Na and this increase in the activity was positively parallel with the increase of dose levels. The enzyme XO is related to the group of enzymes known as the molybdenum containing hydroxylase. The enzyme XO plays a crucial role in the production of uric acid, catalysing the oxidation of hypoxanthine and xanthine. Uric acid acts as a scavenger of hydroxyl radicals, singlet oxygen, hypochlorous acid, oxoheme oxidants, and hydroperoxyl radicals. During the reoxidation of XO, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. Consequently, XO considered an important biological source of superoxide radicals. When acting as a NADH oxidase, XO is a generator of superoxide, a powerful ROS. Due to their highly reactive nature, these ROS affect various molecular components of the cell, with excess amounts leading to cell degeneration and death (Harrison, 2002; Kundu *et al*., 2007).

To understand the cause of this increase in XO activity, Hegazi *et al.* (2010) elucidated the biochemical nature of this enzyme. This enzyme (xanthine oxidoreductase, XOR) exists in separate but inter-convertible forms, with dehydrogenase (XD, EC 1.17.1.4) and oxidase (XO, EC 1.1.3.22) activity. This inter conversion occurs either reversibly by the oxidation of sulfhydryl residues or irreversibly by proteolysis. The two forms act on the same substrate, and convert hypoxanthine to xanthine and xanthine to uric acid. However, the native XD form, in contrast to XO form, utilizes NAD+, producing NADH instead of superoxide anions ($O^{2\bullet-}$). The oxidase form (XO) uses molecular oxygen instead NAD+ as the electron acceptor and releases substantial amounts of superoxide anions ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2). The end product of XD, uric acid, has a potential physiologic function as a protective agent against oxidative damage. Uric acid acts as a scavenger of hydroxyl radicals, singlet oxygen, hypochlorous acid, oxoheme oxidants, and hydroperoxyl radicals. In alcohol consumption, tissue hypoxia, and ischemia-reperfusion, glutathione depletion, and oxidizing agents such as hydrogen peroxide promote the conversion of XD to XO. This mechanism was initially described in rat organs such as the liver, kidney and brain under these circumstances. Therefore, the present increase in the activity of XO may be attributed to an imbalance occurring in the physiological XD/XO ratio that augments XO formation in rats' administrated different concentrations of BSA-Na which, increase ROS production, leading to an ultimate oxidative stress.

The rats administrated different concentrations of BSA-Na after exposure to sunlight for 5, 9, and 31 days, showed a significant increase in SOD, CAT, GST, GR, and XO activities in liver and brain. The degraded product BSA-Na displayed a significant state of oxidative stress in liver and brain of male growing rats. For our knowledge, the biological effects of BSA-Na on rats' oxidative state had not been previously studied and the present investigation is the first trial in this regard. However, food colour additives, especially those containing azo dyes and aromatic amine structures, are cytotoxic compounds because they are metabolized by intestinal bacteria that produce oxygen and O^{2-} free radicals (Bansal *et al.,* 2005; Fouad *et al*., 2013). The levels of ROS are controlled by antioxidant enzymes and non-enzymatic scavengers. Amin *et al*. (2010) were suggested that various azo dye products are genotoxic, not through N-hydroxylation and esterification, which is characteristic of many aromatic amines but rather through a mechanism involving oxygen radicals and the superoxide free radical was produced by the azo dyes only after reduction by the intestinal bacteria. Moreover, Siraki *et al*. (2002) found that incubation of hepatocytes with aromatic amines caused a decrease in the mitochondrial membrane potential before cytotoxicity ensued. Aromatic amines can generate ROS as part of their metabolism by an interaction of these amino groups with nitrite or nitrate containing foods or in the stomach. The ROS such as superoxide anion, hydroxyl radical and H_2O_2 could be produced in the metabolism of nitrosamines and increase oxidative stress (Bansal *et al*., 2005).

The activity of SOD and CAT in liver and brain increased in rats treated with BSA-Na and the increase in the activity was parallel with the increase of doses. Although

liver and brain SOD activities were significantly increased, there are several reports in the literature of decreased hepatic SOD activity in rats treated with SY (Amin *et al*., 2010; Fouad *et al*., 2013; Cemek *et al*., 2014). This increase may occur when cells are exposed to oxidative stress leading to increasing the activity and expression of antioxidant enzymes including CAT and SOD as a compensatory mechanism to better protect them from the damage induced by free radicals (Rodriguez *et al*., 2004). Superoxide (O^{2-}) and hydrogen peroxide (H2O2) represent arguably the best-known and most produced ROS, scavenged by SOD and by CAT (Buettner, 2011; Kirkman and Gaetani, 2007). The SOD family represents the only enzymes able to specifically scavenge O^{2-}, catalysing it into O2 and H2O2. The elevated activity of CAT is due to the adaptive response to the generated free radicals. CAT is predominantly located in peroxisomes of all types of mammalian cells except for erythrocytes (Lei *et al*., 2016). The activities of GST and GR in liver and brain increased in rats treated with BSA-Na and the increase in the activity was positively parallel with the increase of doses. Such results agree with previous studies but on different organs (AbdElhelim and Hegazi, 2016).

Oxidative stress is responsible for an increase in the accumulation of the ROS in cells, which may subsequently lead to an increase in the expression of genes encoding antioxidant enzymes (Michiels *et al*., 1994). The antioxidant enzymes (SOD and CAT), which constitute the major defence system against oxidative stress, are expected to be effective in eliminating the oxidative stress induced by BSA-Na. The SODs are a group of metalloenzymes that catalyse the conversion of reactive superoxide anions into H_2O_2, which is an essential ROS. SODs are regarded as essential antioxidant enzymes and have been observed in all aerobic organisms examined to date (Kim *et al*., 2015). The nuclear encoded primary antioxidant enzyme Cu/Zn SOD is considered the most important isoform for anti-oxidation.

The administration of different concentrations of BSA-Na had significant up- regulation of Cu/ Zn SOD gene transcription by 6.14, 13.83, and 28.59-folds. This is because oxidative stress activated the antioxidant response element, which increased Cu/Zn SOD gene transcription, which in turn resulted in increased SOD activity. When these data are coupled with variations in SOD activity, we can speculate that the organism coped with increased ROS via an increase in SOD activity. To enable SOD enzyme to overcome extremely high ROS levels as a result of higher concentrations of BSA-Na, the organism had to activate higher SOD activity via elevation of the gene transcription of Cu/Zn SOD to counteract the excess superoxide free radicals. This result in agreement with previous studies (Pallavi *et al*., 2003; Sun *et al*., 2014; Kim *et al*., 2015).

Conclusion:

The synthetic organic azo dye SY which widely used in food products, are not stable in sunlight or in dark, particularly when common food acids (e.g. citric acid and ascorbic acid) are present. There are many products degraded products from SY including BSA-Na. The study evidenced that the degraded product of SY called BSA- Na has the ability to cause oxidative stress in two vital organs such as liver and brain and growth rate retardation of growing male rats. The increase in oxidative stress leads to increase in antioxidant-related gene Cu/Zn SOD.

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تأثير الملح الصوديومي لحمض بنزين سلفونيك على الانزيمات المضادة للأكسدة والاجهاد التأكسدي في ذكور الجرذان

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تستخدم أصفر غروب الشمس كمادة مكسبه للطعم واللون في المشروبات الغازية. هذه المادة من مركبات الأزو المصنعة من مواد بترولية. ولقد وجد ان مادة أصفر غروب الشمس تتحلل عند تعرضها للأشعة البنفسجية في اشعة الشمس وخاصة في وجود أحماض مثل حمض الستريك وحمض الأسكوربيك في المشروبات الغازية. وتحلل هذه المادة إلى مركبات الأمينات العطرية مثل: 2-نفثالين حمض السلفونيك, 5 - أمينو6-هيدروكسيل والملح الصوديومي لحمض السالفانيلك. ومع تعرض هذه المركبات الناتجة لأشعة الشمس فإنها تقوم بدروها بنزع مجموعة الأمين لتكون الأمونيا وملح الصوديوم لحمض بنزين سلفونيك كنواتج نهائية. لذلك تم تصميم هذا العمل لدراسة تأثير مادة الملح الصوديومي لحمض بنزين سلفونيك على الحالة الفسيولوجية والإجهاد التأكسدي لفئران التجارب والتي عوملت بتركيزات مختلفة من هذه المادة (34.8، 69.4، 153.6، 242.8، و 485.6 ميكروجرام/كجم من وزن الجسم). وقد أظهرت النتائج إلى حدوث زيادة لها دلاله معنويه في نشاط الانزيمات المضادة للأكسدة: سوبر اوكسيد دسميوتيز(SOD)، كاتايز(CAT)، جلوتاثيون ترانسفيز (GST)، جلوتاثيون ريدكتيز(GR)، زانثين اوكسيدز(XO) بالإضافة إلى حدوث زيادة لها دلاله معنويه في مستوي الدهون المؤكسدة (MDA) في كبد ومخ الجرذان. وقد أظهرت النتائج وجود نقص له دلاله معنويه في معدل نمو الجرذان. بالإضافة إلى ذلك وجود زيادة ذات دلاله معنويه في التعبير الجيني لجين نحاس/خارصين سوبر أوكسيد دسميوتيز (Cu/Zn SOD) في الجرذان. في النهاية ومن هذه النتائج يمكن استنتاج ان ملح الصوديوم لحمض بنزين سلفونيك من الممكن ان تؤدي الى حدوث تأثيرات سلبية على شكل اجهاد تأكسدي وزيادة نشاط الانزيمات المضادة للأكسدة. ولهذا فمن الضروري توعية المستهلكين عن مدى خطورة الاضرار الناجمة عن تناول المشروبات الصناعية المعرضة لأشعة الشمس لاحتوائها على تركيزات تماثل الجرعات المستخدمة في التجربة من مادة الملح الصوديومي لحمض بنزين سلفونيك.