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Investigation of potential protective effects of Betanin on experimental Monosodium Glutamate–induced toxicity in elderly rats

Investigación de los posibles efectos protectores de la Betanina sobre la toxicidad experimental inducida por Glutamato Monosódico en ratas ancianas

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ABSTRACT

This study was conducted to investigate the protective effects of Betanin active ingredient in red beetroot plant (*Beta vulgaris*) in elderly rats exposed to chronic toxicity of monosodium glutamate (MSG). A total of 48 elderly rats were randomly divided into 4 different groups. At the end of the 28-day study, the rats were sacrificed under deep anesthesia. Total antioxidant capacity (TAC), total oxidant capacity (TOC), paraoxonase (PON), thiol, malondialdehyde (MDA), and nitric oxide (NO) levels were investigated in rat blood serum using the spectrophotometric method. Oxidative Stress Index (OSI) was calculated by dividing TOC by TAC. Total bilirubin was measured with the colorimetric method using an ELISA kit. Liver tissues were stained with hematoxylin–eosin (HE) for histopathological examination. The difference in serum levels of TAC, TOC, OSI, PON, MDA, and thiol was statistically significant between the groups ($P<0.05$). The difference in serum levels of NO and total bilirubin was not statistically significant between the groups ($P>0.05$). The analysis of histopathological findings revealed uncommon mild hydropic degeneration in the MSG group and almost normal histological appearance in the MSG+Betanin group. This study demonstrated that betanin could increase the antioxidant effect and reduce the histopathological damage caused by MSG.

Key words: Monosodium glutamate; aged rats; biochemistry; betanin; histopathology

RESUMEN

Este estudio se llevó a cabo para investigar los efectos protectores de betanina ingrediente activo en la planta de remolacha roja (*Beta vulgaris*) en ratas de edad avanzada expuestos a la toxicidad crónica de glutamato monosódico (MSG). Un total de 48 ratas ancianas fueron divididas aleatoriamente en 4 grupos diferentes. Al final del estudio de 28 días, las ratas fueron sacrificadas bajo anestesia profunda. Se investigaron los niveles de capacidad antioxidante total (TAC), capacidad oxidante total (TOC), paraoxonasa (PON), tiol, malondialdehído (MDA) y óxido nítrico (NO) en el suero sanguíneo de las ratas mediante el método espectrofotométrico. El índice de estrés oxidativo (OSI) se calculó dividiendo el TOC por el TAC. La bilirrubina total se midió con el método colorimétrico utilizando un kit ELISA. Los tejidos hepáticos se tiñeron con hematoxilina–eosina (HE) para el examen histopatológico. La diferencia en los niveles séricos de TAC, TOC, OSI, PON, MDA y tiol fue estadísticamente significativa entre los grupos ($P<0,05$). La diferencia en los niveles séricos de NO y bilirrubina total no fue estadísticamente significativa entre los grupos ($P>0,05$). El análisis de los hallazgos histopatológicos reveló una degeneración hidrópica leve poco común en el grupo MSG y un aspecto histológico casi normal en el grupo MSG+betanina. Este estudio demostró que la betanina podía aumentar el efecto antioxidante y reducir el daño histopatológico causado por el MSG.

Palabras clave: Glutamato monosódico; ratas envejecidas; bioquímica; betanina; histopatología

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INTRODUCTION

Oxidative stress is defined as a disruption in the oxidative balance resulting from the increase in reactive oxygen species (ROS) formed during cellular metabolism and the insufficiency of antioxidants responsible for their detoxification [1, 2]. ROS have different chemical structures such as hydroxyl radical, superoxide radical ($O_2^{\cdot-}$), and hydrogen peroxide. Numerous experimental data have shown an increase in intracellular oxidative stress during aging. This is partly due to the gradual reduction of intracellular ROS scavenging during the aging process [3].

Monosodium glutamate (MSG), which is used as a flavor enhancer all over the World due to its well-known umami taste [4, 5] causes the formation of ROS that cause oxidative damage [6]. However, MSG has several adverse effects on various organs, including the brain [5], kidney [7, 8] and liver [7, 9]. The study by Nnadozie *et al.* [7] administering MSG Wistar albino rats for one year to observe mortality, fertility, major organ functions, and histopathological effects showed inflammation in the histological findings and a significant increase in biochemical parameters of the MSG group compared to the control group [7]. In the study by Hazza *et al.* [10], MSG was administered to Wistar albino rats (*Rattus norvegicus*) for 30 days. The study found that MSG caused oxidative stress [10]

Vegetables significantly contribute to healthy nutrition with the antioxidants they contain. Red beetroot is one of these important vegetables [11, 12]. A study investigating the protective properties of betanin on many diseases in rats revealed positive results by suppressing oxidative stress, inflammation, and apoptosis [13]. Antioxidants are chemical substances that inhibit or delay undesirable oxidation reactions. They prevent cell damage by presenting their electrons to free radicals. Antioxidants protect the substrate from irreversible damage and prevent its oxidative conversion via reactive species. Antioxidants in food delay, control, or prevent oxidation and deterioration of food quality. Betanin ($C_{24}H_{26}N_2O_{13}$) is a good free radical scavenger with an antioxidant effect [14]. In line with this information, recent studies have emphasized the antioxidant property of Betanin.

In summary, the biochemical changes that occur with aging and the additional burden of MSG, which is a food additive, lead to pathological liver damage, an increase in oxidant parameters, and a decrease in antioxidant parameters. Likewise, Betanin, which has an antioxidant effect, prevents or decreases liver damage, decreases oxidant parameters, and increases antioxidant parameters. However, these changes have not yet been fully elucidated in elderly rats (*Wistar Albino*).

This study aimed to investigate the effects of Betanin on Monosodium Glutamate-induced toxicity in blood serum and liver in elderly rats with biochemical and histopathological methods.

MATERIALS AND METHODS

Ethics and animals

The present study was reviewed and approved by the institutional ethics committee of the Aydin Adnan Menderes University Animal Experiments Local Ethics Committee in January 2020 (IRB Approval Number: 64583101/2020/05).

Forty-eight male *wistar albino* rats of minimum 15 month old and about 350 g body weight (rat -*wistar albino*- Hatay Mustafa Kemal University-Turkey) were used in this study. Rats were purchased from laboratory animal house of Hatay Mustafa Kemal University. They were acclimatized for 10 days (d) before the beginning of the experiment. All rats fed standard rat diet, allowed to water *ad libitum*, and kept under normal daylight/dark cycle and room temperature during study.

Experimental design

Rats were weighed and assigned into four experimental groups. It is planned to have 12 rats in each group against the risk of death of aged rats. Application took 28 d.

The 3R (Replacement, Reduction and Refinement) has become guiding principles for the ethical use of animals in research [15].

The present study was reviewed and approved by the institutional ethics committee of the Aydin Adnan Menderes University Animal Experiments Local Ethics Committee in January 2020 (IRB Approval Number: 64583101/2020/05).

MSG was obtained from Sigma Company Germany. Betanin was obtained from Sigma Aldrich.

- Control group: Fed standard rat diet, allowed to water *ad libitum*
- MSG group: 120 mg·kg^{-1}·day^{-1} MSG was administered by gavage
- Betanin group: 20 mg·kg^{-1}·day^{-1} betanin was administered by gavage
- MSG+Betanin group: 120 mg·kg^{-1}·day^{-1} MSG and 20 mg·kg^{-1}·day^{-1} Betanin was administered by gavage

Analyses

ELISA kits (Rel Assay Diagnostic-Turkey) were used for biochemical analyses [16]. The colorimetric method is based on the measurement of the UV absorption of the test material or the spectral determination of a colored compound in the visible field as a result of its reaction with a reagent. Micro pellet reader devices show parallelism in many experiments. The number of cells and events are reflected by the color and severity of the reaction [17].

TAC and TOC measurements were performed by means of kits (Rel Assay Diagnostic-Turkey) developed by Erel [1, 18]. MDA in rat blood was determined according to the spectrophotometric method described by Placer *et al.* [19] and the results obtained were expressed as nmol·mL^{-1} [19]. No was analysed using the modified cadmium-reduction method described by Navarro-Gonzalves *et al.* [20]. Paraoxonase activities measurements were performed in the absence (basal activity) and presence of NaCl (salt-stimulated activity). Paraoxonase activity was expressed as U·L^{-1} serum [21].

Pathological evaluation

Liver samples taken for histopathological examination were fixed in 10% buffered formalin (pH 7.2-7.4). After fixation, they were reduced to 4-mm-thick tissue sections and washed overnight with running tap water. After passing through a graded series of alcohol (50, 80, 96 and 100%), xylol, and paraffin according to routine methods, they were blocked in paraffin. Sections of 4-micron thickness taken from the tissue blocks that were prepared in this way with a microtome (Leica RM2125 RTS, Nusslock, Germany) were first placed in a water bath set at a temperature of 37°C, and then taken to slides and dried in an oven at 54°C for 30 min. After these sections were deparaffinized in xylol and immersed through alcohol series of 100, 96 and 80, and 70%, they were stained with Hematoxylin Eosin (HE). They were again

passed through a graded series of alcohol (80, 96 and 100%) and xylol and closed with a coverslip with the help of a glue substance (Entellan). After examination under a light microscope (Olympus CX31, Münster, Germany), microphotographs (Olympus DP12, Hamburg, Germany) were taken. Histopathological changes were scored according to the following criteria: Grade 0, histopathological changes below 5% of the entire area; Grade 1, mild histopathological changes in 5–33% of the entire area; Grade 2, moderate histopathological changes in 33–66% of the entire area; Grade 3, severe histopathological changes in more than 66% of the entire area [22, 23].

Statistical evaluation

Statistical evaluation was performed using the IBM SPSS Statistics 21 package. The test for conformity to normal distribution was evaluated with the Shapiro Wilk Test. One-way ANOVA method determined whether the changes were significant ($P<0.05$). Post-hoc test (Duncan) was used for significant data.

RESULTS AND DISCUSSION

Biochemical Results

As shown in TABLE I, there was a significant difference between the groups in terms of TAC, TOC, OSI, PON, thiol, and MDA ($P<0.05$). The comparison of the MSG group with the Betanin group showed a statistically significant increase in the TAC and PON values in the Betanin groups ($P<0.05$). This result supports the antioxidant property of Betanin. The comparison of the MSG group with the Betanin group showed a statistically significant increase in the TOC, OSI, and MDA values in the MSG groups ($P<0.05$). This result supports the oxidant property of MSG. The comparison of the control group with other groups revealed no significant difference between the groups in terms of total bilirubin and NO ($P>0.05$).

Pathological results

In the control and Betanin groups, the liver had a normal histological structure (FIG. 1A). Mild hydropic degeneration, which was uncommon in hepatocytes, was noted in the MSG group (FIG. 1B). In addition, focal foci of mononuclear cell infiltration were observed in the portal tracts (FIG. 1C). The MSG+Betanin group had an almost normal histological appearance (FIG. 1D).

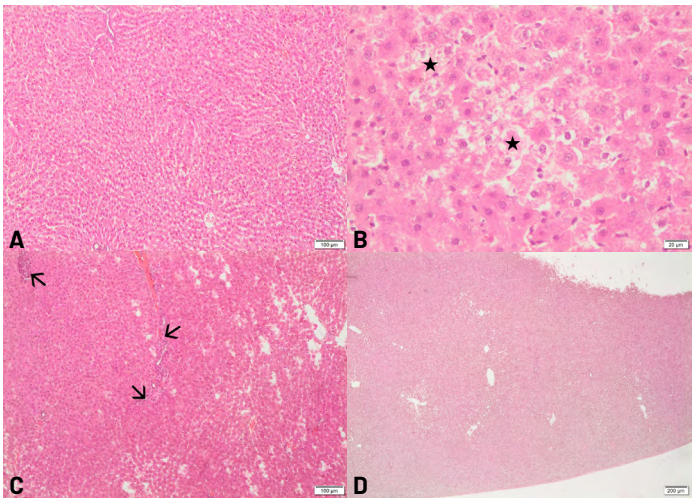

FIGURE 1. Hepatic Histopathological Findings. A) Normal histological structure of the liver, Control Group, HE. B) Hydropic degeneration of hepatocytes (stars), MSG, HE. C) Focal mononuclear cell infiltration of the portal tract (arrows), MSG Group, HE. D) Normal histological structure of the liver, MSG+Betanin Group, HE

The comparison of the control group with the MSG group revealed a significant difference ($P<0.05$), while the comparison of the control group with the Betanin group showed no significant difference ($P>0.05$). When the MSG group and the betanin group were compared, the difference was statistically significant ($P<0.05$). Focal mononuclear cell infiltration of the portal tract was caused by MSG (FIG. 2).

TABLE I
Serum TAC, TOC, OSI, Total Bilirubin, PON, Thiol, NO and MDA Levels

	Control X̄±SD	MSG X̄±SD	Betanin X̄±SD	MSG+Betanin X̄±SD	*P*-value
TAC (mmol Trolox equiv·L^{-1})	2.00±0.20^{a}	1.47±0.13^{ab}	1.94±0.13^{b}	1.77±0.18^{b}	$P<0.05$
TOC (μmol H_2O_2 equiv·L^{-1})	9.93±1.33^{a}	14.14±0.16^{ab}	10.05±0.93^{bc}	12.05±1.16^{abc}	$P<0.05$
OSI [(TOC/TAC)×100]	50.24±9.63^{a}	96.71±10.40^{ab}	51.89±6.49^{bc}	68.62±8.97^{abc}	$P<0.05$
Total Bilirubin (U·L^{-1})	0.029±0.012	0.031±0.014	0.033±0.013	0.024±0.011	$P>0.05$
PON (U·L^{-1})	297.69±13.92^{a}	222.87±32.18^{ab}	300.00±21.38^{b}	280.19±13.63^{b}	$P<0.05$
THIOL (μmol·L^{-1})	283.63±10.35^{a}	301.05±10.74^{ab}	287.66±8.90	279.49±8.64^{b}	$P<0.05$
NO (μmol·L^{-1})	13.68±4.65	17.07±7.70	18.50±5.40	19.74±5.15	$P>0.05$
MDA (mmol·L^{-1})	4.07±0.58^{a}	6.07±0.95^{ab}	4.43±0.74^{b}	5.06±0.49	$P<0.05$

$P<0.05$ (statistically significant). $P>0.05$ (statistically non significant). ^{a,b,c}: groups with the same letters on the same line are different from each other

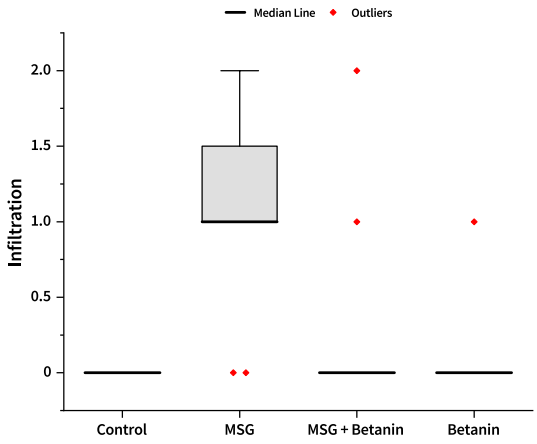

FIGURE 2. Mononuclear Cell Infiltration

The comparison of the control group with the MSG group showed a significant difference ($P<0.05$), while the comparison of the control group with the Betanin group revealed no significant difference ($P>0.05$). Likewise, the comparison of the MSG group with the Betanin group showed a significant difference ($P<0.05$). Hydropic degeneration of hepatocytes was caused by MSG (FIG. 3).

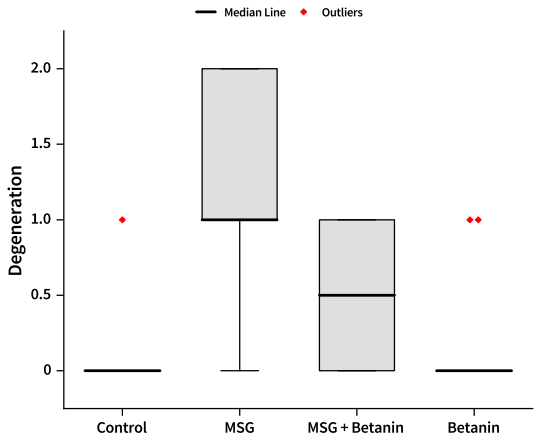

FIGURE 3. Hydropic Degeneration of Hepatocytes

Aging is a physiological process characterized by molecular and anatomical changes leading to progressive loss of functions [24]. Therefore, elderly people who are sensitive to external factors are adversely affected by food additives. A better understanding of biochemical parameters and how the liver is pathologically affected can minimize oxidative damage. Accordingly, it is important to understand the biochemical and pathological changes in aging metabolism. In this context, it is even more important to use natural antioxidants in food technology in order to detoxify the negative effects of food additives. This study aimed to investigate the protective effects of Betanin against the toxic effects of MSG in Elderly rats.

Exposure to the food additive MSG with age can further increase oxidative stress. Moreover, the elimination of oxidative stress or the reduction of its effects can be ameliorated with betanin, which is also used in the food industry and has an antioxidant effect. Biochemical and pathological investigation of these changes is important in terms of gaining new perspectives. Accordingly, in this study, it was investigated TAC, TOC, Thiol, PON, NO, MDA, and total Bilirubin expressions in the serum of elderly rats along with pathological evaluation of the liver. İt was examined the OSI obtained by dividing TOC by TAC.

Thiols, which are organic sulfur derivatives containing sulfhydryl residues (–SH) at their active sites and can be oxidized by disulfide (–S–S–) bond formation, easily react with oxygen-containing free radicals to form disulfides [25]. Paraoxonases (PONs) comprise a gene family consisting of three members (Paraoxanase 1, 2, 3) with diverse roles in multiple biochemical pathways, including inflammation [26]. Nitric oxide (NO) functions as an oxidative biological signaling molecule. Overproduction of NO induces neural injury, leading to neurological disorders [27]. MDA is an important biomarker to determine the extent of tissue injury [28]. In a study on Wistar Albino rats, MSG was administered orally, and MDA values were examined as an indicator of oxidative stress. MDA values were statistically significantly increased in the MSG group [29]. MDA levels and their effects on liver morphology were evaluated in mice fed with a standard diet or a high-fat diet. MSG was found to have effects on oxidative status and organ morphology [30, 31]. In the present study, the MDA serum levels showed an increase in all groups compared to the control group. However, only the increase in the MSG group was statistically significant. Likewise, the results of the present study showed that MSG caused degeneration and infiltration in the liver tissue.

A study by Da Silva *et al.* [32] evaluated the effect of betanin intake for 20 d on oxidative stress in Wistar albino rats. The results of the study showed reductions in MDA levels of betanin-treated rats and reversal of hepatic injury by histological analysis [32]. In a study investigating whether betanin was protective against hepatic injury in rats, pathological hepatic injury and MDA levels were lower in rats treated with Betanin than in rats treated with the chemical alone [33]. In the present study, the highest MDA value was observed in the MSG group. This result clearly shows that MSG causes lipid peroxidation in rats. Other studies also support this result. Furthermore, the current study showed that Betanin reduced the inflammatory (physiological response) and degenerative changes in the liver induced by MSG.

Bilirubin is constantly produced by liver, spleen, or bone marrow macrophages with the breakdown of heme from senescent red blood cells [34]. In their study, Tawfik and Al-Badr [35] found that MSG administration statistically significantly reduced the total bilirubin levels in Wistar albino rats [35]. In the current study, the differences in total bilirubin serum levels were not statistically significant ($P>0.05$). There was an increase in serum levels of total bilirubin in the MSG group and the Betanin group compared to the control group. However, the increase was not statistically significant. There was a decrease in serum levels of total bilirubin in the MSG+Betanin group compared to the control group. However, the decrease was not statistically significant.

A study by Helal *et al.* [36] designed to investigate its therapeutic role in MSG-induced male reproductive system disorders in male rats reported a statistically significant increase in NO in the MSG group [36] The present study showed no significant difference in NO serum levels between the groups ($P>0.05$).

There is no study in the literature administering MSG to elderly rats and investigating the thiol levels. In the present study, the difference in serum levels of thiol was statistically significant between the groups (P<0.05). The comparison of the control group and other groups in terms of serum levels of thiol showed a statistically significant increase in the MSG group. The comparison of the MSG group with the Betanin and MSG+Betanin groups revealed a decrease in thiol levels, which was statistically significant only in the MSG+Betanin group. It is believed that the simultaneous administration of MSG and Betanin may cause toxic effects.

In their study, Elbassuoni *et al*. found significantly lower TAC levels in the MSG group compared to the control group [37]. A study investigating the effect of Betaine supplementation on cadmium–induced oxidative impairment in rat kidney reported that Betanin administration alleviated the reduction in TAC in cadmium+Betanin–treated rats compared to the cadmium group alone [38]. The results of the present study showed that the difference in TAC serum levels was statistically significant between the groups (P<0.05). The comparison of the control group and other groups in terms of serum levels of TAC revealed a decrease in the TAC serum level of all groups. The lowest TAC value was found in the MSG group. Following the control group, the highest TAC value was found in the Betanin group. The result of the lowest TAC level in the MSG group suggests that MSG may have an oxidant effect. Betanin is thought to increase antioxidant capacity. In addition, when administered with MSG, it is believed to reduce the oxidant effect of MSG. In the present study, the difference in serum levels of TOC was statistically significant between the groups (P<0.05). The highest TOC value was observed in the MSG group. Following the control group, the lowest TOC value was found in the Betanin group. Due to the oxidant effect of MSG, the highest TOC value was noted in the MSG group. Due to its antioxidant properties, Betanin reduces the oxidant effects of MSG. The results of the comparison of the TOC values in the MSG+ Betanin group and the MSG group support this result. The difference in serum levels of OSI was statistically significant between the groups (P<0.05). The highest OSI value was observed in the MSG group. Following the control group, the lowest OSI value was noted in the Betanin group. Considering the OSI values, the antioxidant property of Betanin has been proven once again. Serum levels of thiol are presented in TABLE I.

In a cell culture model study by Esatbeyoglu *et al*. [39], which aimed to systematically evaluate Betanin's free radical scavenging activity by electron spin resonance spectroscopy and spin capture, the antioxidant activity of Betanin was determined in terms of PON induction. Betanin was observed to significantly induce PON transactivation in the cell culture model [39].

In the present study, the difference in serum levels of PON was statistically significant between the groups (P<0.05). The highest PON value was observed in the Betanin group. The lowest PON value was noted in the MSG group. These results show that Betanin increases the activity of PON, which is an antioxidant. On the contrary, MSG with toxic and oxidant properties reduces the activity of PON.

The present study revealed the toxic effects of MSG once again. This is the first study on elderly rats with MSG–induced toxicity. The comparison of the MSG groups with the control group showed significantly higher TOC, OSI, THIOL, and MDA levels but significantly lower TAC and PON levels in the MSG group. The histological comparison of the MSG groups with the control group revealed that the liver tissue of the control group was normal, while the MSG group had hydropic degeneration and mononuclear cell infiltration in hepatocytes. On the other hand, the Betanin groups had a normal histological structure of the liver.

CONCLUSION AND IMPLICATIONS

MGS is widely consumed as a food additive in the World. The results of this dissertation study once again revealed the negative health effects of MSG. Therefore, more attention should be paid to the consumption of foods containing this food additive. Foods containing low amounts of MSG or not containing MSG should be consumed. The results of the present dissertation study support the antioxidant property of Betanin. These results indicate that Betanin reduces the oxidant effects of MSG. Accordingly, it is believed that adding red beetroot to diets will have positive effects. The elderly are known as a vulnerable group in society. The results of this study on elderly rats once again demonstrated the sensitivity of this group. The elderly need to be more careful when consuming foods containing MSG. MSG–free foods should be chosen. Betanin can be preferred as a nutrient enhancer in the food industry.

There is a need for further studies to determine the antioxidant effects of the long–term use of Betanin at different doses on the aging process.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

BIBLIOGRAPHICS REFERENCES

[1] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. 2005; [Internet]. 38(12):1103–1111. doi: https://doi.org/dzjwc5

[2] Shrivastava A, Mishra SP, Pradhan S, Choudhary S, Singla S, Zahra K, Aggarwal LM. An assessment of serum oxidative stress and antioxidant parameters in patients undergoing treatment for cervical cancer. Free Radic. Biol. Med. [Internet]. 2021; 167:29–35. doi: https://doi.org/mmwt

[3] Giorgio M, Trinei M, Migliaccio E, Pelicci PG. Hydrogen peroxide: a metabolic by–product or a common mediator of ageing signals? Nat. Rev. Mol. Cell Biol. [Internet]. 2007; 8:722–728. doi: https://doi.org/c7cctz

[4] Niijima A, Togiyama T, Adachi A. Cephalic–phase insulin release induced by taste stimulus of monosodium glutamate (umami taste). Physiol. Behav. [Internet]. 1990; 48(6):905–908. doi: https://doi.org/db2g4v

[5] Hermawati E, Sari DCR, Partadiredja G. The effects of black garlic ethanol extract on the spatial memory and estimated total number of pyramidal cells of the hippocampus of monosodium glutamate-exposed adolescent male Wistar rats. Anat. Sci. Intern. [Internet]. 2015; 90:275–286. doi: https://doi.org/f7pwn6

[6] Anbarkeh FR, Baradaran R, Ghandy N, Jalali M, Nikravesh MR, Soukhtanloo M. Effects of monosodium glutamate on apoptosis of germ cells in testicular tissue of adult rat: An experimental study. Intern. J. Reprod. Biomed. [Internet]. 2019; 17(4):261–70. https://doi.org/10.18502/4

[7] Nnadozie JO, Chijioke UO, Okafor OC, Olusina DB, Oli AN, Nwonu PC, Mbagwu HO, Chijioke CP. Chronic toxicity of low dose monosodium glutamate in albino Wistar rats. BMC Res. Notes. [Internet]. 2019; 12(1):593. doi: https://doi.org/gmdw4t

[8] Sharma A. Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: A mini-review. J. Biomed. Sci. [Internet]. 2015; 22(1):1–6. doi: https://doi.org/gk47qv

[9] El-Meghawry EL-Kenawy A, Osman HEH, Daghestani MH. The effect of vitamin C administration on monosodium glutamate induced liver injury. An experimental study. Exp. Toxicol. Pathol. [Internet]. 2013; 65(5):513–521. doi: https://doi.org/mmwv

[10] Hazzaa SM, Abdelaziz SAM, Eldaim MAA, Abdel-Daim MM, Elgarawany GE. Neuroprotective potential of allium sativum against monosodium glutamate-induced excitotoxicity: Impact on short-term memory, gliosis, and oxidative stress. Nutrients. [Internet]. 2020; 12(4):1–17. doi: https://doi.org/mmww

[11] El Gamal AA, Alsaid MS, Raish M, Al-Sohaibani M, Al-Massarani SM, Ahmad A, Hefnawy M, Al-Yahya M, Basoudan OA,Rafatullah S. Beetroot (*Beta vulgaris* L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model. Mediators Inflamm. [Internet]. 2014; 2014(983952):1–12. doi: https://doi.org/f6pc2x

[12] Krajka-Kuźniak V, Szaefer H, Ignatowicz E, Adamska T, Baer-Dubowska W. Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats. Food Chem. Toxicol. [Internet]. 2012; 50(6):2027–2033. doi: https://doi.org/f3zrgx

[13] Raish M, Ahmad A, Ansari MA, Alkharfy KM, Ahad A, Khan A, Ali N, Ganaie Ma, Hamidaddin MAA. Beetroot juice alleviates isoproterenol-induced myocardial damage by reducing oxidative stress, inflammation, and apoptosis in rats. Biotech. [Internet]. 2019; 9(4):1–11. doi: https://doi.org/mmwx

[14] Ramirez-Velasquez IM, Velez E, Bedoya-Calle A, Caro-Lopera FJ. Mechanism of Antioxidant Activity of Betanin, Betanidin and Respective C15-Epimers via Shape Theory, Molecular Dynamics, Density Functional Theory and Infrared Spectroscopy. Molecules. [Internet]. 2022; 27(6):2003. doi: https://doi.org/mmwz

[15] Strech D, Dirnagl U. 3Rs missing: Animal research without scientific value is unethical. BMJ Open Sci. [Internet]. 2019; 3(1):1–4. doi: https://doi.org/mmw2

[16] Kayode AA, Kayode OT, Oridota OJ. Alterations in the biochemical indices in Wistar rats exposed to an overdose of codeine and dextromethorphan. J Taibah Univ. Med. Sci. [Internet]. 2021; 16(2):198–208. doi: https://doi.org/gmdvs9

[17] Mauriz E. Clinical applications of visual plasmonic colorimetric sensing. Sensors (Switzerland). [Internet]. 2020; 20(21):1–31. doi: https://doi.org/mmw3

[18] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. [Internet]. 2004; 37(4):277–85. doi: https://doi.org/frb5dh

[19] Placer Z, Veselková A, Rath R. Kinetik des Malondialdehydes im Organismus. Experientia. [Internet]. 1965; 21(1):19–20. doi: https://doi.org/chzjqv

[20] Navarro-Gonzálvez JA, García-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin. Chem. [Internet]. 1998; 44(3):679–681. doi: https://doi.org/mmw4

[21] Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Ame. J. Hum. Genet. 1983; 35(6):1126–1138. Cited in: PubMed; PMID 6316781.

[22] Guvenc M, Cellat M, Gokcek İ, Ozkan H, Arkali G, Yakan A, Özsoy SY, Aksakal M. Nobiletin attenuates acetaminophen-induced hepatorenal toxicity in rats. J. Biochem. Mol. Toxicol. [Internet]. 2020; 34:e22427. doi: https://doi.org/mmw5

[23] Ozkan Hu, Kutlu T, Yakın A, Ozsoy SY. Molecular, biochemical and histopathological effects of long term low and high percentage fructose consumption on liver in rats. Ankara Üniv. Vet. Fak. Derg. [Internet]. 2021; 69(4):1–24. doi: https://doi.org/mmw6

[24] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging Europe PMC Funders Group. Cell. [Internet]. 2013; 153(6):1194–1217. doi: https://doi.org/f42f5g

[25] Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin. Biochem. [Internet]. 2014; 47(18):326–332. doi: https://doi.org/f6r6dk

[26] Erenler AK, Kocabaş R, Doɨan T, Erdemli HK, Yetim M. Paraoxanase as an indicator of myocardial ischemia and its utility in determining extension of ischemia. Ame. J. Emerg. Med. [Internet]. 2016; 34(1):45–48. doi: https://doi.org/f73bhb

[27] Förstermann U, Boissel J, Kleinert H. Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). FASEB J. [Internet]. 1998; 12(10):773–790. doi: https://doi.org/mmw7

[28] Nazifi S, Razavi SM, Kianiamin P, Rakhshandehroo E. Evaluation of erythrocyte antioxidant mechanisms: Antioxidant enzymes, lipid peroxidation, and serum trace elements associated with progressive anemia in ovine malignant theileriosis. Parasitol. Res. [Internet]. 2011; 109(2):275–281. doi: https://doi.org/b899nh

[29] Paul MVS, Abhilash M, Varghese M V., Alex M, Nair HR. Protective effects of α-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. Toxicol. Mech. Methods. [Internet]. 2012; 22(8):625–630. doi: https://doi.org/mmw8

[30] Onaolapo AY, Ayeni OJ, Ogundeji MO, Ajao A, Owolabi AR, Onaolapo OJ. Subchronic ketamine alters behaviour, metabolic indices and brain morphology in adolescent rats: Involvement of oxidative stress, glutamate toxicity and caspase-3-mediated apoptosis. J. Chem. Neuroanat. [Internet]. 2019; 96:22–33. doi: https://doi.org/mmw9

[31] Onaolapo A. A Histological Study of the Hepatic and Renal Effects of Subchronic Low Dose Oral Monosodium Glutamate in Swiss Albino Mice. Br. J. Med .Med. Res. [Internet]. 2013; 3(2):294–306. doi: https://doi.org/mmxb

[32] Da Silva DVT, Pereira AD, Boaventura GT, Ribeiro RSDA, Verícimo MA, De Carvalho-Pinto CE, Baião DDS, Aguila EMD, Paschoalin VMF. Short-term betanin intake reduces oxidative stress in wistar rats. Nutrients. [Internet]. 2019; 11(9):1–16. doi: https://doi.org/mmxc

[33] Han J, Zhang Z, Yang S, Wang J, Yang X, Tan D. Betanin attenuates paraquat-induced liver toxicity through a mitochondrial pathway. Food Chem. Toxicol. [Internet]. 2014; 70:100–106. doi: https://doi.org/f6b64r

[34] Smith GS, Walter GL, Walker RM. Clinical Pathology in Non-Clinical Toxicology Testing. In: Haschek WM, Colin G. Rousseaux, Wallig MA, editors. Haschek and Rousseaux's Handbook of Toxicologic Pathology. 3rd ed. Cambridge (MA): Academic Press; 2013. p. 565–594.

[35] Tawfik MS, Al-Badr N. Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E. Food Nutr. Sci. [Internet]. 2012; 3(5):651–659. doi: https://doi.org/mmxj

[36] Helal AM, Abdel-Latif MS, Abomughaid MM, Ghareeb DA, El-Sayed MM. Potential therapeutic effects of Ulva lactuca water fraction on monosodium glutamate–induced testicular and prostatic tissue damage in rats. Environ. Sci. Pollut. Res. [Internet]. 2021; 28(23):29629–29642. doi: https://doi.org/mmxk

[37] Elbassuoni EA, Ragy MM, Ahmed SM. Evidence of the protective effect of L-arginine and vitamin D against monosodium glutamate-induced liver and kidney dysfunction in rats. Biomed. Pharmacother. [Internet]. 2018; 108:799–808. doi: https://doi.org/mmxm

[38] Hagar H, Al Malki W, Al W. Betaine supplementation protects against renal injury induced by cadmium intoxication in rats: Role of oxidative stress and caspase-3. Environ. Toxicol. Pharmacol. [Internet]. 2014; 37(2):803–811. doi: https://doi.org/f523rk

[39] Esatbeyoglu T, Wagner AE, Motafakkerazad R, Nakajima Y, Matsugo S, Rimbach G. Free radical scavenging and antioxidant activity of betanin: Electron spin resonance spectroscopy studies and studies in cultured cells. Food Chem. Toxicol. [Internet]. 2014; 73:119–126. doi: https://doi.org/f6q4pk