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Alteration in pancreas of rats treated with individual and combined food additives

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ABSTRACT

The impact of individual and combined treatment of artificial food additives on oxidative stress, antioxidant parameters and pancreatic disruptor in male albino rats will be examined in this study. Six groups of young albino rats (80 - 100 gm) were examined Group I: (Control untreated group), Group II, III, IV and V: treated with ADI of MSG (300 mg / kg), Sodium benzoate (5 mg / kg), Carmoisine (4 m g / kg), and EDTA (2.5 mg / kg) respectively. Group VI: treated with mixture of tested materials simultaneously., The treatments were carried out orally for 28 days. The obtained results revealed production of malondialdehyde MDA and protein carbonyl PC indicators of oxidative stress in individually treated MSG and sodium benzoate and remarkable in mixture group. Significant reduction in catalase, total antioxidant capacity, and glutathione content were recorded in individually treated groups and pronounced in mixture group. Elevation in serum glucose level and alteration in pancreatic enzymes amylase and lipase, as well as insulin, were recorded.

Keywords: Food additives; Oxidative stress; antioxidants; pancreatic enzymes; Insulin.

1. INTRODUCTION

Food added substances are natural or manufactured essence that inserted to foods (Emerton and Choi, 2008). They are grouped into antimicrobial agents, antioxidants, stimulated colors, stimulated flavors and flavor stimulated, chelating agents thickening and stabilizing agents (El-Samrages, 2012) adding food additives to length shelf-life of the industrial foods by preventing growth of bacteria, fungi and other microorganisms (Renha and Dharman, 2011). The symbol added by European Union - EU is the prefix 'E,' Tartrazine (E102), Quinoline Yellow (E104), Carmosine (E122) and Amaranth (E123) (Inetianbor et al., 2015). Among the currently used preservatives, sodium chloride (known as salt) is probably the oldest preservative. Natural acids, for example, acidic corrosive (E260), benzoic corrosive (E210), and propanoic corrosive (E280) also, sorbic corrosive (E200), sodium benzoate (E211) were widely used in the production of canned soda; over 50% of benzoate goes into the soft drink industry (Al-Shammari et al., 2014). Monosodium glutamate (MSG) E621 is a flavor and flavor enhancer used in different foods and food commodities. It makes food taste better, or to give them a specific taste (Branen, 2001). Carmoisine or Azorubineis E122 is one of the most common food coloring. It could be manufactured ruddy nourishment dye from the azo color gather or ruddy to maroon powder.

Artificial food coloring added in food industries to prevent color loss of foods due to store or transformation, perform color diversity and enhancement of food form to encourage consumers demand carmoisine show in nourishment like swiss roll, Jams, yoghurts and cheesecake blend with (Amin, 2010). Common antioxidant used through food industry as Ethylenediamine Tetra Acetic Acid (EDTA E385) and its salts (Lanigan and Yamarik, 2002). It is added to oily food to prevent oxidation and increase their palatabilitya long time (Dalton, 2002). Simultaneous consuming for food additives induced harmful effects such as headaches, lopsidedness in vitality level, also impact on mental concentration and immune response in addition to allergies, these compounds induce stomach ache, vomiting, breathing asthma, hives and skin rashes. Benzoates are one of the worst additives that produce skin rashes, asthma and perhaps brain deterioration (Abdulmumeen et al., 2012). Some artificial antioxidants induced health hazard like hyperactivity in children, lungs, liver, and kidneys injury and may led to cancer (Tran, 2012). MSG induced extensive oxidative stress represented by elevation in MDA companied by reduction in GSH, catalase and SOD antioxidant enzymes (Hussein et al., 2017). Moreover, Helal et al., (2017) reported that rats administrated with mixture of food additives MSG, NaNO2 and annatto (flavoring, preservative and coloring) with different doses increase fasting blood glucose companied with decreased cellular insulin sensitivity.

This research work aimed to examine the effects of individual and combined treatment with different artificial food additives on oxidative stress and antioxidant parameters as well as pancreatic disrupter in male albino rats.

2. MATERIALS AND METHODS

Tested Materials

Monosodium glutamate (MSG), Sodium benzoate: white, Carmoisine (4 mg / kg b.wt / day), and Ethylene diamine tetra acidic acid (EDTA) (2.5 mg / kg b.wt / day) were obtained from Sigma chemical Co (USA)

Experimental Animals

Young male experimental rats from species Rattus norvigecus weighing around 80-100 g. Thirty rats were collected from the culture of the King Fahd Medical Research Center (KFMRC) Jeddah, KSA., and acclimatised to a temperature of 25 ± 1ºC and humidity of 55%. The rats were controlled with 12 h light/dark cycles at KFMRC's Animal Facility Breeding Colony and maintained with ad libitum access to a standard pellet diet and tap water. The animals were cared for according to the guidelines for animal experiments, which were approved by the Ethical Committee of KFMRC, Jeddah, KSA. Approval number (163-18).

Experimental Design

After the acclimatisation period (one week), the animals were randomly assigned divided into 6 groups five rats for each and were orally treated daily for 28 days. Group I: control untreated group each rat was given distilled water. Group II, III, IV, and V: rats were orally treated with ADI of MSG (300 mg / kg b.wt) (Geha et al., 2000), Sodium benzoate (5 mg / kg b.wt) (Shahmohammadi et al., 2016), Carmoisine (4 mg / kg b.wt) (Larsen et al., 2009) and EDTA (2.5 mg / kg b.wt) (Van et al., 2014) respectivily. Group VI: rats were orally treated with mixture of ADI of tested materials simultaneously.

Blood sample collection

Blood sample were taken from orbital plexus vein at the end of 28 days of treatment. Collected in centrifuge tubes and centrifuged at 3600 rpm for 15 min. Serum samples were preserved at -20 ºc till used for analysis of biochemical parameters.

Biochemical analyses

Malondialdehyde (MDA) marker of lipid peroxidation, protein carbonyl, catalase, total antioxidant capacity (TAC), glucose, lipase, alpha amylase, total GSH, oxidized and GSSG were measured in serum respectively were evaluated spectrophotmetrically using Biovision Kit, CA. USA .While, insulin was determined using ELISA Kits Kamiya Biomedical Co.,CA, USA).

Statistical analysis

Determination the degree of variance between trials data statistically studied with (SPSS) program (version 23) by ANOVA - one way at $p< 0.05$ significant level. The demonstrated results were showed as Mean ± S.Eof 10 rats/group.

3. RESULTS

Table (1) depicted that treatment with each of monosodium glutamate MSG and sodium benzoate induced remarkable induction in lipid peroxidation (MDA) and oxidized protein (PC) biomarkers of oxidative stress is significant compared with control. However, treatment with food coloring Carmosine and ethylenediamine tetra acetic acid salt EDTA induced significant decrease in MDA when compared with control and other groups. PC recorded non-significant change with the same treatments. On the other hand, rats treated with mixture of the previous fore mentioned food additives (MSG, sodium benzoate, Carmosine and EDTA) remarkable significant elevation in both examined parameters (MDA & PC) against control and all treatments. Reduction in the activity of catalase enzyme in serum of all treated groups pronounced reduction in catalase activity was recorded in Carmosine, EDTA and mixture groups. This reduction was significant against control and other groups. Main while, total antioxidant capacity (TAC) level in serum of MSG, Carmosine, and mixture treated groups' recorded significant decrease versus control and between groups.

Table 1 Effect of MSG, Sodium Benzoate, Carmosine, EDTA and Their Mixture on Oxidative stress and Antioxidant Markers in Serum of Male Albino Rats

Parameters groups	Lipid peroxide (nmol/ml)	PC (nmol/ml)	Catalase (U/L)	TAC (mmol/ml)	total GSH (mmol/ml)	GSSG (mmol/ml)	reduced GSH (mmol/ml)
Control	4.49 ± 0.17	4.11 ± 0.16	276.72 ± 9.07	0.20 ± 0.003	9.22 ± 0.11	0.65 ± 0.03	8.62 ± 0.12
MSG (300mg/Kg)	6.96 ± 0.15[a]	8.20 ± 0.415[a]	240.07 ± 12.06	0.02 ± 0.001a	4.51 ± 0.27a	1.31 ± 0.06a	3.43 ± 0.38a
Sodium benzoate (5mg /kg)	5.90 ± 0.17[a]	4.41 ± 0.21[ab]	232.82 ± 11.10	0.19 ± 0.002b	8.32 ± 0.50b	0.81 ± 0.04b	7.51 ± 0.48[ab]
Carmosine (4mg/kg)	2.93 ± 0.16[ab]	4.15 ± 0.23[abc]	71.911 ± 3.63abc	0.02 ± 0.001ac	7.75 ± 0.43ab	0.51 ± 0.04bc	7.16 ± 0.43ab
EDTA (2.5mg/kg)	3.99 ± 0.27[abcde]	4.49 ± 0.15[abcd]	126.90 ± 46.65abc	0.205 ± 0.003bd	7.85 ± 0.08ab	0.77 ± 0.05bd	7.04 ± 0.09ab
Mixture	5.64 ± 0.18[abcde]	7.56 ± 0.44[abcd]	215.75 ± 4.72ade	0.11 ± 0.01bcd	5.67 ± 0.34abcde	1.32 ± 0.10acde	4.31 ± 0.27acde

Values expressed as mean ± SE,

a significance variance against control at $P<0.05$.

b significance variance against MSG at $P<0.05$.

c significance variance against Sodium benzoate at $P<0.05$.

d significance variance against Carmosine at $P<0.05$.

e significance variance versus EDTA at $P < 0.0$

Furthermore, significant depletion in total glutathione content (GSH) was recorded all through the experimental groups pronounced in mixture treated group significant against control and between groups. Remarkable elevation in serum oxidized glutathione GSSG in most of the treated groups pronounced in mixture group. Significant elevation in GSSG was recorded against control and other groups. Reduction in GSH levels in serum of treated rats with each of MSG, sodium benzoate, Carmosine, EDTA and their mixture was declared, concomitant with elevation of GSSG. Difference between the ratios of GSSG / GSH level reflects the pronounced reduction in total reduced glutathione as previously demonstrated.

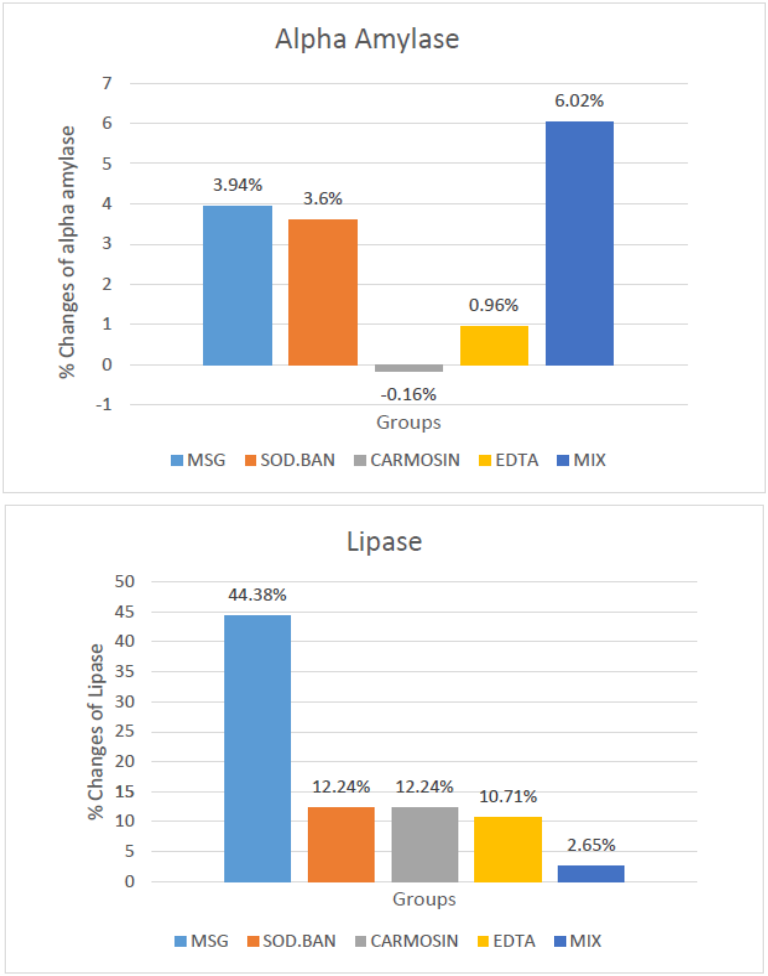
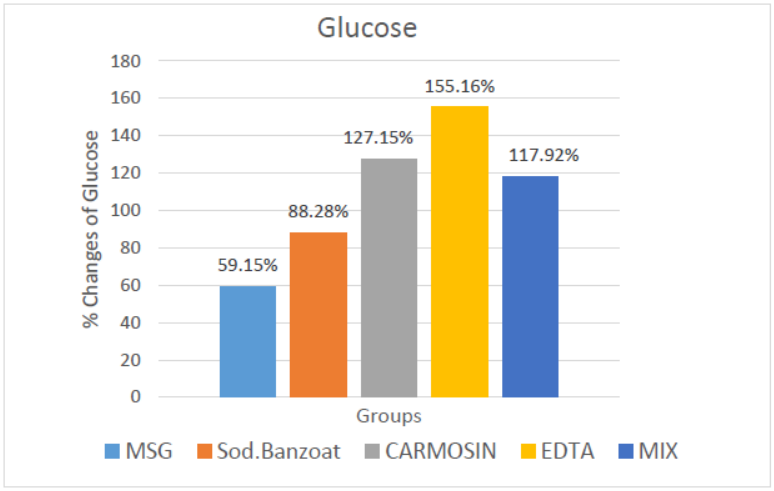


Figure 1 % changes from control of serum pancreatic enzymes levels in different groups of rats treated with individual and combined food additives.



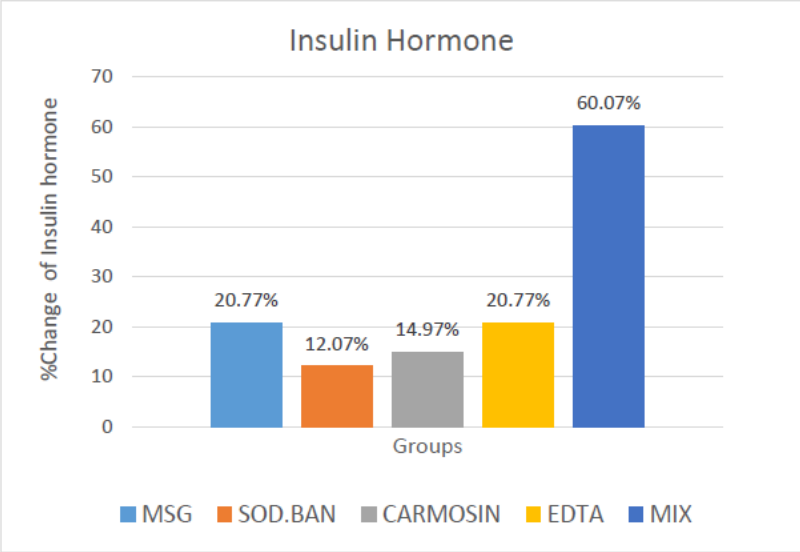
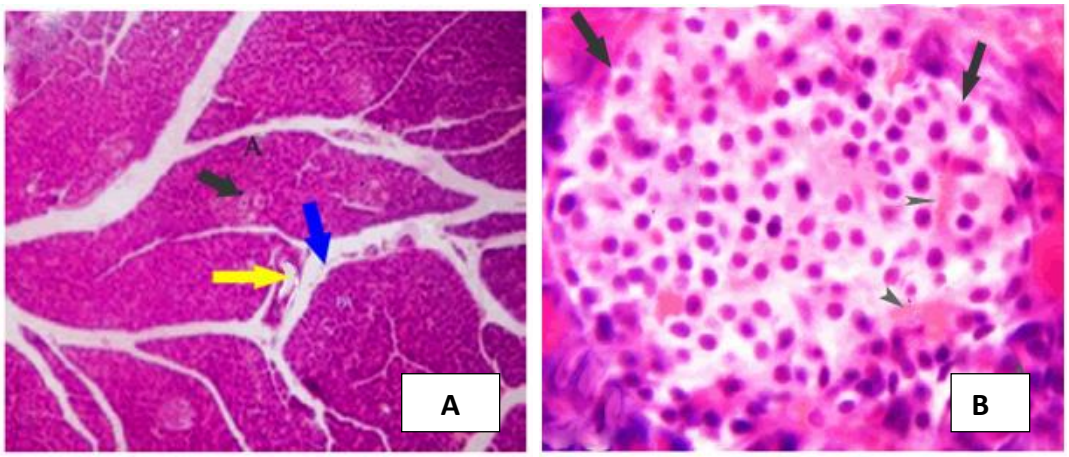


Figure 2 % changes from control of serum glucose and insulin levels in different groups of rats treated with individual and combined food additives.

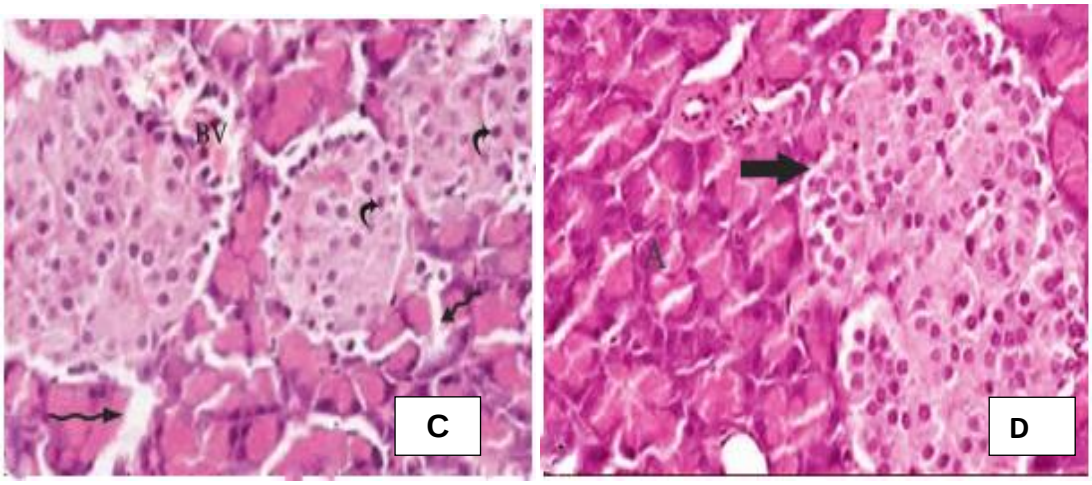
Expressed data in Figure (1) declared that treatment with individual and mixed food additives exhibited slight but significant changes in Serum alpha amylase enzyme all through the individually treated groups with % changes 3.94%,3.6%,-0.16%,and 0.96% from control in MSG, sodium banzoate, carmosin and EDTA groups respectively. On the other hand, Mixture treated group showed noticeable elevation in amylase activity with about 6% from control statistically significant versus control and all other groups. Furthermore, the results of serum lipase enzyme activity depicted in Figure (1) recorded slight elevation throughout the individually treated experimental groups. Noticeable significant increase versus control recorded in MSG group with 44.38% from control. Synergistic effect of the combined food additives was recorded in mixture treated group where the elevation in enzyme activity was 2.65% from control only. As regards to the serum glucose level induced significant elevation in all treated groups was recorded with % changes 59.15%, 88.28%, 127.15%, 155.16%, and 117.92% from control in all treated groups respectively. Considering, the effect of individual and combined treatment with food additives on level of serum total insulin the results showed statistically significant changes in MSG and EDTA groups with 20.77% from control. However, mixture treated groups' recorded pronounced significant increase in serum insulin level versus control as well as all other groups as expressed in Figure (2).

Histopathological Results

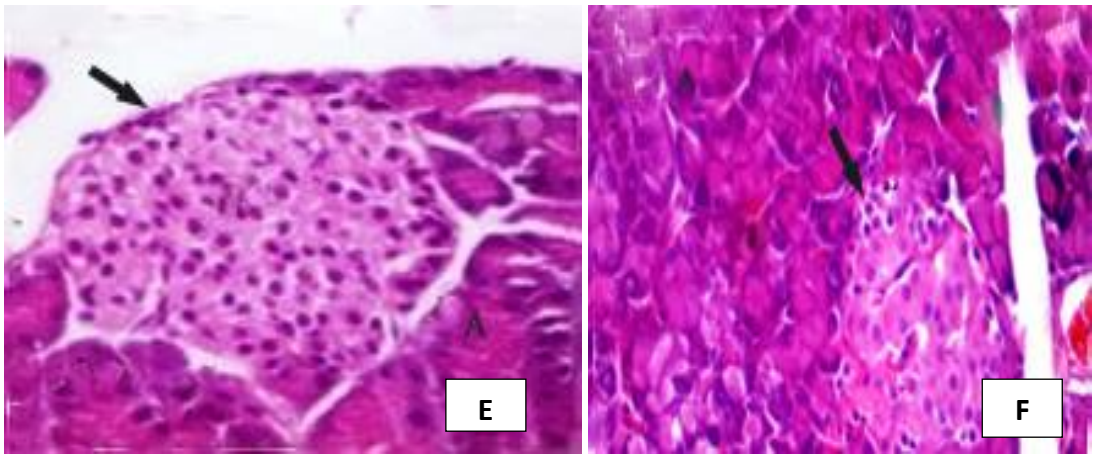


Photomicrograph (A and B) of pancreatic section from control group showing normal architecture of the pancreas; normal islets of Langerhans (black arrows) in between normal pancreatic acini (A). The islet cells are seen interspersed between the acinar cells and arranged in cords separated by blood capillaries (head arrows). The islets appeared lightly stained than the surrounding acinar cell with interlobular connective tissue (blue arrow) and interlobular duct (yellow arrow). Left figure (H&E x100), right figure (H&E x400)

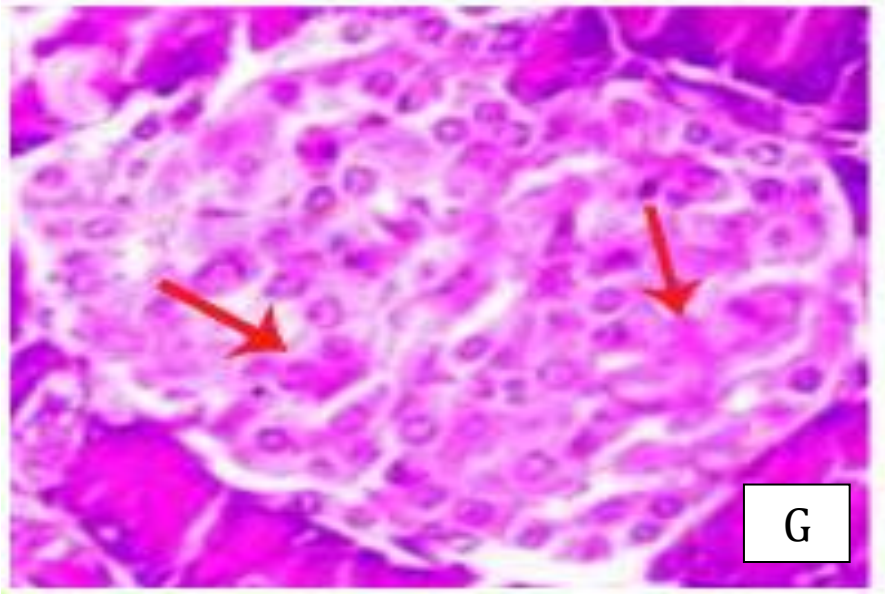
Pancreas is a gland having both exocrine and endocrine parts. Exocrine part in pancreas, secrets digestive enzymes like trypsin, amylase and lipase in duodenum. The endocrine parts in pancreas consist of groups of cells called islets of Langerhans. That contains alpha, beta cells and delta cells for glucagon, insulin and somatostatin secretion. Under light microscope, photomicrograph (A&B) the pancreas sections of normal rats (control group) has a thin cover of loose connective tissue from which septa pass into the gland, subdividing it into many small lobules. Each lobule is composed of several rounded or tubular groups of pancreatic cells called acini. Most of the pancreas is exocrine acini. The acini cells secrete the pancreatic juice. The acinar cells are formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm. Among the acini are the scattered the islets of Langerhans which appeared lightly stained than the surrounding acinar cells. Islets of Langerhans are composed of cords of endocrine cells with rounded vesicular nuclei and pale acidophilic cytoplasm. However, photomicrograph (C) of MSG group pancreatic tissues showed marked histological alterations in the architecture of the islets, their number was decreased, and some β-cells showed pyknotic nuclei. The acini cells showed loss of normal architecture, the presence of edema between the acini and the presence of some congested dilated blood vessels. On the other hand, Photomicrograph (D, E and F) pancreas sections of sodium benzoate, Carmosine and EDTA groups' rats showed normal appearance of the pancreas. Photomicrograph (G) pancreas sections of mixture treated group rats showed focal necrosis of the islets of Langerhans.



photomicrograph (C) of pancreatic section from (MSG group) showing loss of architecture of islets of Langerhans associated with decreased number and pyknotic nuclei of β-cells (curved arrows), loss of normal architecture of acini cells, the presence of edema between the acini (zigzag arrow) and congested dilated blood vessels (BV). (H&E x400). photomicrograph D of pancreatic section from (Sodium benzoate group) showing normal appearance of the pancreas. Normal pancreatic cells the acinar cells (A) which stained strongly are arranged in lobules with prominent nuclei. The islet cells (black arrow) are seen embedded within the acinar cells and surrounded by a fine capsule. (H&E x400)



photomicrograph (E) of pancreatic section from Carmosine group showing normal pancreatic structure. Normal appearance of islets of Langerhans (arrow) and acinar cells (A). photomicrograph (F) of pancreatic section from EDTA group showing normal pancreas; normal islets of Langerhans (arrow) in between normal pancreatic acini(A).



Photomicrograph (G) of pancreatic section from mixture group showing focal necrosis of the islets (red arrows). (H&E x400)

4. DISCUSSION

Results of the present study revealed that treatment with each of monosodium glutamate and sodium benzoate induced remarkable elevation in lipid peroxidation (MDA) and Oxidized protein (PC), concomitant with reduction in some measured antioxidant markers Catalase, total antioxidant capacity as well as total glutathione content and reduced glutathione. Okwudiri et al. (2012) reported that male Wistar Rats treated with MSG at a dose of 4g/kg body weight for ten days showed elevation in lipid peroxidation may be refer to a direct effect of propagation of ROS resulting from MSG treatment. Also, Villagarcia et al. (2016) recorded significant increase in protein carbonyl in newborn male pups of Wistar rats treated with 4mg/g B.W. They also reported reduction in GSH and antioxidant enzymes in MSG treated Wister Rats. Meanwhile, Khoshnoud et al. (2017) reported significant elevation in MDA and reduction in GSH levels in mice administrated Sodium benzoate 0.56, 1.125, and 2.25 mg/ml for 4 weeks. On the other hand, food color Carmosine and antioxidant EDTA induced decrease in serum MDA and non-significant changes in protein carbonyl as reported by Cemek et al., (2014) color and antioxidant food additives did not induce elevation in oxidative stress markers and in total glutathione and antioxidant enzymes. However, treatment with mixture of MSG, sodium benzoate, Carmosine and EDTA induced remarkable significant elevation in oxidative stress examined parameters (MDA & PC) in addition to the reduction in antioxidant enzymes and GSH, these findings runs with that recorded by Helal et al, (2017) induction in oxidative stress biomarkers after examining MSG, NaNO2 and annatto food additives. Significant alterations in antioxidant enzyme activity were explained by Sivaramakrishnan et al., (2008) glutathione is important to preserve the reduced state of cells and to abolish all the damage effects of oxidative stress. GSH may be a key in a large number living exercises including that detoxification about endogenous and exogenous mixes. Over production in ROS is the results of increased consumption of food additives and reduction of antioxidants, such as glutathione Chaves et al., (2008) reduced form of glutathione (glycyl-cysteinyl-γ-glutamate) the major intracellular antioxidant detoxifying agent because ROS induced oxidation of GSH into glutathione disulfide (GSSG). There is a balance between de novo synthesis of GSH and recycling from GSSG by glutathione reductase. Under normal conditions 95% of the intracellular glutathione is present in its reduced GSH form (Reid and Jahoor, 2001). The oxidized GSSG form can either be recycled to GSH or removed from the intracellular environment by certain transportation mechanism that explain the increase in serum GSSG and reduction in GSH recorded in the present study. Regarding to serum glucose level treatment with individual and combined different food additives induced pronounced elevation in serum glucose level significant versus control and groups each other at $p < 0.05$. Elshaikh (2014) reported that Wistar albino rats treated with MSG 240 mg/kg B. wt or synthetic color (tartrazine and sulfonic acid) and 75mg/kg of amaranth for four weeks showed significant increase in plasma glucose. In addition, Helal et al. (2017) reported that young rats received NaNO2 with annatto and MSG had remarkable sounded elevation in glucose level. Significant increases in each of amylase and lipase pancreatic enzymes were recorded in the present study. Statistically elevation in total insulin level was recorded in individually treated groups and pronounced in mixture treated group. Lennerz et al. (2015) reported that mice treated with color and banzoate food additives had no effect on insulin level. Increment of glucose elucidated the prompting of glycogenolysis by liver accompanied with fluctuation in the function of the pancreas inducing hyperglycemia (AL-Shinnawy and Elkattan, 2013). Rats administrated MSG,

the obvious impact of MSG in enhancing gluconeogenesis from glutamate to glutamine. Another clarification to the elevation in glucose in MSG groups may be due to the reduction in cellular sensitivity to insulin although the recorded hyperinsulinemia (Macho et al., 2000). In addition, leaking of glucose from tissues to blood may be due to gluconeogenesis, as well as changes in antioxidant enzymes and induction of oxidative stress which induce disturbance in metabolic processes (Bansal et al., 2005). These biochemical findings were confirmed with histopathological examination of pancreas where marked histological alterations in the structure of the pancreatic islets, their number was decreased, and some β-cells showed pyknotic nuclei were present in MSG and mixture treated groups. Hu et al. (2015) and Lennerz et al., 2015 reported that mice treated with color and benzoate food additives had no effect on insulin level. Blood glucose is controlled by different organs that affect insulin secretion these organs are pancreas, liver and kidneys (Creutzfeldt, 2001). Elevation of glucose levels can be explained by stimulation of glycogenolysis by the liver with the temporary loss of endocrine functions of pancreas that leads to hyperglycemia (Al-Shammari, 2014). In the MSG given groups, it is believed that these effects may be caused as result of MSG toxicity which leading to stimulation for gluconeogenesis from glutamate and glutamine. It has been also suggested a possible deterioration of glucose tolerance in rats following MSG administration that could be attributed to decrease cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG (Macho et al., 2000). Under conditions of hyperinsulinemia, cells could switch to pathways that favor gluconeogenesis to compensate for the increased insulin release (Okwudiri et al., 2012). In addition, leaking of glucose from tissues to blood may be due to gluconeogenesis as well as changes in antioxidant enzymes and induction of oxidative stress which induce disturbance in metabolic processes (Bansal et al., 2005).

5. CONCLUSION

Regular consuming of individual and combined food additive induced vehement disturbance in antioxidant enzymes system that stimulate oxidative stress and all tested biochemical metabolites including glucose in addition to pancreatic enzymes amylase and lipase. The obvious impacts of these additives to food reside in its hormonal effect that was remarkable on insulin hormone that can lead to health problems and induction of diabetes so, due to the above impact of these additives; it is prescribed to certain their uses.

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Conflicts of Interest: The authors declare no conflict of interest.

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