IVERMECTIN PROTECTS AGAINST MONOSODIUM GLUTAMATE-INDUCED EXCITOTOXICITY IN RATS

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ABSTRACT

Monosodium glutamate (MSG), an established excitotoxic food additive, has been found to induce oxidative stress in all tissues. To examine the protective effects of ivermectin on MSG-induced excitotoxicity, twenty-eight male albino rats were randomized into group 1, the control, which received 1 ml oral administration of distilled water; group 2, aqueous solution of MSG (4 mg/kg body weight/day); group 3, co-administered with the same dose of MSG and 0.4 mg/kg body weight of ivermectin; group 4, orally administered with the same dose of MSG for 2 weeks after which ivermectin was orally administered for 1 week. Oral administration of MSG for 21 days, and for only 14 days followed by oral administration of ivermectin for 7 days, significantly increased ($p<0.05$) glutathione-S-transferase, nitric oxide synthase, superoxide dismutase and catalase activities as well as malondialdehyde and intracellular Ca^{2+} concentrations while the activities of Na^{+}-K^{+}-ATPase, Ca^{2+}- Mg^{2+}-ATPase, acid phosphatase and alkaline phosphatase were significantly reduced ($p< 0.05$) when compared with the control. However, co-administration of MSG and ivermectin for 21 days showed no significant difference ($p>0.05$) in all the parameters investigated when compared with the control. This result suggests that ivermectin may exert protection against MSG-induced excitotoxicity in rats.

KEYWORDS: Monosodium glutamate, ivermectin, excitotoxicity, oxidative stress

1 INTRODUCTION

It is known that under normal physiological conditions, an efficient Ca^{2+}-buffered system occurs following neurotransmitter-induced opening of glutamate, N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels, and voltage-dependent Ca^{2+} channels (VDCC) which maintain Ca^{2+} intracellular concentration in response to the release of glutamate [1]. Many chemicals have been found to possess the ability to disrupt this buffering system, resulting in a rise of postsynaptic calcium levels, thereby producing toxic effects on neurons and other excitable tissues [2]. Excitotoxicity is the pathological process by which nerve cells are damaged and killed through excessive stimulation by neurotransmitters, such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors), such as the NMDA and AMPA receptors, are over-activated. One of these chemicals is monosodium glutamate (MSG), a widely used food additives as flavor enhancer. It is found in significant amounts in processed foods, and has a chemical makeup similar to the brain's glutamate neurotransmitters. This amino acid can act on multiple receptor types. The receptors are divided into two main groups: ionotropic glutamate receptors (iGluR) and metatropic glutamate receptors (mGluR) [3, 4]. MSG is an established neuro- and excitotoxin [5, 6]. Administration of MSG to neonatal rats during the first week of life induces a neurodegenerative process, which is presented by several neurochemical alterations of surviving neurons in the brain [6]. Although the mechanism of action of MSG as excitotoxin is not well explained yet, Bojanić *et al*. [7] hypothesized that a high concentration of MSG could alter permeability of the neural membrane for calcium. Many compounds which are antagonists of the glutamate type receptors, such as diltiazem and some macrolide antibiotics, have demonstrated protective effects against MSG toxicity when co-administered to rats [7].

Ivermectin (22,23-dihydroavermectin B1a), a member of the avermectin class of macrocyclic lactones (macrolides) isolated from *Streptomyces avermitilis*, is a potent antihelminthic used to treat onchocerciasis (river blindness) [8] and *Lymphatic filariasis* (elephantiasis) [9] in humans. It is a white to yellowish-white powder with a melting point of about 156 °C, freely soluble in methanol with each tablet containing additionally cellulose, pregelatinized starch, magnesium, hydroxyanisole, and citric acid powder. Being a macrocyclic lactone, ivermectin produces a flaccid paralysis of the somatic worm musculature and inhibits feeding of the parasite through blockage of pharyngeal pumping [10, 11] suggesting that a

disruption of ingestion is the primary action of this compound [12]. The half maximal effective concentration (EC_{50}) for the biological activity of ivermectin is strongly correlated with its ability to activate glutamate-sensitive channels and their binding affinity for nematode membrane preparations [13]. Ivermectin has been reported to induce the release of Ca^{+} from the sarcoplasmic reticulum (SR) [14]. This may be due, in part, to the activation of the glutamate-sensitive channels via ryanodine receptor (RyR) Ca^{+} channels in excitable tissues. An interesting observation is the ability of macrolide antibiotics to exert protective effects against glutamate excitotoxicity [15]. Manev *et al*. [15] suggested that this protection is structure-dependent with larger ringed macrolides being more effective. However, there is paucity of information regarding the potentiality of compounds which are not antibacterial but possess the macrocyclic ring structure to exhibit such a protective property. For this purpose, we evaluated the protective potential of ivermectin against monosodium glutamate-induced excitotoxicity in rats.

2 MATERIALS AND METHODS

2.1 Chemicals

AMP-buffered sodium thymolphthalein monophosphate substrate used for ALP was a product of Teco Diagnostics, Lakeview Avenue, Anaheim, Canada. Ivermectin and monosodium glutamate were products of Merck and Co. England, and Ajinomoto, Chicago, respectively. All other reagents used were of analytical grade, obtained from Merck and Co. England, and were prepared in all-glass distilled water. The reagents were stored in clean, airtight reagent bottles.

2.2 Experimental animals

A total of 28 female albino rats (*Rattus novergicus*) of average weight 169.32 g $\pm$5.22 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad-libitum*. The studies adhered to the Principles of Laboratory Animal Care [16].

2.3 Experimental design

The rats were divided into four groups with each group consisting of seven rats. Group 1 served as the control and received 1 ml oral administration of distilled water. Group 2 was orally administered with aqueous solution of MSG (4 mg/kg body weight/day). Group 3 received co-administration of 4 mg/kg body weight of MSG and 0.4 mg/kg body weight of ivermectin (IVER) while rats in Group 4 were orally administered with 4 mg/kg body weight of MSG for 2 weeks after which ivermectin (0.4 mg/kg body weight) was orally administered for 1 week. The treatments lasted for 21 days, and the rats were sacrificed by decapitation, 24 h after the last administration. The brains were collected and perfused in normal saline after which tissue homogenates were prepared.

2.4 Biochemical analysis

Acid phosphatase (ACP) activity was assayed using the method of Armstrong [17]. The method of Bassey *et al*. [18] as modified by Wright and Plummer [19] was employed while assaying for alkaline phosphatase (ALP) activity. ATPase activities were assayed using the method of Ronner *et al.* [20] as modified by Bewaji *et al.* [21], and catalase (CAT) by the method of Sinha [22]. The method described by Varshney and Kale [23] was used to assay for malondialdehyde (MDA) concentration. Glutathione-S-transferase (GST), nitric oxide synthase (NiOS) and superoxide dismutase (SOD) activities were assayed by the methods of Habig *et al.* [24], Bredt *et al*. [25] and Oyanagui [26], respectively. Reduced glutathione (GSH) and calcium ion concentrations were determined by the methods of Moron *et al*. [27] and Chu *et al*. [28], respectively.

2.5 Statistical analysis

Comparisons were made using Duncan's multiple range test [29], and values were considered to be significant at $p<0.05$.

3 RESULTS

Co-administration of MSG and ivermectin for 21 days to rats showed no significant difference ($p>0.05$) in the concentration of brain GSH and MDA, and in the activity of GST, when compared with the control. However, administration of MSG alone for 21 days, and its administration for 14 days followed by administration of ivermectin for 7 days, showed significant decrease ($p<0.05$) in the levels of these parameters when compared with the controls (Table 1).

A similar pattern of results as in Table 1 was observed on the effect of ivermectin and MSG on ALP and ACP activities of the brain tissue (Table 4).

There was a significant increase ($p< 0.05$) in brain calcium ion concentration of rats administered MSG alone, as well as those administered MSG and then ivermectin, when compared with the control but groups co-administered MSG and ivermectin showed no significant difference ($p> 0.05$) to the control. Additionally, the activities of Na^{+}- K^{+} and Ca^{2+}-Mg^{2+} ATPases in the brain tissue of the rats in group 2 (MSG alone for 21 days) and group 4 (MSG for 14 days and ivermectin for 7 days) were significantly increased while co-administration of both chemical substances showed no significant change ($p>0.05$) (Table 2).

The brain activity of superoxide dismutase (SOD), nitric oxide synthase (NiOS) and catalase of rats in groups 2 and 4 increased significantly ($p< 0.05$) while rats co-administered with MSG and ivermectin (group 3) showed no significant difference ($p>0.05$) when compared to the control as represented in Table 3.

TABLE 1 - Effect of administration of monosodium glutamate (MSG) and ivermectin (IVER) on reduced glutathione (GSH), glutathione S-transferase (GST) activity, and malondialdehyde formation in rat brain.

Groups	GSH μg g^{-1} protein	GST nmol min^{-1} mg^{-1} protein	MDA nmol mg^{-1} protein
Control	1.70 ± 0.11^{a}	72.51 ± 1.86^{a}	0.61 ± 0.04^{a}
MSG	0.55 ± 0.03^{b}	111.94 ± 7.50^{b}	9.57 ± 0.55^{b}
MSG+IVER	1.91 ± 0.12^{a}	74.37 ± 4.30^{a}	0.74 ± 0.03^{a}
IVER after MSG	0.49 ± 0.02^{b}	114.37 ± 9.82^{b}	10.00 ± 0.90^{b}

Values are means (n=7) ± SEM. Values with different superscripts in each column are significantly different.

TABLE 2 - Effect of administration of monosodium glutamate (MSG) and ivermectin (IVER) on Ca^{2+} level and activities of Na^{+}-K^{+} and Ca^{2+}-Mg^{2+} ATPases in rat brain.

Groups	Ca^{2+} mg 100 ml^{-1}g wet $weight^{-1}$	Na^{+}-K^{+} ATPase μmol mg^{-1} protein hr^{-1}	Ca^{2+}-Mg^{2+} ATPase μmol mg^{-1} protein hr^{-1}
Control	8.12 ± 0.71^{a}	0.47 ± 0.02^{a}	1.21 ± 0.11^{a}
MSG	19.57 ± 1.10^{b}	0.16 ± 0.01^{b}	0.97 ± 0.07^{b}
MSG+IVER	7.07 ± 0.41^{a}	0.51 ± 0.03^{a}	1.19 ± 0.10^{a}
IVER after MSG	17.97 ± 1.52^{b}	0.18 ± 0.01^{b}	0.99 ± 0.07^{b}

Values are means (n=7) ± SEM. Values with different superscripts in each column are significantly different.

TABLE 3 - Effect of administration of monosodium glutamate (MSG) and ivermectin (IVER) on the activities of nitric oxide synthase (NiOS), superoxide dismutase (SOD) and catalase (CAT) in rat brain.

Groups	NiOS *Unit mg^{-1} protein	SOD μmol min^{-1} mg^{-1} protein	CAT μmol min^{-1} mg^{-1} protein
Control	94.11 ± 7.40^{a}	1001.32 ± 61.42^{a}	0.35 ± 0.01^{a}
MSG	122.48 ± 10.11^{b}	1189.24 ± 65.91^{b}	2.33 ± 0.10^{b}
MSG+IVER	90.41 ± 6.50^{a}	998.17 ± 21.66^{a}	0.29 ± 0.02^{a}
IVER after MSG	121.12 ± 11.14^{b}	1178.15 ± 71.70^{b}	2.21 ± 0.19^{b}

Values are means (n=7) ± SEM. Values with different superscripts in each column are significantly different.
* One unit of NiOS is defined as the amount of the enzyme that oxidizes 1 mol of $NADPH^{+}$ per hour at 37 °C.

TABLE 4 - Effect of administration of monosodium glutamate (MSG) and ivermectin (IVER) on the activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) in rat brain.

Groups	ACP μmol mg^{-1} protein min^{-1}	ALP μmol mg^{-1} protein min^{-1}
Control	14.45 ± 1.04^{a}	89.30 ± 6.51^{a}
MSG	6.18 ± 0.51^{b}	22.73 ± 2.11^{b}
MSG+IVER	15.16 ± 1.31^{a}	91.17 ± 7.21^{a}
IVER after MSG	7.01 ± 0.43^{b}	23.11 ± 2.01^{b}

Values are means (n=7) ± SEM. Values with different superscripts in each column are significantly different.

4 DISCUSSION

It has been demonstrated that administration of high concentrations of monosodium glutamate (MSG) induces oxidative stress in different organs [30]. Oxidative stress is a characteristic feature in a number of neurodegenerative disorders, such as stroke, Parkinson's disease and Alzheimer's disease [31]. The brain is particularly vulnerable to oxidative stress injury because of its high rate of oxidative metabolic activity [32], high content of polyunsaturated fatty acids, relatively low antioxidant capacity, the abundance of redox-active transition metal ions [33], and non-replicating nature of its neuronal cells [34]. Oxidative stress results from an imbalance between the concentration of free radicals (reactive oxygen species) and the antioxidant system [35]. In order to scavenge reactive oxygen species (ROS), different defense systems exist in the brain, such as enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (glutathione) ones [32]. Another possible action of MSG is through activation of the glutamate receptor channels which induce the flux of Ca^{2+} into the cytosol from the stored vesicles and/or outside the cell. Elevated Ca^{2+} concentration can activate various Ca^{2+}-dependent degradative enzymes (e.g., phospholipases, proteases, endonucleases), which may contribute to cell death [36]. This mechanism can also occur through oxidative stress which may result in impairment of Ca^{2+} transport and subsequent perturbation of intracellular Ca^{2+} homeostasis, thus resulting in a sustained increase of the cytosolic Ca^{2+} level.

In addition to the brain anti-oxidants, the blood-brain barrier is an important neuro-protective device regulating the movement of substances into the brain [37]. P-glycoprotein is an important component of blood-brain barrier (BBB). Many macrocyclic lactones interact differently with *p*-glycoprotein and have different *p*-glycoprotein efflux potential. The efflux activity of mammalian multidrug resistance protein 1 (mdr1) appears to be critically important for preventing central nervous system toxicity by ivermectin and other avermectins [38]. Despite the wide range of biological effects, the cellular and molecular mechanisms of action for macrocyclic lactone ring-containing compounds on neuro-protective effects is unknown but it is possible that they are partly mediated by altered Ca^{2+} transport across the endoplasmic reticulum, similar to those effects reported in the sarcosplasmic reticulum [14]. Another possibility is that interaction of ivecmetin with *p*-glycoproteins may impair the transport function of the BBB [34], thereby preventing the movement of toxins across the barrier.

The significant increase in GST activity and drop in GSH concentration of the rats administered MSG alone for 21 days, and also rats that later received oral administration of ivermectin, is an indication that free radicals-induced oxidative stress is, in part, the mode of action of excitotoxic effects of MSG. Reduced glutathione is a substrate for conjugation reaction, an important step in detoxification process with its thiol group being a reducing agent, and offers protection against ROS [40]. GSH can diminish oxidative stress either through protection of the detoxifying enzymes by increasing the efficacy of NADPH, or by helping in the elimination of compounds which produce peroxidation in the cell membranes [41]. This could be one of the reasons for the decreased level of brain GSH in the present study. The decrease in GSH concentration recorded herein is in accordance with the report of Neveen and Iman [32]. However, the effect of co-adminitration of MSG and ivermectin on the rats is possibly due to interaction of ivermectin with the BBB *p*-glycoproteins, thus preventing the increased accumulation of MSG in the brain. Glutamate does not easily pass the blood brain barrier but, instead, is transported by a high-affinity transport system [42]. Since, it has been proposed that ivermectin may be a *p*-glycoprotein substrate [43]. Therefore, since *p*-glycoprotein may have higher affinity for ivermectin, it may continue to mediate the efflux of ivermectin, thus preventing MSG influx and subsequent accumulation in the brain. This is supported by the non-significant change in all the parameters studied for this group of rats, indicating the absence of oxidative stress or excitotoxicity due to MSG.

Glutathione-S-transferases (GST) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional properties [44]. An increase in the level of any of these enzyme substrates increases its activities [45]. Thus, the increase in GST activity in the group administered MSG alone, and that administered ivermectin after 14-days administration of MSG, may be attributed to the substrate effect of MSG or ivermectin on the high-affinity transport system [42].

Peroxidation of lipids yields malondialdehyde (MDA) as a major aldehyde product. It is an autocatalytic mechanism leading to oxidative destruction of cellular membranes [46]. The brain is particularly prone to oxidative injury because the membrane lipids are essentially rich in polyunsaturated fatty acids [47], resulting in increased peroxidation. An increase in MDA in groups 2 and 4 is an indication of increased peroxidation of lipids in the brain, and, consequently, of increased ROS levels. This change was not observed in group 3 rats, possibly due to the prevention of MSG influx as a result of ivermectin-*p*-glycoprotein interaction.

The effects of oral administration of MSG and ivermectin on calcium transport were evaluated by assaying for intracellular Ca^{2+} level, and Na^{+}-K^{+} and Ca^{2+}-Mg^{2+} ATPase activities. The significant increase in Ca^{2+} level observed in both MSG- treated and ivermectin-after MSG-treated groups can be attributed to two reasons; glutamate induction of calcium release from the endoplasmic reticulum inside the cell, and oxidative stress-induced impairment of Ca^{2+} transport and subsequent perturbation of intracellular Ca^{2+} homeostasis, resulting in a sustained increase in cytosolic Ca^{2+} concentration. The latter is supported by the significant decrease in Na^{+} - K^{+} and Ca^{2+} - Mg^{2+} ATPase, reducing the active efflux of Ca^{2+} ions from the cell. This corroborates the report that MSG causes damage to mitochondria from excessively high intracellular Ca^{2+} [48].

Nitric oxide synthase (NiOS) catalyses the formation of nitric oxide (NO) from arginine. NO is an important cellular signaling molecule, believed to function as a retrograde neurotransmitter. It is produced by NiOS as a defence mechanism. The induction of high-output of NiOS usually occurs in an oxidative environment, and thus, high levels of NO have the opportunity to react with superoxide leading to peroxynitrite formation and cell toxicity [49]. The non-significant change observed in the NiOS activity of rats in group 3 indicated the non-oxidative state of the brain tissue. This is further justified by non-significant change in SOD and catalase activity of the rats. The prevention of influx of MSG by ivermectin-*p*-glycoprotein interaction may also be attributed to this observation. The increased activities of SOD and CAT enzymes in rats administered MSG, and those that received ivermectin for 7 days after MSG, in the present study, was in accord with the reports of Farombi and Onyema [50].

The significant reduction in the brain ACP and ALP of both MSG- and ivermectin after MSG- treated groups is suggestive of possible lysosomal membrane and plasma membrane damage, respectively. This may be a consequence of leakage in the membranes resulting into loss of theses enzymes into the surrounding fluids. The significant increase in MDA level observed in this study maybe

an indication of alteration of membrane integrity. Administration of MSG to neonatal rats during the first week of life has been reported to induce a neurodegenerative process, which is presented by several neurochemical alterations of surviving neurons in the brain [6]. Bojanić *et al*. [7] also hypothesized that high concentration of MSG could alter permeability of neural membrane, and cause possible derangement. The effect of co-administration of MSG and ivermectin on ACP and ALP activities is in line with earlier observations that ivermectin in the presence of MSG may have caused reduced influx of MSG into the brain cells through the *p*-glycoprotein of the BBB.

4 CONCLUSION

The present study suggests that ivermectin may exert an important role in protection against neuro- and excitotoxin when co-administered. This may occur possibly through the interaction of ivermectin with the *p*-glycoprotein of the blood brain barrier, thus causing reduced influx of MSG into the brain. However, this study gives ways for further investigations on the effects of ivermectin on brain cell glutamate channels, and the Ca^{2+} pump under conditions of excitotocity induced by MSG.

The authors have declared no conflict of interest.

REFERENCES

[1] Rao VR, Finkbeiner S (2007) NMDA and AMPA receptors: old channels, new tricks. Trends Neurosci. 30(6) 284-91

[2] Jaiswal MK, Zech WD, Goos M, Leutbecher C, Ferri A, Zippelius A, Carrì MT, Nau R, Keller BU (2009) Impairment of mitochondrial calcium handling in a mtSOD1 cell culture model of motoneuron disease. BMC Neurosci 10: 64.

[3] Hinoi E, Takarada T, Ueshima T, Tsuchihashi Y, Yoneda Y (2004) Glutamate signaling in peripheral tissues. Eur. J. Biochem. 271: 1-13.

[4] Ganor, Y and Levite, M. 2012. Glutamate in the immune system: glutamate receptors. In: Nerve-Driven Immunity. Mia Levite (ed) Springer-Verlag/Wien; New York. pp121-124.

[5] Jászai J, Farkas L M, Gallatz K, Palkovits M (1998) Effects of glutamate-induced excitotoxicity on calretinin-expressing neuron populations in the area postrema of the rat. Cell and Tissue Research 293(2): 227 - 233

[6] Ureña-Guerrero ME, Orozco-Suárez S, López-Pérez SJ, Flores-Soto ME, Beas-Zárate C (2009) Excitotoxic neonatal damage induced by monosodium glutamate reduces several GABAergic markers in the cerebral cortex and hippocampus in adulthood. Int. J. Dev. Neurosci. 27: 845-855.

[7] Bojanić V, Bojanić Z, Najman S, Savić T, Jakovljević V, Najman S, Jancić S (2009) Diltiazem prevention of toxic effects of monosodium glutamate on ovaries in rats. Gen Physiol Biophys. 28 Spec No: 149-54.

[8] Campbell, WC (1989) Ivermectin and Abamectin. Springer-Verlag, New York.

[9] Ottesen, EA and Ramachandran, CP (1995) Lymphatic filariasis in tropical countries. Parasitol. Today 11: 129 – 131.

[10] Kotze, AC (1998) Effects of macrocyclic lactones on ingestion in susceptible and resistant *Haemonchus contritus* larvae. J. Parasitol. 84:E631-635.

[11] Martin, RJ, Gardon, J and Stapely, EO (1996) An electrophysiological preparation of pharyngeal muscle reveals a glutamate-gated chloride sensitive to the avermectin analogue milbemycin D. Parasitol. 120:587-594.

[12] Sangster, NC, Sangster, CM and Leill, J (1999) Pharmacology of anthelmintic resistance. Parasitol. Today 15:141-146.

[13] Arena, JP, Liu, KK, Paress, PS, Frazier, EG, Cully, DF, Mrozik, H and Schaeffer, JM (1995) The mechanism of action of avermectins in Caenorhabditis elegans: correlation between activation of glutamate-sensitive chloride current, membrane binding, and biological activity. Journal of Parasitology. 81: 286—294.

[14] Ahern GP, Junankar PR, Pace SM, Curtis S, Mould JA and Dulhunty AF (1999) Effects of ivermectin and midecamycin on ryanodine receptors and the Ca^{2+}-ATPase in sarcoplasmic reticulum of rabbit and rat skeletal muscle. Journal of Physiology. 514.2: pp. 313—326

[15] Manev, H, Favaron, M, Candeo, P, Fadda, E, Lipartiti, M and Milani, D (1993) Macrolide antibiotics protect neurons in culture against the N-methyl-D_aspartate (NMDA) receptor-mediated toxicity of glutamate. Brain Research 624, 331—335.

[16] Derenlanko, M.J. 2000. Guiding Principles in the use of Animals in Toxicology In: Toxicologist's Pocket Handbook. CRC Press Boca Raton, London pp. 1-7.

[17] Armstrong, K (1964) Enzymes: In: Text of Clnical Chemistry. Tietz, NW (ed.) WB Saunders Company. pp 704-757.

[18] Bassey, OA., Lowry, OA and Brock, MD (1946) Appearance of specific acid phosphatase isoenzymes in the synovial fluid of patients with rheumatoid arthritis. Nature 216 : 274 – 277.

[19] Wright, PJ and Plummer, DT (1974) The use of urinary enzyme measurement to detect renal damage caused by nephrotoxic compounds. Biochem. Pharmcol.12:65. Enzymologia 42: 317-327.

[20] Ronner, P, Gazzotti, P and Carafoli, E (1977) A lipid requirement for the Ca^{2+}, Mg^{2+}- ATPase of erthrocyte membranes. Arch. Biochem. Biophys. 179: 578-583.

[21] Bewaji, CO, Olorunsogo, OO and Bababunmi, EA (1985) Comparison of the membrane bound (Ca^{2+} + Mg^{2+}) ATPase in erythrocyte ghosts from some mammalian species. Comp. Biochem. Physiol. 82B: 117-122.

[22] Sinha, KA (1971) Colorimetric assay of catalase Anal. Biochem. 47: 389-394.

[23] Varshney, R and Kale, RK (1990) Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsome. Int. J. Rad. Biol. 58: 733-743.

[24] Habig, WH, Pabst, MJ and Jacoby, WB (1977) Glutathione-S- transferase. The first enzymic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

[25] Bredt, DS, Hwang, PM and Snyder, SH (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347, 768-770.

[26] Oyanagui Y (1999) Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal Biochem. 142(2) 290-6.

[27] Moron MA, Depierre JW, Mannervick B (1979) Levels of glutathione, glutathione reductase and glutathions-S-transferase activities in rat lung and liver. Biochem Biophys Acta. 582:67–78.

[28] Chu, A, Dixon, MC, Saito, A, Seiler, S, Fleischer, S (1989) Isolation of sarcoplasmic reticulum fractions referable to longitudinal tubules and junctional terminal cisternae from rabbit skeletal muscle. Methods in Enzymology. 157: 36—50.

[29] Duncan, D.B. (1955) Multiple range and multiple F-tests. Biometrics 11: 1-42.

[30] Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Jevtovic-Stoimenov T, Cekic S, Velickovic D (2007) Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Mol Cell Biochem. 303(1-2) 161-6.

[31] Ji B-S, Ji H, Liu G-Q (2004) Doxepin protects cultured neurons against oxidative stress-induced injury. Acta Pharmacol Sin 25: 297-300.

[32] Neveen, AN, Iman, MM (2010) Evaluation of Antioxidant Effect of *Nigella sativa* oil on Monosodium Glutamate-Induced Oxidative Stress in Rat Brain. Journal of American Science. 6(12) 13 - 20

[33] Butterfield DA, Stadtman ER (1997) Protein oxidation processesin aging brain. Adv. Cell Aging Gerontol. 2: 161–191.

[34] Evans PH (1993) Free radicals in brain metabolism and pathology. Br Med Bull. 49: 577-587

[35] Allen RG (1991) Oxygen-Reactive Species and Antioxidant Responses during Development: The Metabolic Paradox of Cellular Differentiation. Proc Soc Exp Biol Med: 196:117—129

[36] Orrenius S, Burkitt MJ, Kass GEN, Dypbukt JM, Nicotera P (2004) Calcium ions and oxidative cell injury. Annal of Neurology. 32(1) 33 - 42

[37] Rubin, LL and Staddon, JM (1999) The cell biology of the blood-brain barrier. Ann. Rev. Neurosci. 22:11-28.

[38] Sezler P (2009) Antiparasitic and antibacteria drug discorvery: from molecular target to drug candidates. First edt. Willey Blackwell, Germany.

[39] Xu D, Thiele GM, Beckenhauer JL, Klassen LW, Sorrell MF, Tuma DJ (1998) Detection of circulating antibodies to malondialdehyde-acetaldehyde adducts in ethanol-fed rats. Gastroenterology. *115:686-92.*

[40] Pompella, A, Visvikis, A, Paolicchi, A, De Tata, V, Casini, AF (2003) The changing faces of glutathione, a cellular protagonist. Biochemical Pharmacology. **66** (8) 1499–503

[41] Machlin L, Bandich A (1987) Free radical tissue damage: Protective role of antioxidant nutrients. FASEB. J. 1: 441-445.

[42] Smith, QR (2000) Transport of glutamate and other amino acids at the blood-brain barrier. J. Nutr. 130 (4S Suppl) 1016S–1022S.

[43] Redondo, PA, Alvarez, AI, Garcia, JL, Larrode, OM, Merino, G, Prieto, JG (1999). Presystemic metabolism of albendazole: experimental evidence of an efflux process of albendazole sulfoxide to intestinal lumen. Drug. Metab. Dispos. 27: 736- 740.

[44] Yousef, MI (2004) Aluminum-induced changes in hematobiochemical parameters, lipid peroxidation and enzyme activities of male rabbits: Protective role of ascorbic acid. Toxicology. 199: 47-57.

[45] Allocati N, Federici L, Masulli M, Di Ilio C (2009) Glutathione transferases in bacteria. *FEBS J.* 276 (1) 58–75.

[46] Cheeseman, KH (1993) Mechanisms and effects of lipid peroxidation. Mol. Aspects Med. 14: 191-197.

[47] Halliwell, B, Gutteridge, JMC (1989) Free radicals in Biology and Medicine. Mol. Aspects. Med. 8:89-93.

[48] Manev H, Favaron M, Guidotti A, Costa E (1989) Delayed increase of Ca^{2+} influx elicited by glutamate: role in neuronal death. Molecular Pharmacoloy 36(1): 106-112.

[49] Mungrue IN, Husain M, Stewart DJ (2002) The role of NOS in heart failure: lessons from murine genetic models. Heart Fail Rev **7** (4): 407–22.

[50] Farombi EO, Onyema OO (2006) Monosodium glutamate – induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum. Exp. Toxicol., 25: 251-259.

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