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Original Article

The ameliorative role of *Moringa oleifera* on the abnormalities induced by aspartame in rats: I. Monoamine neurotransmitters and oxidative stress levels in the rat brain

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ABSTRACT

This study aimed to investigate the ameliorative effect of *Moringa oleifera* (MO) aqueous leaves extract against the damage induced by the sweetener aspartame (AM) on brain monoamines, oxidative stress and creatine kinase (CK) in adult male albino rats. AM and MO were administered (75 and 500 mg/ Kg body weight) as a daily oral dose, respectively. Rats were divided into five groups; namely, saline control (Sc), AM, Protection (Pr), Improvement (Im) and treatment (Tr). Results showed a diminishing neurotransmitter, total antioxidant capacity levels and catalase and CK activities with a compatibility with increase in the malondilaldehyde level in the AM administered groups. The groups of rats administered MO extract by different routes of administration (Pr, Im or Tr designs) exerted a beneficial effect on all parameters. Whereas, the rats administered AM and MO under the Tr design had the significantly highest values of all the estimated parameters ($P<0.01$) compared with the other experimental groups (Pr and Im). The aqueous leaves extract of MO administration could trigger the damage induced in brain of rats induced by AM.

Key words: aspartame, *Moringaoleifera*, neurotransmitters, brain creatine kinase, brain oxidative stress.

INTRODUCTION

Aspartame (L-aspartyl-Lphenylalaninemethylester) (AM) is a dipeptide artificial sweetener that is widely used as a non-nutritive sweetener in various food products (Rencuzogullari et al., 2004). AM represents 62% of the intense sweetener market in terms of its world consumption (Fry, 1999). Sweeteners have a primary importance in nutritional guidance for diabetes, a disease with increasing incidence in developing as well developing countries (Gougeon et al., 2004). AM is used as a sweetener in food products including dry beverage mixes, chewable multi-vitamins, breakfast cereals, chewing gums, pudding and fillings, carbonated beverages, refrigerated and non-refrigerated ready to drink beverages, yoghurt type products and pharmaceuticals (Rencuzogullari et al., 2004). Upon ingestion of AM, it is immediately absorbed from the intestinal lumen and metabolized to phenylalanine (Ph), aspartic acid and methanol. Thus the concentrations of its metabolites are increased in the blood (Abhilash et al., 2011). Ph may cross the blood-brain barrier (BBB) and cause severe changes in the production of very important neurotransmitters. Methanol breaks down into formate, which in turn is very cytotoxic and can even cause blindness. That the excessive AM ingestion is involved in the pathogenesis of certain mental disorders and also in comprised learning and emotional functioning (Humphries et al., 2008). *Moringa oleifera* (MO) belongs to the Moringaceae family, which has 14 species classified in single gene, namely *Moringa adams* (Gupta et al., 2005). MO is a fast growing tree, originally found in sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine (Anwar et al., 2007). However, it is at the moment distributed all over the world (Bakre et al., 2013).

A large number of reports on the nutritional aspects of this tree now exist in both scientific as well as popular literature. MO leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than orange and more potassium than bananas (Bennett et al., 2003) and protein quality of MO leaves rivals that of milk and eggs (Debnath et al., 2011). In addition, the leaves of this plant have been reported to be a rich source of anti-nutrients such as alkaloids, tannins, phenolics, saponins and steroids (Bambishaiye et

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al., 2011). Despite the above mentioned pharmacological beneficial profile of MO leaves, its efficacy in relation to the cellular effects of aspartame on the brain in particular, has not been studied earlier. The present study was planned to determine the effect of aqueous MO leaves extract on the alterations in brain monoamines and oxidative stress induced by the administration of daily oral dose of AM to rats.

MATERIALS AND METHODS

Chemicals:

- AM was purchased from Ajinnomoto sweeteners Europe.
- Dopamine (DO), serotonin (SE) and noradrenaline (NO) assay Eliza kits were purchased from Wkea Med supplies, India.
- Alanine aminotransferase (ALT), Asparate aminotransferase (AST) and creatine kinase (CK) assay enzymatic kits were purchased from Spinreact Co., Spain.
- All the other chemicals were of analytical grade obtained from Gamma Trade Co., Cairo, Egypt.

Plant material and extraction: The MO dried leaves were obtained from the local market. They were crushed into a course powder using a laboratory electric blender. The powder was soaked in distilled water at room temperature for 24 h. It was then filtered using sieve cloth, then filter paper and the filtrate was used for the oral daily administration of rats (Aja et al., 2013).

Experimental animals and design: Adult male albino rats "Sprague-Dawely strain" weighing 140-180 g were obtained from Research of Bilharzias Institute. Academic of Scientific Research and Technology.Cairo, Egypt. Rats were acclimatized to laboratory conditions for 3 days, maintained at constant 24 C with 12 h light-dark cycle and fed with balanced rodent pellet diet and water *ad libitum*. After acclimatization, the rats were randomized into five experimental groups (n = 12); namely, saline control (Sc), AM, protection (Pr), improvement (Im) and treatment (Tr) groups. AM mixed in sterile saline was orally daily administered (75 mg/ kg body weight) (Ashok et al., 2013) for 2 weeks. The MO aqueous extract was orally daily administered (500 mg/ kg body weight) (Aja et al., 2013) for 2 weeks.

Sc group: Rats were administered orally daily saline for 2 weeks.

AM group: Rats were administered orally daily AM dose for 2 weeks.

Pr group: Rats were administered orally daily MO dose for 2 weeks, and then AM orally daily dose for another 2 consecutive weeks.

Im group: Rats were administered orally daily AM dose for 2 weeks, then MO orally daily dose for another 2 consecutive weeks.

Tr group: Rats were administered orally daily MO and AM doses for 2 weeks.

Sample collection: At the end of the experimental duration, rats were killed for blood and brain samples collection. Blood was centrifuged (10 min, 3000 rpm, 4ºC) for serum samples. The brain was immediately removed and washed with ice-cold phosphate buffered saline and the cerebellum region was dissected according to the method given by Glowinski and Iverson (1996). The homogenate (10% w/v) was prepared using ice-cold TrisHcl (pH 7.4) buffer and centrifuged in refrigerated centrifuge at 3000 rpm for 15 min (Ashok et al., 2013). The supernatant was used for analyzing the parameters in this study.

Biochemical determinations: The values of neurotransmitters; DO, SE and NO were determined in serum samples according to the methods of (Kim et al., 2008; Trofs et al., 2012 and Westermann et al., 2002), respectively. CK activity was determined in the brain homogenate according to the method of Okinaka et al. (1964). The level of lipid peroxidation in brain homogenate was expressed as nanomoles malondialdehyde (MDA) as described in the method of Draper and Hadley (1990). The total antioxidant capacity (TAC) content of the brain homogenate was estimated according to the method of koracevic et al. (2001). The brain catalase (CAT) activity was estimated by the method of Aebi (1984).

Statistical analysis: The data were statistically analyzed by SPSS version 9.0 statistical packages. Data were presented as a mean ± S.D.; statistical differences between groups were performed using student's t-test. Differences considered significant when $P < 0.01$.

RESULTS

The serum monoamine neurotransmitters (DA, SE and NO) levels of adult male albino rats administered either MO and/or AM by different routes were shown in table (1). The rats which were administered AM had the lowest significant neurotransmitter values ($P<0.01$) compared with all of the groups. Whereas the highest significant values ($P<0.01$) were recorded in the serum of rats employed for the treatment design. Serum DA values were significantly elevated ($P<0.01$) in the rats of the Pr and Tr designs when compared with those on the Sc or Am groups. However, there was no significant difference between the values of rats employed for the Sc and Im groups. The serotonin

of rats administered AM and MO either by Pr, Im or Tr designs had a significantly higher values (P<0.01) than the rats of the Sc group. Whereas there was no significant difference (P<0.01) between the serotonin values of rats in both of the Sc and AM groups. There was a highly significant difference (P<0.01) among all the values of serum noradrenaline of all the treated groups either by AM or MO and with those of the Sc group.

Table (2) showed that the significantly highest (P<0.01) Ck activity was recorded in the cerebellum region of brain of the Sc group of rats. There was no significant difference (P<0.01) between the cerebellum Ck activities of the rats administered AM and those administered MO of the Pr design. The cerebellum oxidative stress markers of the male albino rats were reported in table (3). There was a highly significant difference (P<0.01) between MDA content of cerebellum of all groups of rats administered either AM or different designs of MO compared with that of the Sc group of rats. The highest significant value (P<0.01) was recorded in the cerebellum of rats administered the AM, whereas the lowest value (P<0.01) was recorded in the cerebellum of the Sc group of rats. The Sc group of rats had the highest significant (P<0.01) cerebellum TAC content and CAT activity, however the rats administered AM had the lowest significant (P<0.01) values. There were a highly significant difference (P<0.01) among all the cerebellum activities of CAT of rats in different experimental designs. There was no significant difference (P<0.01) between the TAC content of the cerebellum of both groups of rats administered MO either in the Pr or Im designs.

DISCUSSIONS

This study documented that the AM administration inhibits significantly the synthesis of the monoamine neurotransmitters (DO, SE and NO) in adult male albino rats. The explanation may be related to the high content of Ph released as a breakdown of AM in the alimentary tract then cross the brain and alter the neurotransmitters. This result was correlated to Abdel-Salam et al.(2012) who concluded that the administration of AM (22.5 and 45 mg/Kg) inhibited brain DO, SE and NO. Also the intraperitoneal injection of doses of 200-800 mg/ kg body weight decreased both of DO and SE of rats (Goerss et al., 2000). The aqueous extract of MO was investigated on novelty in recover and overcome the inhibitory effect of AM administration on the monoamine neurotransmitters (DO, SE and NO) of adult male albino rats. The results revealed that, the greatest effect of MO administration was recorded when it was administered parallel to AM (Tr group), which was superior than the saline control group (Sc). This result may related to the high quality protein content in the MO leaves ,as described previously by Debnath et al. (2011),which can antagonist the effect of excess Ph released from the ingestion of AM.

It has previously been reported that MO leaf possesses nootropic activity and hence can enhance memory (Mohan et al., 2005) probably by altering brain monoamines levels and electrical activity (Ganguly and Guha, 2008). Also, the methanol extract from the leaves has been shown to provide protection against convulsion induced by maximum electric shock seizure test and pentylenetetrazole-induced seizures (Amrutia et al., 2011). Several lines of evidence also suggest that colchicine-induced Alzheimer`s disease can be ameliorated by ethanolic extract of MO by modifying the brain monoamines and electrical activity in a rat model (Obulesu and Rao, 2011). Also, an oral ethanolic extract dose of 250-2000 mg/ kg body weight enhanced learning memory and increased anxiogenic effect. While the administration of 2000 mg/ kg body weight protect mice against convulsion induced by pentylenetetrazol (Bakre et al., 2013). In addition, the aqueous MO leaf extract protect ulcer formation by modulating serotonin secretion in gastrointestinal tract (Debnath et al., 2011).

Ph not only plays a role in amino acid metabolism and protein structuring in all tissues, but is also a precursor for tyrosine, dopamine, norepinephrine, epinephrine (Ganong, 1997), phenylethylamine (Young, 1988) and phenylacetate (as phenylacetate interferes with brain development and fatty acid metabolism) (Humphries et al., 2008). Phenylalanine also plays an important role in neurotransmitter regulation. Phenylalanine is either converted into tyrosine or cross the BBB via a large neutral amino acid transport (NAAT) as it is. Tyrosine is converted into dihydroxyphenylalanine (DOPA) once it is in the brain, by the enzyme tyrosine hydroxylase, with the help of the co-factors oxygen, iron and tetrahydrobiopterin (THB) (Caballero and Wurtman, 1988).

Dopamine, a catecholamine, is formed from DOPA by an aromatic amino acid decarboxylase. Tyrosine hydroxylase activity is inhibited by high concentrations of dopamine through its influence on the THB co-factor (negative feed- back). This system is very necessary to prevent large amount of dopamine being produced, as dopamine isan inhibitory neurotransmitter. However, if phenylalanine, as the main part of aspartame, competes with tyrosine for NAAT, a compromised dopamine production will result because

phenylalanine will bind more frequently and freely than tyrosine owing to its higher concentration, and thus lead to lower concentrations of dopamine in the brain ***(Humphries et al., 2008)***. The dopaminergic system is active in maintaining normal motor behavior, and loss of dopamine is related to Parkinson's disease, in which the muscles are rigid and movement is difficult (Kolb and Whishaw, 2003).

Serotonin, an indolamine, causes powerful smooth muscle contraction (Ganong, 1997). Physiologically, it is also important for behavior and control of sleep, temperature, appetite and neuroendocrine functions. Tryptophan, independently utilized for synthesis of serotonin in the brain, is transported across the BBB via NAAT. Therefore, if NAAT is occupied with phenylalanine, tryptophan will not be adequately carried across the BBB and serotonin production can ultimately be compromised (Humphries et al., 2008). Furthermore, disruptions of the serotonergic pathways due to excess or inadequate activation of specific 5-HTreceptors during development are implicated in the pathogenesis of developmental disorders such as autism (Gaspar et al., 2003).

Noradrenaline plays an important role in regulating attention during postnatal development, as noradrenergic cells are exquisitely sensitive to environmental stimuli, especially powerful emotional events (Panksepp, 1998). With low noradrenaline activity, individuals tend to perseverate on a task despite changes in stimulus contingencies because of attention deficits. Such individuals are prone to act impulsively rather than deliberately. Depletion of noradrenaline during the perinatal period can also result in subtle dendritic changes and possibly also alterations in cortical differentiation that may lead to behavioral changes (Berger-Sweeney and Hohmann, 1997). It is also known that noradrenaline dampens the background 'noise' or cortical neural activity irrelevant to a given task (Panksepp, 1998). This makes the influence of specific incoming signals more prominent in the cortex, namely the ratio of the signal to background noise is increased. Thus, it is suspected that with high noradrenaline activity, individuals can better process information that already has access to the cortex (Humphries et al., 2008).

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phosphocreatine kinase is an enzyme expressed by various tissues and cell types. CK catalyzes the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP). This CK enzyme reaction is reversible, such that also can generate ATP. Creatine kinase isoenzymes play an important role in the maintenance of ATP level in the CNS tissue (Wallimann et al., 1998). Active site of CK isoenzymes contains an essential cysteine residue and tyrosine residues, which could be the targets for oxidative modifications (Koufen and Stark, 2000). Therefore, CK is likely to be one of the primary targets for ROS, which is over-produced during AM intake and thus justify the decrease in its activity. Lipid peroxidation and protein oxidation can impair the function of numerous cellular components including creatine kinase. However, the decrease in CK might alter the brain energy metabolism could not be over looked and must considered as serious issue (Westermann et al., 2002).

In this study AM administration decreased the activity of CK in the cerebellum homogenate, however the MO aqueous leaves extract ameliorate the activity when administered parallel to AM in a greater extent than after AM administration. The decrease in CK activity was correlated to the increase in peroxidation level in cerebellum (MDA) and a decrease of both of TAC content and CAT activity. These disturbances were all enhanced by the administration of MO at different extents by different designs (Pr, Im and Tr). That could be due to the abundant active components (Bennett et al., 2003; Bamishaiye et al., 2011 and Debnath et al., 2011) found in MO leaves, which could alleviate peroxidation.

Lipid peroxidation (LPO) is a free-radical-mediated process. In the entire rat brain regions after aspartame consumption, a marked in- crease in LPO was noted, which also supports the generation of free radicals. Generally, when the generation of reactive free radicals overwhelms the antioxidant defense, there is a possible loss of membrane integrity. Moreover, reactive-oxygen-mediated damage could not be overlooked particularly to proteins, which has been shown to deleteriously affect cellular functions (Sohal et al., 2002).

The alteration in the free-radical-scavenging enzymes in the aspartame-administered animals clearly indicates that free radical generation may be due to the methanol which is one of the metabolic products of aspartame. Even after chronic aspartame administration, there was detectableblood methanol in the aspartame-treated animals. Methanol is metabolized by three enzyme systems, namely, the alcohol dehydrogenase system, the catalase peroxidative pathway and the microsomal oxidizing systems. Among these, the microsomal oxidizing system is reported to be responsible for free radical generation (Iyyaswamy and Rathinasamy, 2012). Exposure to methanol

causes oxidative stress by altering the oxidant/antioxidant balance in lymphoid organs of rat (Parthasarathy et al., 2006). In addition to that, oxidative stress may lead to the generation of superoxide, peroxyl and hydroxyl radicals.The increase in free radicals could not beignored as cells can be injured or killed when the ROS generation overwhelms the cellular antioxidant capacity. Particularly, the brain is more vulnerable to oxidative damage due its high oxygen consumption and due to the presence of high levels of polyunsaturated fatty acids (Ashok et al., 2014).
The major groups of phytochemicals contributing to the total antioxidant capacity of plant foods include polyphenols, carotenoids and antioxidant vitamins such as vitamins C and E. Leaves of MO have been reported to have sufficient amount of these antioxidants and are also used as green leafy vegetables of high total antioxidant capacity (260 mg/100 g). Leaves have also been found to be rich in flavonols such as total polyphenols (250 mg/100 g), quercetin (100 mg/100 g) kaempferol (34 mg/100 g) and β-carotene (34 mg/100 g) (Siddhurgiu and Becker, 2003 and Jaiswal et al., 2013). Dried powder of drumstick leaves is a rich source of Vitamin A, phenolics, glutathione, α-tocopherol and β-carotene. Its leaves extract has been reported to exhibit good antioxidant activity in the linoleic acid peroxidation system. The antioxidant activity of MO in vitro and in vivo, against hydroxyl radicals generated by Fenton reaction was documented (Rao et al., 2001 and Kumar and Pari, 2003).
This study demonstrated the enhancing effect of MO leaves extract might occur partly via the decreased oxidative stress and the enhanced neurotransmitters system. However, other mechanisms concerning the vasodilation effect (Dangi et al., 2002) which in turn increased regional blood flow and the suppression of monoamine oxidase (MAO) which gave rise to the enhanced dopaminergic function (Prabsattroo et al., 2012) induced by MO leaves extract might also play the pivotal role in the cognitive enhancing effect of MO leaves extract. However, a previous finding concluded that the neuronal dysfunctions and neurodegeneration could be improved by flavonoids (Youdim and Joseph, 2001 and Sutalangka et al., 2013).

CONCLUSION

It could be concluded that the neuro-protective and enhancing effects of the rats administered the aqueous MO leaves extract against the damage induced by the administration of AM might occur partly via the flavonoids and the high protein quality in the extract. However, these ameliorative effects differ according to the different routes of AM and MO administration. The superior effects in neurotransmitter system were recorded for rats administered AM and MO together, that their results exceed those of the saline control group. This finding requires further investigation by using different doses of MO extracts lower than that used in this study.

Table I. Serum monoamine neurotransmitter values of adult male albino rats administered different experimental doses of AM and /or MO

Group	Dopamine (ng/ L)	Serotonin (ng/ L)	Noradrenaline (ng/ L)
Sc	57.98 ± 0.849	2113.78 ± 3.29	2066.57 ± 8.75
AM	53.40 ± 0.489	2102.45 ± 11.47	1651.29 ± 8.64
Pr	60.61 ± 0.698	2390.72 ± 5.14	1987.42 ± 7.31
Im	57.10 ±1.10	2374.75 ± 4.29	1697.63 ± 7.70
Tr	66.62 ± 1.19	2654.13 ± 9.87	2408.16 ± 9.48

Values are the mean ± S.D., n = 12.

Table II. The cerebellum creatine kinase activity of adult male albino rats administered different experimental doses of AM and /or MO

Group	Creatine kinase (IU/ g)
Sc	16.28 ± 0.690
AM	11.51 ± 0.435
Pr	10.99 ± 0.602
Im	13.01 ± 0.422
Tr	15.01 ± 0.369

Values are the mean ± S.D., n = 12.

Table III. The cerebellum oxidative stress markers of adult male albino rats administered different experimental doses of AM and /or MO

Group	Malondialdehyde (nmol / g)	Total antioxidant capacity (mM / g)	Catalase (U /g)
Sc	91.14 ± 0.674	1.89 ± 0.119	35.11 ± 0.935
AM	190.58 ± 0.982	0.764 ± 7.37X10^{-2}	19.12 ± 1.08
Pr	131.65 ± 0.937	1.02 ± 0.111	27.96 ± 0.663
Im	166.72 ± 0.988	0.981 ± 7.58X10^{-2}	22.79 ± 0.531
Tr	119.91 ± 0.837	1.45 ± 0.139	31.21 ± 0.497

Values are the mean ± S.D., n = 12.

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