Ameliorative effects of Punica granatum juice and Bee pollen against monosodium glutamate induced toxicity in adult male albino rats.

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Abstract

Monosodium glutamate (MSG) is a worldwide consuming food additive and recently reported to be health hazardous. In this study, we aimed to investigate the possible ameliorative effects of Punica granatum juice (PJ) and Bee pollen (BP) against hematological and splenic toxicities. Thirty adult male albino rats were divided randomly into five groups (n = 6 each). In the normal group, rats were administered orally with distilled water for 10 weeks. All other groups were orally administered with MSG (2.4g/kg b.w.) daily for 4 weeks. After that, the third group was treated orally with PJ (4ml/kg b.w.) daily for 6 weeks, the fourth group was orally treated with (BP) (200 mg/kg b.w.) daily for 6 weeks and the fifth group was treated orally with (PJ, 4ml/kg b.w. + BP, 200mg/kg b.w.) daily for 6 weeks. By the end of the experiment the rats were sacrificed for collecting the blood and tissue samples for hematological assays and histopathological studies. The hematological results revealed that MSG administration induced significant reduction in RBCs, Hb, HCT, WBCs, neutrophils, lymphocytes and Platelets (PLTs) values. At the same time MCV and MCH recorded significant increase. Also, our results showed sever disorders in the splenic oxidative state biomarkers as manifested by a significant reduction in its total antioxidant capacity (TAC) and a remarkable increase in its malondialdehyde (MDA) levels as well as severe splenic histopathological abnormalities. Concomitant administration of PJ and BP alleviated all these altered hematological disorders besides clear ameliorations in splenic histopathological alterations.

Keywords: Bee pollen; hematological abnormalities; Monosodium glutamate; Punica granatum juice; splenic toxicity.

1. Introduction

Food additives: organic chemicals added in little amounts to almost all synthetic food for improving its taste quality. However, they are considered to be harmful, especially if they are continuously consumed. MSG, naturally occurring L-form of glutamic acid, is one of the most openly used food additives as a flavor enhancer known as fifth taste (umami). It is included in a lot of packaged foods like prepared flavored chips, marinated meats, seasoned chicken, vegetarian burgers, luncheon chicken and sausages without appearing on the label (Abd-Ella and Mohammed, 2016). Glutamate has many receptors in the body organs, thus daily intake of high amounts of it in food in the form of MSG impairs many hazardous alterations in the bone marrow and peripheral blood elements as well as toxic effects on different organs including spleen, liver and kidneys. Glutamate in high doses produce oxidative damage in the body organs that leads to biochemical, physiological and histological disturbances in animal models
The spleen histopathology is vital in evaluation of the immune system efficiency (Onkar and Govardhan, 2013); as the metabolites and xenobiotics immune toxicity on lymphocytes populations is represented in the spleen; the largest secondary lymphoid greatly involved in host immune response (Mebius and Kraal, 2005). Unfortunately, new data showing that synthetic pharmaceutical antioxidants have toxic effects on human cells, thus fueling an intense search for new natural, available, nontoxic and potentially efficient antioxidants. Punica granatum, commonly known as pomegranate, is an important fruit that belongs to the family Punicaceae and grown widely in many tropical and subtropical countries (Qnais et al., 2007). It is used in this work as fruit juice. It possesses a wide range of biological roles among which, its antioxidant activities that relates to its high contents of antioxidants of polyphenolic class that includes tannins, anthocyanins as well as flavonoids (Ricci et al., 2006). Bee pollen (BP), honey bee product, is a mixture of pollen grains from various plant sources, which are collected by the honeybee workers and mixed with enzymes, nectar, honey, beeswax and honeybee salivary secretions, so this mixture contains almost all nutrients present in different botanical sources and required essentially by human organism. It has not only antibacterial and antifungal biological activities but also strong antioxidant abilities (Reynaldi et al., 2010; Saral et al., 2016). Therefore, this study provides new insights on MSG overconsumption toxicological effects and its hazardous changes in hematological parameters as well as splenic biochemical and histopathological alterations with clarification of the possible ameliorative roles of PJ and BP as natural available antioxidants against MSG toxicity.

2. Materials and Methods

2.1. Drugs and chemicals

MSG, its purity (99.7%), was purchased from AVI-CHEM LABORATORIES Pvt. Ltd. (A-221, Amargain Industrial Complex, Opp. S.T. stand, LBS marg, Khopat Thane (w) Mumbai, Maharashtra, India). MSG orally administered to the different groups of the rats at dose 2.4 g/kg b.w. (15% of LD₅₀ of MSG (Shukry et al., 2020). Punica granatum were subjected to peel off, then squeezed for collecting its fresh juice. PJ doses were prepared freshly daily in labeled containers and were administered at a dose of 4 ml/kg b.w., chosen as an optimal concentration according to (Arkoub et al., 2022). Bee pollen (BP) were purchased from faculty of Agriculture-South Valley University, Qena, Egypt. BP was orally administered at a dose of 200 mg/kg b.w. (in distilled water) chosen as an optimal concentration according to (Shaldoum et al., 2021).

2.2. Animals

This study included 30 adult male albino Wister rats aged 13–14 weeks were obtained and housed in the Animal House, Faculty of Science, South Valley University, Qena, Egypt. Their body weights ranged from 190 to 210 gm. The rats were housed in polypropylene cages under a controlled environment (23 ±2 °C, 55% relative humidity and a 12-h light/dark cycle) and were provided with standard commercial pellets, which were used as food, and water was provided ad libitum. Other conditions pertaining to the health of the animals were maintained during the entire course of the study. All experimental protocols were performed in accordance with the local institutional guidelines and approved by the Animal Ethical Committee (published by the Faculty of Science, South Valley University under code No. 007/01/24), Qena, Egypt.

2.3. Experimental design

After two weeks of acclimatization, rats were randomly divided into 5 groups, (n=6 each). The normal group orally administered with distilled water daily for 10 weeks, MSG group orally
administered with MSG (2.4g/kg b.w.) daily for 4 weeks, MSG+PJ group orally administered with MSG (2.4g/kg b.w.) daily 4 weeks and then orally administered with PJ 4 ml/kg b.w. for 6 weeks, MSG+BP group orally administered with MSG (2.4g/kg b.w.) daily for 4 weeks and then orally administered with BP (200 mg/kg b.w. in distilled water) daily for 6 weeks, MSG+PJ+BP group orally administered with MSG (2.4g/kg b.w.) daily for 4 weeks and then orally administered with (4 ml/kg b.w. of PJ and 200 mg/kg b.w. of BP) for 6 weeks.

2.4. Sample collection
The blood samples were collected immediately from the retro-orbital sinus using microcapillary tubes Twenty-four hours after treatment with the last dose. The was taken in labeled EDTA containing tubes from every animal for the examination of complete blood picture. Then rats were euthanized by cervical dislocation for spleen specimens collection. The spleen was carefully dissected out and washed well using chilled saline (0.9 % NaCl) and blotted dry quickly samples of it were frozen and stored at -80 °C for subsequent oxidative stress assays. The later samples of spleen were fixed in 10% neutral buffered formalin to be used in the histopathological examinations.

2.5. Hematological analysis
All CBC parameters were determined using automated hematology analyzing machine (Dirui BCC-3000B).

2.6. Malondialdehyde (MDA) and Total antioxidant capacity (TAC) assays of spleen tissue homogenate
For Total antioxidant capacity and malondialdehyde determinations, homogenization of frozen spleen tissue was performed using chilled Tris-HCl buffer (pH 7.4) and then centrifugation (4000 r/min for 30 min at 4 °C). Then supernatant from the tissue homogenate was used for colorimetric assays of oxidative state biomarkers using commercial kits supplied by (Bio-Diagnostic, Egypt) using (UV-Visible Spectrophotometer, T60).

2.7. Histopathological studies
The neutral buffered formalin-fixed spleen samples were washed in 70 % ethanol to get rid of the fixative before subsequent processing. Then the samples dehydrated in ascending ethanol series (70 %, 80 %, 90 %, 100 I, 100 % II), cleared in methyl benzoate and embedded in paraffin wax (Abd-Elhafeez and Soliman, 2017). Paraffin sections were cut at 5μm in thickness and stained with Hematoxylin and Eosin stain for general histopathological examinations.

2.8. Statistical analysis
All data were analyzed using one-way ANOVA analysis of variance (prism computer program); the variability degree of results was expressed as Means ± Standard Deviation of means (Mean ±S.D). And the least significant difference (L.S.D) was used to test the difference between b nm treatments. Results were considered statistically significant when P < (0.05).

3. Results
3.1 Hematological parameters among the studied groups
The mean ± SD values of the RBCs, Hb, HCT, WBCs, Lymphocytes, Neutrophils and PLTs in the MSG group were significantly lower than those in the normal group, while the mean ± SD values of the MCV and MCH in MSG group were significantly higher than those in the normal group  (p˂0.05 for all. However, the mean ± SD values of the RBCs, Hb, HCT, WBCs, Lymphocytes, Neutrophils and PLTs in the MSG+PJ, MSG+BP and MSG+PJ+BP groups were significantly higher than those in the MSG group (p<0.05 for all. However, the mean ± SD values of the MCV and MCH in the MSG+PJ, MSG+BP and MSG+PJ+BP groups were significantly higher than those in the MSG group (p<0.05 for all). But when compared with the normal group,
all the results in MSG+PJ, MSG+BP and MSG+PJ+BP recorded remarkable improvements with non-significant differences. (Figure 1).

Figure (1). Hematological parameters including RBCs, Hb, HCT, MCV, MCH, WBCs, Lymphocytes, Neutrophils and Platelets in normal, MSG, MSG+PJ, MSG+BP and MSG+PJ+BP groups. Data are expressed as mean ±S.D. of 6 rats in each group.

Figure (2). Splenic MDA and TAC in normal, MSG, MSG+PJ, MSG+BP and MSG+PJ+BP groups. Data are expressed as mean ±S.D. of 6 rats in each group.
3.2. Histopathological findings of the spleen among the various studied groups

The spleen of the rats in normal group, which orally received distilled water daily for 10 weeks displayed normal structure of splenic parenchyma, where white pulp showed lymphocyte sheaths surrounding a central arteriole and normal structure of red pulp forms (Fig. 3, a). The spleen of the rats in MSG group, which orally received MSG (2.4 g/kg b.w.) daily for 4 weeks, suffered from severe necrosis in splenic tissues with hemosiderin and proliferation and activation in mononuclear cells through the splenic tissues, with depletion in white pulp (Figs. 3 (b, c)), besides thickening in wall of splenic blood vessels with depletion in WBCs (Fig. 3, d). The spleen of the rats in MSG+PJ group, which orally received MSG (2.4 g/kg b.w.) daily for 4 weeks plus PJ (4 ml/kg b.w.) daily for 6 weeks showed mild necrosis in splenic tissues with normal white pulp with hemosiderin scattered the red pulp (Fig. 3, e). The spleen of the rats in MSG+BP group, which orally received MSG (2.4 g/kg b.w.) daily for 4 weeks plus BP (200 mg/kg b.w.) daily for 6 weeks appeared apparently normal structure of white pulp and red pulp with hemosiderin scattered the red pulp (Figs. 3 (f, g)). The spleen of the rats in MSG+PJ+BP, which received MSG (2.4 g/kg b.w.) daily for 4 weeks plus PJ (4 ml/kg b.w.) and BP (200 mg/kg b.w.) daily for 6 weeks, showed mild necrotic changes in splenic tissues with mild depletion in white pulp and hemosiderin still present (Fig. 3, h).
It is well known that, HCT measures the volume of RBCs relative to the total blood volume, also RBCs contain mainly Hb. On this basis, any disturbances in the RBCs count reflects directly on HCT and Hb values. These observed results run in full agreement with (Abu-Taweel et al., 2016). Additionally, the significant increase in MCV and MCH values run in full agreement with (George and Kumaran, 2016; Abdulsalam et al., 2018). This increased MCV indicates the RBCs...
to be macrocytic, that is observed in megaloblastic and pernicious anemia, while the increased MCH is seen in macrocytic anemia; thus in our study pernicious macrocytic anemia is more specifically observed, that is might be due to gastric mucosa atrophy induced by the acidic effect of L form of glutamic acid (MSG), leading to suppression in the synthesis of the intrinsic factor, and so on poor absorption of vitamin B12 that is the main cause of pernicious anemia. Besides, the significant fall in the PLTs count after MSG administration might also be due to bone marrow disturbances induced by MSG (Al-Harbi et al., 2014; Abu-Taweel, 2016). Moreover, the significant decrease in WBCs, lymphocytes and neutrophils count after MSG-administration might be due to these cell lyses and bone marrow destruction, as other ways of MSG toxicity and immunosuppression (Abdulsalam et al., 2018; Shukry et al., 2020). This the immune-suppressant effect of MSG may be due to its hazardous effects on the spleen as it is observed in the splenic histopathological findings of the MSG-treated rats in our current study, this is in the same line with (Tan et al., 2000 and Xochelli et al., 2015). Additionally our current results revealed that, MSG resulted in a significant increase in splenic MDA and a significant reduction in its TAC levels, relative to the normal ones, we can relate this to the oxidative injury caused by MSG as glutamate receptors are widely distributed in peripheral organs including the liver, kidneys and spleen and its overconsumption resulting in overstimulation of these receptors by glutamate that is poorly transported across cell membranes and accumulate intracellularly initiating the lipid peroxidation, an autocatalytic mechanism leading to oxidative destruction of cellular membranes by altering the redox potential of the cell, resulting in the formation of aldehyde products, such as MDA that can possibly diffuse from their destinations of cause to achieve far off intracellular and extracellular targets, resulting in oxidative injury (Gill and Pulido, 2001; Banerjee et al., 2021). Consequently, these changes can lead to spleen functional impairments suggesting disturbances in cellular functions and extreme decline in the antioxidant enzymes activities (GSH, SOD and CAT) resulting in inhibition of the total antioxidant capacity (TAC), this is in full agreement with (Khodier et al., 2020).

On the other hand, PJ can significantly ameliorate all the hematological alterations and can significantly reduce splenic MDA and elevate splenic TAC. These improvements in RBCs, Hb and HCT values, after administration of PJ, are in accordance with (Manthou et al., 2017) who relate this RBCs improvement to increasing erythropoiesis or prevention of RBCs degradation. It is worth to mention that PJ contains high contents of polyphenols and its supplementation could elevate GSH levels in RBCs supporting the RBCs resistance to oxidative stress (Matthaio et al., 2014). It has been determined that PJ flavonoids can bind to Hb preventing its oxidation by ROS (Gebicka and Banasiak, 2012). Besides, the HCT improvement may be due to RBCs restoration after PJ supplementation. Furthermore, MCV and MCH levels recorded significant reduction after PJ supplementation compared with the MSG-treated ones, MCV and MCH are highly related to the RBCs, HCT as well as Hb values. So, any improvements in these hematological parameters reflect positively on MCV and MCH values. Besides, PJ restoration of PLTs may relate to the amelioration in the bone marrow that produces them.

Above all, our results showed that, BP significantly ameliorate all disrupted hematological constituents as well as significantly reduction in splenic MDA and elevation in its TAC, this is in accordance with (Shaldoum et al., 2021) who proved the amelioration role of BP at dose 200 against doxorubicin toxicity at the same dose of our BP dose (200mg/kg b.w.). Also, this restoration in the RBCs count agrees with (Attia et al., 2011). BP is rich in many nutrients such as proteins,
amino acids, vitamins and trace elements, that stimulate immune cells proliferation and differentiation; so on it plays vital roles in restoration the WBCs, Lymphocytes and Neutrophils counts, depending upon its antioxidant chemical components. According to Wang et al. (2005b), Polysaccharides in BP promote T lymphocytes production, cell proliferation and activity in the spleen, as well as in the thymus and bursa, which produce T and B lymphocytes, respectively. All of these effects result in a better immune status in our experimental rats. BP contains Fe, Cu, Zn, Cr and Mn; as well as catalase, superoxide dismutase, peroxidase and antioxidants (Oliveira et al., 2013). Also, it has been highly associated with increased humoral immune cells, increased RBCs number, accelerated antibody production and delayed disappearing antibodies (Zuo and Xu, 2003). As well as BP restoration of PLTs may relate to the amelioration in the bone marrow that produce them. Additionally, we can relate the Hb restoration after BP treatment to preventing of Hb oxidation and denaturation by the antioxidant components present in BP. Besides, the HCT improvement may be due to RBCs restoration after BP supplementation. The restoration of RBCs, Hb and HCT reflect mainly on restoration of MCV and MCH values. Above all, our splenic histopathological findings prove these splenic ameliorations of PJ and BP (reduction in splenic MDA and elevation in its TAC levels), either separately or both together, against MSG hematological disorders and splenic toxicity.

4. Conclusion

In conclusion, our outcomes showed that MSG overconsumption induces hematological and splenic toxicities. On the other hand, the treatment with PJ and BP, either each separately or both together, individually, expressed potentially effective antioxidant and ameliorative effects against all these MSG hazardous effects. So, we recommend that we should reduce our daily MSG consumption as much as possible, also our current findings lead us to recommend daily consuming of PJ and BP in our daily usual diet as available, strong natural antioxidants.

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Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate
Not applicable

Consent for Publication
Not applicable.

Conflicts of Interest
The authors disclosed no conflict of interest.

5. References


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