

EFFECTS OF ETHANOLIC GINGER EXTRACT ON OXIDATIVE STRESS AND SOME TRACE ELEMENTS IN CADMIUM – INDUCED TOXICITY IN MALE RABBITS

Ahlam A. AL-Rikabi

Ala AL-Deen H. Jawad

College of Veterinary Medicine , University of Basra, Basrah, Iraq.

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ABSTRACT

This study was conducted to evaluate the antioxidant activity of ginger (*zingiber officinale*) in preventing oxidative stress ,lipid peroxidation and changes in trace elements induced by cadmium toxicity in male rabbits .

For this purpose twenty four of adult male rabbits were divided randomly in to four groups (6 in each). Group 1 was given distilled water and considered as control group, group 2 received (10mg/kg B.w) of cadmium chloride , group 3 received (100mg/ kg B.w) of ginger extract plus (10mg/kg B.w) of cadmium chloride , group 4 received (200mg/ kg B.w) of ginger extract plus of cadmium chloride(10mg/kg B.w) . All treatments were orally given a single dose for 35 days. The water and food were provided *add libitum*.

After the end of the treatment blood samples were taken for biochemical analysis to estimate serum Malondialdehyde (MDA) , Antioxidant activity (Glutathione , Superoxide dismutase and Catalase) and Trace elements (Zinc, Copper and Iron) .

The result showed that the administration of cadmium chloride resulted in a high concentration of malondialdehyde (MDA) and caused a significant decrease in both superoxide dismutase and catalase and reduced glutathione. Serum levels of trace elements were also significantly decreased. While , ginger extract administration and cadmium chloride increased and restored their levels to near normal in comparing with cadmium chloride treated rabbits.

INTRODUCTION

Ginger (*zingiberofficinale*) is a spice well known medicinal and as a culinary spice and cell- protective effect in animals and humans body , it exhibits anti-inflammatory and antioxidant activities and other action (1). Ayurvedic and Unani-Tibb medicines, since antiquity, for a wide array of ailments that include arthritis , rheumatism , sore throats, hypertension, vomiting and indigestion (2,3). Ginger increased the activities of antioxidant enzyme may attributed to the ginger rhizome contains vitamins and flavonoids which their antioxidant roles have been thoroughly been proved(4)

Ginger contains polyphenols which administrated a higher chelatoforming capacity with regard to Fe +3 , leading to the prevention of the hydroxyl radicals initiation which are known as inducers for lipid peroxidation(5)

Material and Methods

The preparation of plant material.

The fresh rhizomes of Chinese ginger (*zingiberofficinale*) were bought from the local market in Basra city /Iraq, the plant material was identified and authenticated at College of Science / University of Basra . The fresh rhizomes was washed with distilled water and then dried at room temperature for two days under the shade , the dried rhizome were cut into small pieces and ground into powder by using electric mill for 3 minutes, 50g of powder were put in the round bottal flask, 200ml of ethanol (70%) were added to flask and extracted for 12 hrs at 70c°. the extract was filtered by using whatman No.31 filter paper , then the extract were put in the petri dish and left at room temperature under the shade . the resultant was viscous substance with brown color the collection extracts were kept in tight closed container and stored at 4c° until using.

Experimental Animal.

Twenty four of mature domestic male rabbits (*Lepuscuniculus*) , weight between 1-1.5 kg and six months age were used in this study. The animals were bought from local market in Basra city they were housed in the animal house of the

College of Veterinary Medicine / University of Basra. They were housed in well ventilated standard environment condition at temperature $25 \pm C^{\circ}$ and photoperiod 12 hrs light /dark cycle.They were supplied with standard pellet and water *add libitum* , they were allowed for two weeks for adapting with new environment before experimentation . The animals were given anticoccidiosis (Amprolium) through the drinking water daily for two weeks (1g / L). Finally the rabbits were randomly and equally divided into four groups. The ginger extract and Cadmium chloride dissolved in distilled water and introduced as single daily doses administered orally . The treatment expended for 35days.

The Biochemical Measurements.

1) The serum malondialdehyde determination(MDA):

Measurement of malondialdehyde as one of the main endoprodut of lipid peroxidation will be carried out in serum according to the method of (6). Thiobarbituric acid (TBA) reacts with MDA to form thiobarbituric acid reactive substance (TBARs) . the absorbance of the resultant pink product can be measured at 535nm wave length against reagent blank, which was containing all the reagents minus the serum.

2) The measurement of serum antioxidants:

The serum antioxidant are divided in to enzymatic and non enzymatic antioxidants and measured by the following :

1-The serum glutathione determination:The glutathione content of the serum was determined utilizing the method descrbed by (7). The method based on the reduction of 5,5 dithiobis(2- nitrobenzoic acid – DTNB) with glutathione (GSH) to product a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412nm wave length.

2-The serum superoxide dismutase activity estimation (SOD):

The endogenous SOD activity was determined by using a modified photochemical nitroblueteterzolium (NBT) and utilizing sodium cyanide as

peroxidase inhibitor (8) . the rate of antioxidant was estimated by recording the increase in the absorption at 560 nm wave length.

3- The serum catalase activity estimation (CAT):

The activity of CAT in serum was measured by method (9) . based on the estimated of amount of hydrogen peroxide decreased. The read absorbance at 240 nm wave length.

4- Analysis of trace elements:

The content of zinc , copper and iron in serum sample were first released from protein matrix by wet digestion method (10), and their concentrations were determined using atomic absorption / Flame- Emission spectrophotometer at wave length of 214nm for zinc, 247nm fro iron and 324nm for copper (11).standard solutions elements were used to prepare calibration curve for quantitative analysis.

The Statistical analysis

The results of the present study were analyzed by using variance (ANOVA) test. The statistical analysis was performed by using the program. The data were expressed as a means \pm SD. *P* values less than 0.05 ($P < 0.05$) were considered to be significant for all data of this study.

RESULTS

Malondialdehyde concentration (MDA) in table (1) results indicate a significant increase ($P < 0.05$) in the animals treated with cadmium chloride as compared with control group. While the groups treated with ethanolic ginger exteact showed a significant reduction ($P < 0.05$) in serum MDA concentration compared with group that treated with Cadmium chloride.

Table (1)Serum MDA level control group and groups treated with cadmium chloride and ethanolic ginger extract.

Parameter	Control D.W	CdCl ₂ 10mg/kg B.W	G.Ex 100 mg/kg plus CdCl ₂ 10mg/kg B.W	G. Ex 200 mg/kg plus CdCl ₂ 10mg/kg B.W
Malon Dialdehyde μ mol /L	0. 52 \pm 0.03 C	3.25 \pm 0.26 A	1.26 \pm 0.02 B	0.55 \pm 0.03 C

The difference in the letters means statistical difference at (P<0.05) level as compared with control group.

Antioxidant activity (GSH,SOD and CAT) in table(2) the result showed that the average of GSH,SOD and CAT significantly decrease(p< 0.05) in group treated with cadmium chloride compared with control group.However, animals received ethanolic ginger extract showed a significant increase (p< 0.05) in average of GSH,SOD and CAT concentrations compared with cadmium chloride group .The elevated in serum GSH,SOD and CAT were more prominent at dose 200mg/kg of ethanolic. ginger extract .

Table (2) Serum GSH,SOD and CAT levels of control group and groups treated with cadmium chloride and ethanolic ginger extract.

Parameters	Control D.W	Cd Cl ₂ 10mg/kg B.W	G.Ex 100 mg/kg plus CdCl ₂ 10mg/kg B.W	G. Ex 200 mg/kg plus CdCl ₂ 10mg/kg B.W
Serum GSH µmol/L	9.500.29 ± A	4.79 ±0.18 C	7.70±0.50 B	9.39±0.32 A
Serum SOD U/ml	8.19±0.10 B	3.63±0.21 D	6.48±0.35 C	8.27±0.01 A
Serum CAT U/L	65.29 ±3.77 A	30.35±0.17 C	52.99 ±1.93 B	66.69 ±1.84 A

The difference in the letters means statistical difference at (P<0.05) level as compared with control group.

Trace elements concentrations (Zn,Cu and Fe) are given in table(3). The results indicate that there is a significant decrease (p< 0.05) in serum Zn,Cu and Fe concentrations in animals treated by cadmium chloride compared with control group. Whereas the average Zn,Cu and Fe concentrations were elevated significantly (p<

0.05) in group treated with ethanolic ginger extract compared with cadmium chloride group.

Table (3) Serum Zn ,Cu and Fe levels of control group and groups treated with cadmium chloride and ethanolic ginger extract.

Parameters	Control D.W	Cd Cl ₂ 10mg/kg B.W	G.Ex 100 mg/kg plus CdCl ₂ 10mg/kg B.W	G. Ex 200 mg/kg plus CdCl ₂ 10mg/kg B.W
Serum Zn ppm	0.90 ±0.02 A	0.40±0.02 C	0.77±0.02 B	0.91±0.02 A
Serum Cu ppm	1±0.01 B	0.43±0.02 D	0.83±0.02 C	1.1±0.01 A
Serum Fe ppm	1.56±0.03 A	0.78±0.02 C	1.42±0.02 B	1.55±0.02 A

The difference in the letters means statistical difference at (P<0.05) level as compared with control group.

DISCUSSION

The results in table (1) showed that there was a significant increase in the level malondialdehyde concentration in rabbits treated with cadmium as compared with normal control ,this may indicate that the administration of cadmium stimulated lipid peroxidation production and deterioration of cell membrane. The results are consistent with other studies(12,13,14,15) these authors emphasized in this studies that cadmium induced oxidative stress and change in the structure and/ or function of cell membranes .

(16) stated that oxidative stress is an important mechanism of cadmium – induced toxicity possibly due to depletion of glutathione (GSH) and changes in the activity of antioxidant enzymes. While (17) concluded that the cadmium – induced

oxidative stress through the cadmium induce inflammation in the tissue include produce inflammatory mediators which in turn stimulates generation of free radicals in the tissue . On the other hand , (18) demonstration that the cadmium – induced reactive oxygen species (ROS) production via iron (Fe) due to the cadmium may displace iron from various cytoplasmic and membrane proteins and consequently , increased concentration of ionic iron stimulates ROS production in tissue . (19) stated that cadmium decreases the activity of antioxidants system elements as well as glutathione content and leads to the production of oxygen reactive forms.

However , Co-treatment of ginger extract with cadmium , there was a significant reduction in the level of malondialdehyde concentration , these results indicate that ginger extract is a potent antioxidant when the correlate with noticed reduction in the malondialdehyde level. The protective effect of ginger extract against cadmium- induced oxidative stress may be attributed to its potent free radical scavenger activity similar results were given by(20).

(21) concluded that ginger- free phenolic and ginger hydrolysed phenolic fractions of ginger exhibited free radical scavenging , inhibited lipid peroxidation , DNA protection and reduced power abilities indicating strong antioxidant properties.

Tables (2) shows that cadmium has significant reduced the average GSH, SOD and CAT levels in rabbits treated with cadmium as compared with normal control. These results indicate that cadmium causes marked alterations of enzymatic (SOD and CAT) and non- enzymatic component of antioxidant (GSH). These results are concordance with (22) who Showed that the effect of cadmium on the antioxidant defense system of the cell is the second mechanism for cadmium – induced oxidative stress which due to cadmium toxicity have been attributed to alteration in the antioxidant activities by inhibiting function SH groups in –enzymes such as SOD, CAT and GPX and non- enzymatic molecule like glutathione which normally protect against free radical toxicity .

(23,24) concluded that the antioxidant enzymes (SOD, CAT and GPS) are potential targets for cadmium toxicity because these enzymes depend on various essential trace elements for proper molecular structure and activity, copper and zinc which are important for the activity of SOD molecule , Fe required for the activity

CAT and selenium is an integral component of GPX. These elements have antagonistic effect with cadmium.

In general cadmium has a very high affinity for glutathione therefore, exposure to cadmium decreases GSH level due to either increased utilization of GSH by the cell to act as scavenger of free radicals caused by toxic chemical agent and formation of metal- GSH complex or enhanced utilization of GSH by GPX under oxidative stress induced by metal or decreased availability of selenium which lead to inefficient disposal of peroxides and results in elevated lipid peroxidation (25,26). Cadmium also inhibits the activity of antioxidant enzymes including catalase, manganese- superoxide dismutase and copper , zinc superoxide dismutase (27).

The exposure to cadmium results in decreased nitric oxide (NO) production in rabbits and inhibition of NO synthesis leads to a marked decreased in the GSH synthesis through down regulation of rate – limiting enzyme (28).

Oral administration of ginger extract with cadmium significantly increased the activities of SOD, CAT and glutathione content when compared to rabbits treated with cadmium alone , this revealed that ginger extract ameliorated cadmium- induced lipid peroxidation by removing ROS once formed , thus preventing radical chain reactions . these results are consistent with the (29) who found that ginger extract significantly lowered lipid peroxidation by maintaining the activity of the antioxidant enzymes, superoxide dismutase, catalase in rats, the enzymes SOD and CAT constitute the first line of defense against free radical induced damage and the maintenance activity of enzymes by ginger may account for their protective effect . The major active phenolic ingredients isolated from zingiberofficinale(zingerone , gingerols , shogaols, gingerdiol and zingibrene) have antioxidant activity (30). The antioxidant activity of natural poly phenols is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, free radical scavenger, singlet oxygen quenchers and metal chelator (31,32).

(33,34) stated that ginger contains a high level of selenium and glutathione antioxidant , thus it can be proved that these ingredients can increase the level of GSH by increased supply of GSH or selenium for the activation of GPX which can capable of reducing peroxides and hydrogen peroxide. On the other hand , studies have shown Co-administration of ginger extract and sodium arsenite produces

significant prophylactic action against sodium arsenite induced oxidative stress by means of lowering lipid peroxidation and increasing SOD , CAT and glutathione content in male rats (35) .

The results in tables (3) showed that serum zinc, copper and iron concentration decreased significantly through cadmium administration alone (10mg/kg B.w) for 35 days compared to normal control . this indicating that the non – enzymatic antioxidant defense mechanism is lowered . these results are reported that showed significant decrease in the concentrations of zinc , copper and iron by cadmium treatment this may be due to interference of cadmium on absorption and transport of these trace elements, which would have resulted in the depletion of these metals in rats (36,37,38). On the other hand in the earlier study , (39) demonstrated that cadmium may inhibit zinc activities at many stages , interfering with absorption, distribution and transport of zinc into cell or into several intracellular structural . also cadmium impairs iron absorption and utilization or both and reduced copper absorption. In supports of this hypothesis the cadmium disrupts zinc homeostasis may have serious consequences on cell growth,development and their function (40). (41) stated that cadmium may have antagonized iron and copper metabolism. Thereby adversely affecting hemoglobin synthesis. However, Co- administration of ginger extract with the cadmium caused a significant increase the level of these trace elements in serum as compared to cadmium intoxicated rabbits . this could be attributed to the interaction and antagonistic action of these trace element (42) who found that ginger root contains trace elements (zinc, copper and iron) and resulted in elevated level of these trace elements this could be attributed to either increased supply of trace elements or which their antioxidant roles have been thoroughly been proved. Another possible explanation for this effect is based on metal chelating ability of poly – phenols and flavonoids which present in ginger extract . this feature plays an important role in antioxidant activity , the poly phenolic compound and flavonoid can chelate metal and may affect its availability for absorption(43)

(44) indicated that adverse effect of heavy metal would be antagonized by administration of antioxidant minerals (zinc , copper and iron).

Other studies have showed that supplementation of zinc, copper and iron offered a protective effects against toxic effects of cadmium (45,14)

تأثير المستخلص الكحولي للزنجبيل على الإجهاد التأكسدي وبعض العناصر النادرة في ذكور الأرانب المعرضة للكاميوم.

احلام علي الركابي علاء الدين حسن جواد

كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

الخلاصة

اجريت هذي الدراسة لتقييم التأثير المضاد للأكسدة للخلاصة الكحولية للزنجبيل وتأثيره على صورة المعادن في ذكور الارانب البالغه مقارنة مع الارانب التي اعطيت كادميوم فقط وحيوانات السيطرة . قسمت الارانب المستخدمة في البحث عشوائيا الى اربعة مجاميع (كل مجموعة تحوي ست حيوانات) . المجموعة الاولى مجموعته السيطرةه والمجموعة الثانية جرعت فموية (10 ملغم / كغم) من كلوريد الكادميوم والمجموعة الثالثة جرعت فموية (100 ملغم / كغم) من خلاصه كحوليه للزنجبيل و (10 ملغم / كغم) من كلوريد الكادميوم والمجموعة الرابعة جرعت فموية (200 ملغم / كغم) من خلاصه كحوليه للزنجبيل و (10 ملغم / كغم) كم كلوريد الكادميوم .

تبين من تحليل النتائج إن هناك فرط الأكسدة وزيادة في تركيز المالونالديهايد وقلة في تركيز مضادات الأكسدة ومستويات العناصر النزرة في الحيوانات المعرضة الى الكادميوم . وقد تبين إن المستخلص الكحولي للزنجبيل تأثيرا مهما ومضاد للإجهاد التأكسدي والذي تبين من انخفاض مستوى المالونالديهايد وزيادة في تركيز مضادات الأكسدة في الدم.

واظهر التحليل الإحصائي للنتائج مايلي: -انخفاض معنوي ($P < 0.05$) في تركيز المالونالديهايد مقترن مع ارتفاع معنوي ($P < 0.05$) في مضادات الأكسدة .

- ارتفاع معنوي ($p < 0.05$) في تركيز العناصر النزرة.

ومن خلال نتائج الذي تم التوصل اليها في هذه الدراسة يمكن الاستنتاج إن استخدام مضادات الأكسدة المتمثلة بمستخلص الكحولي للزنجبيل لها دورا في حماية الانسجة من التأثير التأكسدي للجذور الحرة الذي يسببه الكادميوم في الجسم من خلال خفض نسبة المالونالديهايد وكذلك الالتهاب في الانسجة.

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