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CHEMOPREVENTIVE EFFECTS OF ZINGIBEROFFICINALEEXTRACT AGAINST ASPARTAME INDUCED NEPHROTOXICITY AND OXIDATIVE STRESS IN RAT MODEL

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ABSTRACT

Ginger (Zingiberofficinale) contains active phenolic compounds that have antioxidant, anti-cancer, anti-inflammatory and antithrombotic properties. The aim of the present study was to evaluate the chemo protective effect of Z. officinale roots against aspartame inducedrat nephrotoxicity. The chosen dose of aspartame was adjusted to 0.2ml/ 25g b.wt. and was given three times per week for 12 weeks. Ginger aqueous extract were adjusted to 500mg/kg and were given three times per week for 12 weeks. Aspartame caused a marked rise in serum urea, creatinine and potassium level which improved by ginger extract pretreatment. Sodium level was decline in aspartame treated group that ameliorated through ginger extract pretreatment. Concerning oxidative stress and antioxidant defense system, ginger extract pretreatment successfully improved renal reduced glutathione (GSH) level, glutathione peroxidation (GPx) and superoxide dismutase (SOD) activities compared to aspartame treated group which give inverse results. However, while elevated lipid peroxidation (MDA) was noticed in aspartame treated rats, pretreatment with ginger extract produced a detectable decrease in lipid peroxidation level. The natural plant components may have chemo preventive effect against aspartame that induce nephrotoxicity and oxidative stress. However, further clinical studies are required to assess the safety and benefits of ginger root in human beings.

KEYWORDS: Aspartame, Ginger extract, Nephrototoxicity, Rattusnorvegicus, Antioxidant, Lipidperoxidation

INTRODUCTION

Aspartame is widely consumed by humans who are diabetic and who are under weight loss regime. Aspartame (L-aspartyl-L-phenylalanine methyl ester) also known as NutraSweet, after oral administration to humans and experimental animals, aspartame is rapidly and completely metabolized to 50% phenylalanine, 40% aspartic acid and 10% methanol (Oppermann, 1984). Methanol is being increasingly recognized as a substance that

damages the liver cells where it is oxidized to formaldehyde and later to formate (Oppermann, 1984). These processes are accompanied by elevation of NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation (Parthasarathy et al., 2006). Also, methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals (Castro et al., 2002). And these factors together with the excess of formaldehyde formed during acute methanol intoxication cause significant increase in lipid peroxidation (Parthasarathy et al., 2006). The kidney is one of the organs responsible for the maintenance of constant extracellular environment through the excretion of such purinecatabolite as urea, creatinine, blood urea nitrogen (BUN) and uric acid as well as electrolyte balance. Abnormal concentration of this catabolite and some electrolyte in plasma or serum is a clear indication of renal function impairment (Zanna et al., 2008). The kidney function may be assessed from the level of some electrolyte (such as Na+, and K+,) and metabolite (such as creatinine, urea, uric acid and BUN) in the serum (Atangwho et al., 2007 and Uboh et al., 2009). Soffritti et al. (2006) showed that rats treated with long-term doses of aspartame (equivalent to human aspartame intakes of 0, 4, 20, 100, 500, 2500 and 5000 mg/kg) stated that aspartame is a "multipotential carcinogenic agent" based on increases in malignant tumor-bearing animals. Relatively small amount of aspartame can significantly increase methanol levels (Davoli, 1986). Ashok et al. (2013) evaluated the long-term effect of aspartame (75 mg/kg) on liver and brain antioxidant status with histopathological changes in liver and renal cortex in wistar albino rats. They also concluded that long-term aspartame may be responsible foroxidative stress and the hepato-renal toxicity.

Ginger (Zingiberofficinale Roscoe, Zingiberacae) is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Awang, 1992; Wang and Wang, 2005; Tapsell et al., 2006). Grzanna et al. (2005) was on the use of ginger as an anti-inflammatory agent, while that of Shukla and Singh (2007) dealt with the cancer prevention properties of the crude drug. Today many botanicals natural products are used in therapy of different diseases (Ogungbe and Lawal, 2008). Among the pharmacological effects demonstrated are anti-platelets, antioxidant, anti-tumor, anti-rhino viral, anti-hepatotoxicity, anti-arthritic effect and anti-inflammatory (Sharma et al., 1994; Kamtchoving et al., 2002;

Lantz et al., 2007). The antioxidant activity of gingerol and other constituents of ginger have been confirmed (Aeschbach et al., 1994). Yeh et al. (2014) used two different types of ginger extract (aqueous and ethanolic extracts) and they found that ginger aqueous extracts were more effective in free radical scavenging activities and chelating abilities. Also, the two ginger rhizomes exerted protective effects and could be used as a flavouring agent and a natural antioxidant. Molecular oxygen, while providing efficient energy from ingested food, can generate free radicals and peroxide by-products, which have high intrinsic toxicity. A number of physiological processes in living organisms produce reactive nitrogen species (RNS)and reactive oxygen species (ROS) as by-products. Oxidative stress occurs in a cell or tissue when the concentration of ROS generated exceeds the antioxidant capability of the cell. Oxidative stress induced organ damage has been observed as the dose limiting factor of most of the cancer chemotherapeutic agents when used at higher concentration. Ajith et al. (2008) evaluated the nephroprotective activity of aqueous ethanol extract of Z. officinale and they found that the nephroprotection is mediated by preventing the doxorubicin-induced decline of renal antioxidant status, and also by increasing the activity of glutathione- S- transferase.

Curcumin, an another active component present in ginger, was found to be an antioxidant and anti-inflammatory agent and induced haem oxygenase-1 and protected endothelial cells against oxidative stress (Motterlini et al., 2000). Overall, ginger components might be effective in antioxidant, anti-inflammatory and antimicrobial activities (Dugasani et al., 2009;Jolad et al.,2005; Park, 2008).The antioxidants inhibit the reactive oxygen species (ROS), which are capable of causing damage to DNA, associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Patel et al., 2000). Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems (Sies, 1997). Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O2-, H2O2, and OH-) generated exceeds the antioxidant capability of the cell (Sies, 1997). The status of lipid peroxidation as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress (EFSA , 2006).

Therefore, the present study was designed to determine the chemo preventive role of Z. officinale roots on the nephrotoxicity and oxidative stress induced by aspartame treated male rat.

Materials and Methods

Experimental animals

Adult male albino rats (Rattusnorvegicus) weighing 120-150 g were used in the present study. The animals were obtained from the animal house in the Ophthalmology Research Center, Giza, Egypt. They were kept under observation for two weeks before the onset of the experiment to exclude any inter current infection. The animals were kept at room temperature and exposed to natural daily light-dark cycles. Rats were fed ad libitum and clean water was continuously available. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals stated by the Canadian Council on Animal Care (CCAC, 1993).

Plant

Aspartame was purchased from Amriya pharmaceutical industries, Alexandria, Egypt. All other chemicals used for the investigation were of analytical grade. ZingiberofficinaleL. (Ginger, Family Zingiberaceae) dried roots were obtained from local market of Herbs and Medicinal plants. Authentication of the plant was carried out by staff members of Botany Department, Faculty of Science, Bani-Suef University, Bani- Suef, Egypt.).

Preparation of plant extracts

The watery extract was prepared by soaking 100 g of the dry roots in 500 ml hot distilled water at 40–50 _C with daily shaking for 5 days and kept in a refrigerator. The infusions were filtered by a piece of double layer gauze and filtrate was centrifuged at 3000 rpm for 10 min then water was evaporated in hot air oven at50 _C. Known grams of extract were suspended in distilled water.

Doses and treatment

The dose of aspartame used in this study was 1000 mg/kg body weight. This dose was previously reported to induce an increase in the frequency of cell damage in mammalian systems (Abhilash et al., 2011). The animals were treated with aspartame orally. The chosen dose of aspartame was adjusted to 0.2ml/ 25g b.wt. in distilled water prior to use and was given three times per week for 12 weeks. Ginger aqueous extract were adjusted to 500mg/kg and were given three times per week for 12 weeks (Shati and El said , 2009).

Experimental design

Animals were divided into three groups comprising six animals each:

1- Group 1 (normal control) is orally given the equivalent volume of the vehicle 1 (distilled water) daily for 12 weeks.

2- Group 2 (positive"+ve"control) is given aspartame orally at dose level of 1000mg/kg b.wt (Abhilash et al., 2011) three times per week for 12 weeks.

3. Group 3 (treated with ginger aqueous extract & aspartame) is given ginger extract orally at dose level of 500mg/kg b. w (Shati and El said, 2009) three times per week together with aspartame (orally) 1000mg/kg b.wt three times per week for 12 weeks

Blood and organ sampling

Under diethyl ether anesthesia, 5 ml of blood sample was collected from jugular vein of each animal in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m. at 30ºC for 15 minutes and kept frozen at -30ºC for various physiological and biochemical studies.

kidneys were washed in saline and blotted on filter paper and kept on ice throughout the whole procedure. Renal tissue of 0.5g was homogenized in 5ml 0.9% NaCl (10% w/v) using a teflon homogenizer (Glas-Col, Terre Haute, USA).

Biochemical analyses

Serum urea concentration was determined in serum according to the Urease- modified Berthelot reaction (Patton& Crouch, 1977), using the reagent kits purchased from Diamond Diagnostics, Egypt. Serum creatinine was determined by colorimetric procedure using kits from Biodiagnostic according to Bartles et al. (1972). Serum sodium was determined by colorimetric procedure using kits from Biodiagnostic according to the methods of Trinder (1951). Serum potassium was determined by turbidimetric method using kits obtained from Biodiagnostic according to the method of Sunderman and Sunderman (1958). Kidney glutathione (GSH) was determined according to the method of Beulter et al. (1963). Kidney lipid peroxidation (MDA) was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Preuss et al. (1998). Glutathione peroxidase (GPx) activity in kidney was assayed according to the chemical method of Matkovics et al.

(1998). Renal superoxide dismutase (SOD) activity was determined according to the chemical method of Marklund and Marklund (1974).

Statistical analysis:

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, 1985)followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). F-probability, obtained from one-way ANOVA, expresses the effect between groups.

Results

Biochemical Changes

Changes in different serum variables related to the kidney are presented in tables1and 2. Concerning serum parameters related to the kidney function, the aspartame- administered rats exhibited significant increase ($p<0.01$; LSD) in urea, creatinine and potassium levels(table 1).The pre-treatment of these animals with ginger extract produced a potential decrease ($p<0.01$; LSD) in urea, creatinine and potassium levels. Inversely, the aspartame-administered rats caused a marked decrease ($p<0.01$; LSD) in sodium level while animals with ginger extract pretreatment improve sodium level cause towering of it ($p<0.01$; LSD) as compared to control group(table 1).

Kidney reduced glutathione level (GSH), glutathione peroxidase (Px) and superoxide dismutase (SOD) activities recorded a significant decrease ($p<0.01$; LSD) in aspartame treated group. Ginger extract pretreatment induced a significant increase in reduced glutathione level (GSH), glutathione peroxidase (Px)and superoxide dismutase (SOD) activities far above normal level(table 2). The percentage changes were 52.83% 24.94%, and 26.63%respectively compared to aspartame treated group (-49.09%, -36.59% & -28.68%).In contrast, renal lipid peroxidation was increased as a result of aspartame administration, while ginger extract group recorded a significant decrease compared to control group(table 2). One-way ANOVA revealed that the effect on the variables of oxidative stress and antioxidant system was of $p<0.001$ between groups.

Table 1: Effect of *Zingiberofficinale* on serum urea, creatinine, sodium (Na^+) and potassium (K^+)levels in aspartame treated rats.

Parameters Group	Urea (mg / dl)	% change	Creatinine (mg /dl)	% change	Na+ (mmol/L)	% change	K+ (mmol/L)	% change
G1 Normal	44.8 ± 1.06[b]	-	0.532 ± 0.036[b]	-	148.02 ± 2.16[a]	-	6.27 ± 0.177[b]	-
G2 Aspartame	55.43 ± 2.43[a]	23.73	0.687 ± 0.015[a]	29.14	129.49 ± 3.71[b]	-12.52	8.47 ± 0.550[a]	35.09
G3 Aspartame + ginger extract	33.66 ± 1.69[c]	-39.27	0.547 ± 0.006[b]	-20.38	151.32 ± 1.98[a]	16.86	6.51 ± 0.723[b]	-23.14
F-Probability	P<0.0001	-	P>0.0016	-	P>0.0006	-	P>0.0080	-
LDS at 5% level	5.79	-	0.073	-	8.72	-	1.31	-
LDS at 1% level	8.32	-	0.104	-	12.53		1.88	-

- Data are expressed as mean ± standard error.
- Number of animals in each group is six.
- Mean, which have the same superscript symbol(s), are not significantly different.
- Percentage changes (%) were calculated by comparing normal group with aspartame treated group and pre-treated aspartame groups with aspartame treated group.

Table 2: Effect of *Zingiberofficinale* on kidney glutathione (GSH) level, glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities and lipid peroxidation (MDA) level in aspartame treated rats.

Parameters Group	GSH (nmol/100mg tissue)	% change	GPx (U/g)	% change	SOD (U/g)	% change	MDA (nmol/100mg Tissue/hr)	% change
G1 Normal	61.76 ± 3.03 [a]	-	68.66 ± 3.30[a]	-	2.58 ± 0.201[b]	-	5.31 ± 0.244[c]	-
G2 Aspartame	31.44 ± 2.21[c]	-49.09	43.54 ± 3.79[c]	-36.59	1.84 ± 0.062[a]	-28.68	8.59 ± 0.216[a]	61.77
Aspartame + ginger extract	48.05 ± 2.01[b]	52.83	54.40 ± 0.915[b]	24.94	2.33 ± 0.107[a]	26.63	6.60 ± 0.191[b]	-23.17
F-Probability	P<0.0001	-	P>0.0001	-	P>0.0118	-	P<0.0001	-
LDS at 5% level	7.85	-	9.42	-	0.437	-	0.697	-
LDS at 1% level	11.28	-	13.53	-	0.628	-	1.002	-

- Data are expressed as mean ± standard error.
- Number of animals in each group is six.
- Mean, which have the same superscript symbol(s), are not significantly different.
- Percentage changes (%) were calculated by comparing normal group with aspartame treated group and pre-treated aspartame groups with aspartame treated group.

DISCUSSION

The present study was undertaken to assess the chemo preventive properties of ginger root extract on nephrotoxicity and oxidative stress. Aspartame caused a marked rise in serum urea, creatinine and potassium level which improved by ginger extract pretreatment. Sodium level was lowered in aspartame treated group that ameliorated through ginger extract pretreatment.

These results are in agreement with Parthasarathy et al. (2005) who observed that Methanol-induced free radicals and anim balanced antioxidant system may damage the kidney functionsand probably contribute to the increased serum urea and creatinine. When liver and kidney cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream (Choudhary and Devi, 2014). Their estimation in

the serum is a useful quantitative marker of the extent and type of hepatic and renal cellular damage (Pagana and Pagana, 2002). Disruption of the ordered lipid-bilayer of the membrane structure probably due to the presence of reactive oxygen species produced due to oxidative stress leading to escape of detectable quantity of these enzymes out of the cell into the extracellular fluid. The reactive oxygen species might have oxidized the polyunsaturated fatty acids which make up the lipid bilayer resulting in its disruption. Lower value of serum sodium indicated inability of kidney to conserve sodium. Haemo dilution too may be involved in the fall of sodium value via excess of water intake and or increased production of endogenous water increased in plasma volume and extracellular fluid volume would increase the circulating filling pressure, resulting in an increase in cardiac output and better blood flow to the kidneys. Increase of potassium may be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of methanol metabolite of aspartame induced lesions in renal tubular epithelium. Ashok et al. (2013) concluded that aspartame consumption in a long-term basis may affect the brain and liver, it may be due to its metabolite methanol or aspartame may act as a chemical stressor to alter the antioxidant status and histological pattern. Also, aspartame can induce oxidative stress and hepato-renal toxicity. Ajith et al. (2008) indicated that aqueous ethanol extract of Z. officinale significantly protected doxorubicin DXN-induced nephrotoxicity. The in vitro and in vivo studies using Z. Officinale had reported the significant antioxidant activities (Kikuzaki and Nakatani, 1993; Masuda et al., 2004). The exhibited renal protective activity of Z. officinale might partially be due to its antioxidant property. In the fresh ginger rhizome, the gingerols (polyphenols) were identified as the major active component (Masuda et al., 2004). Presence of volatile oils with many mono and sesquiterpenes and flavonoids were also reported (Kikuzaki et al., 1991). The presence of such active components might be responsible for the exhibited renal protective activity. Recent experimental observations reported that Z. officinale is an effective anticancer agent (Shukla and Singh, 2007). Further, ginger can relieve the chemotherapy associated nausea and vomiting in patients (Ernst and Pittler, 2000). Ginger was one of the components of antrath, a poly herbal preparation which was reported to be effective against DXN-induced cardiotoxicitywithout interfering its antineoplastic activity (Jagetia et al.,2005). Z. officinalesignificantly protected the nephrotoxicity either by enhancing the DXN-induced declined renal antioxidant status or by its direct antioxidant activity. The possible use of Z. officinaleas nutraceutical against oxidative stress induced organ toxicities (Ajith et al., 2008).

Concerning oxidative stress and antioxidant defense system, the present study showed that ginger extract pretreatment successfully improved kidney reduced glutathione (GSH) level, glutathione peroxidation (GPx) and superoxide dismutase (SOD) activities compared to aspartame treated group which give inverse results. However, while elevated lipid peroxidation (MDA) was noticed in aspartame treated rats, pretreatment with ginger extract produced a detectable decrease in lipid peroxidation level.

The current results are in agreement with Choudhary and Devi (2014) who found that the increased level of lipid peroxidation, nitric oxide level is taken as direct evidence for oxidative stress (Matsumoto et al., 1999). The modified enzymatic and non-enzymatic free radical scavenging system with an elevated lipid peroxidation level after aspartame administration clearly indicated the generation of free radicals. This alteration after aspartame administration may be attributed to its metabolite methanol. Methanol is primarily metabolized by oxidation to formaldehyde and then to formate, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide. Lipid peroxidation in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity, which is essential for proper functioning of the cell. This alteration could have been due to the methanol released during aspartame metabolism and the formaldehyde formed during methanol metabolism. Zararsizet al. (2007) recorded a significant increase in lipid peroxidation level in the kidney of rats after treatment with formaldehyde. SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H2O2 and O2-, which are deleterious to polyunsaturated fatty acids and proteins(Murray et al., 2003). Catalase further detoxifies H2O2 into H2O and O2(Murray et al., 2003). Glutathione peroxidase also functions in detoxifying H2O2 similar to CAT. Thus, SOD, CAT and GPx act mutually and constitute the enzymatic anti oxidative defense mechanism against reactive oxygen species (Bhattacharjee and Sil, 2006). The free radical slowly increases due to methanol metabolite of aspartame. To remove the free radical there is increase in both enzymatic and non-enzymatic level initially in order to prevent oxidative cell damage (Vidyasagar et al., 2004).Methanol administration could decrease the enzymatic antioxidant (SOD, CAT and GPx) in the lymphoid organs (Castroet al., 2002). Zararsiz et al. (2007) demonstrated that the renal tissue activity of SOD was significantly decreased in rats treated with form aldehyde. The decline in the activities of these enzymes might be due to their inactivation caused by excess reactive oxygen species production (Pigeoleet al., 1990) normally, the antioxidant enzymes CAT and

GPx protect SOD against inactivation by H2O2. Reciprocally, SOD protects CAT and GPx against superoxide anion. Furthermore, the decrease in SOD and CAT activities may be due to the formation of formaldehyde from the methanol. GSH is an important non-enzymatic antioxidant that plays a critical role in the maintenance of thiol redox potential in cells, keeping sulfhydryl groups of cytosolic proteins in the reduced form, and plays a crucial role in the detoxification of toxic chemicals of endogenous and exogenous origin. Therefore, the depletion of cellular GSH increases cell vulnerability to oxidative stress (Oyama et al. 2002). GSH is a cofactor of formaldehyde dehydrogenase and is responsible for formaldehyde metabolism (Harris et al. 2004). A decrease in glutathione levels reduces formaldehyde metabolism, thereby increasing its toxicity. It has been noticed that a decrease in GSH concentration would also be caused by its rapid reaction with formaldehyde, forming nucleophilic adducts and/or lipid peroxidation products (Sogut et al. 2004). These findings are supported by a recent study by Iyyaswamy and Rathinasamy (2012), where a significant decrease in GSH and protein thiols was noted after aspartame administration. GSH is the electron donor of peroxides in the reaction catalyzed by GPx, a decrease in GSH levels may adversely affect the GPx activity too (Abhilash et al., 2014).

The decrease in GSH activity could be caused by methanol, because methanol metabolism depends upon GSH (Parthasarathyet al., 2006).The decreased GSH levels seems to have been caused by methanol derived from aspartame ingestion because metabolism of methanol depends on GSH (Abhilash et al., 2014).

In conclusion, the aqueous extract of ginger root may cause chemoprotective effect. Therefore, this study recommends that intake of Z. officinale roots as a drink may have chemopreventive effects against aspartame that may cause nephrotoxicity and oxidative stress. However, further clinical studies are required to assess the safety and benefits of ginger root in human beings.

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