BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE
BENHA VETERINARY MEDICAL JOURNAL

ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF GINGER IN RATS

El Shemy, M.A.[a], **Abdalla, A.O.**[b], **and Fararh, K.M.**[a]

[a] *Clinical pathology dept., Faculty of Veterinary Medicine, Benha University,* [b] *Clinical pathology dept., Faculty of Veterinary Medicine, Suez canal University.*

A B S T R A C T

The present study was designed to determine the protective effect of ginger against oxidative damage induced by malathion. Animals were divided into four groups (15 rats per group). Group one was used as a control. Group two was administered 400 mg ginger / kg body weight for 6 weeks. Group three was administered 100 mg malathion/kg body weight. Group four was given ginger for 2 weeks before malathion for 6 weeks. Results revealed that in ginger administered group there was significant increase in the activities of reduced glutathione and glutathione peroxidase in liver homogenate compared to malathion group. Furthermore, there was significant increase in the GSH-PX mRNA expression in liver tissue. Also there was significant increase in the concentration of serum albumin and total protein. On the other hand there was significant decrease in liver MDA, serum ALT and AST. These results revealed that ginger has a strong antioxidant and anti-hepatotoxic effects in rats treated with malathion.

Key words: Anti-hepatotoxic, Antioxidant, Ginger, Malathion, Rat

(BVMJ 22 (2): 9-16; 2011**)**

1. INTRODUCTION

Nowadays free radicals are accepted widely to be responsible in the pathogeneses of many diseases. Also, it interferes with antioxidant scavenging mechanisms [15]. Oxidative stress occurs when the generation of reactive oxygen species in the body exceeds the ability of the body to neutralize and eliminate them. The imbalance can result from lack of antioxidant capacity caused by disturbance in production, by an overload of reactive oxygen species from oxidative stress or by age. It is suggested that age associated decrease in antioxidant body system is the main cause of age related disease [25]. Since free radicals are constantly produced in the body. The body has certain defense mechanisms to stop the harmful effects of free radicals. The highly effective group of protective agents and defense mechanisms which regulate oxidative reactions and prevent damage termed as antioxidants. Under normal circumstances there is a balance between natural antioxidants and pro-oxidants produced in the body [22]. This endogenous enzymatic and non-enzymatic antioxidant system is depleted by excess reactive oxygen species production and by age. Therefore, there is growing need to utilize abundant plant resources available as exogenous source for antioxidant. Ginger is one of the most important exogenous antioxidants that is used for prophylaxis and treatment of various diseases caused by free radicals (8**)**. The oleoresin from rhizomes of ginger contains phenolic substances that have been found to

have anti-inflammatory, analgesic antipyretic, cardiotonic, antioxidant and anti-hepatotoxic effects [2, 24]. Liver disease constitutes a major problem of world wide, especially liver injury due to organophosphorous compounds. Malathion is well-known hepatotoxin that is used to induce hepatotoxicity, inflammation, necrosis, and oxidative stress in hepatocytes of laboratory animals [18]. Therefore, the present study was designed to investigate the antioxidant and hepatoprotective effects of ginger in malathion-induced hepatotoxicity.

2. MATERIAL AND METHODS

2.1. Animals:

60 male albino rats with average body weight 125 gm were used in this study. Rats were obtained from United Co. for Chemical and medical preparation, Cairo, Egypt. Rats were housed in separate metal cages. Fresh and clean drinking water was supplied *ad libtium.* The rats were left for 15 days for acclimatization before the beginning of the experiment. Rats were kept at constant environmental and nutritional condition throughout the period of experiment.

2.2. Ginger dosage:

Ginger was provided as tablet from MEPACO pharmaceutical company and administrated intragasteric using stomach tube at a dose of 400 mg / kg body weight once daily for 6 weeks [6].

2.3. Oxidant:

Malathion was administrated intragasteric using stomach tube at a dose of 100 mg / kg body weight once daily for 6 weeks according to Raja *et al.* [20].

2.4. Sampling:

Blood samples were obtained from the retro-orbital venous plexus at the end of second week, after that the samples were collected by scarification at the 4^{th} and 6^{th} week. Blood was collected into plain centrifuge tube to separate serum for biochemical analysis (ALT, AST, total protein and albumin) according to previous methods [9-16].

At the end of the 4^{th} week and 6^{th} week, the rats were scarified to collect liver tissue. Liver specimens were divided into two portions, the first specimen was rapidly washed with saline to avoid drying, weighted and processed for determination of GSH, GSH-PX, and MDA according to (26 -18]. The second portion was rapidly placed in RNA latter solution then frozen at -80 till determination of GSH-PX using PCR according to standard method [19].

Statistical analysis:

The obtained data were analyzed with the statistical software package SPSS for Windows (version 11.0; SPSS Inc., Chicago, USA). Statistical analysis was carried out with one way ANOVA test [23].

Table 1 Summary of experimental design:

Groups	Control	Ginger	Malathion	Ginger protected
Abbreviation	(C)	(G)	(M)	(G+M)
N	15	15	15	15
Reagents administrated	-	Ginger for 6 weeks	Malathion for 6 weeks	Ginger alone was taken for 2 weeks before malathion then combined together till the end of experiment
Type and time of sampling from all exp. groups	▪ Whole blood and serum were collected at the 2^{nd}, 4^{th} and 6^{th} weeks. ▪ Tissue specimens were collected at the 4th and 6th weeks.			

.

3. RESULTS

Concerning to the changes in MDA level, GSH and GSH-PX, there was significant increase ($p<0.05$) in MDA level accompanied with significant decrease ($p<0.01$) in GSH and GSH-PX at the 4th and 6th week of age in malathion group when compared to control group. Meanwhile, there was significant decrease in MDA level accompanied with significant increase in GSH and GSH-PX at the 4th and 6th week of age in ginger group compared to control group. Also, there was significant decrease in MDA level and there was significant increase in GSH and GSH-PX in ginger protected group when compared to malathion group as shown in Table 2.

Regarding to GSH-PX expression, our results confirmed that there was significant ($p<0.001$) decrease in GSH-PX mRNA expression in malathion group when compared to control group. Meanwhile in ginger group when compared to control group there was significant increase in GSH-PX expression at the 6th week of age. There was significant increase in GSH-PX expression in ginger protected group when compared to malathion group at the 6th week of age (Table 3).

Biochemical parameters showed significant increases ($p < 0.05$) in ALT and AST at the 2nd, 4th and 6th week of age. While, total protein and albumin showed a significant decrease at the 4th and 6th week of age in malathion group when compared to control group. Meanwhile, there were no significant changes in ALT, AST, Total protein and albumin at the 2nd, 4th and 6th week of age in ginger group when compared to control group. In ginger protected group when compared to malathion group there were significant decrease in ALT and AST at the 2nd, 4th and 6th week of age. Total proteins showed significant increase at the 2nd and 4th week of age. Albumin showed significant increase at the 4th and 6th week of age as shown in Table. 4.

Table 2 MDA, GSH and GSH-PX level in liver tissue of rats in control, ginger, malathion and ginger protected groups after 4 and 6 weeks.

Parameters	Treatment groups			
	Control	Ginger	Malathion	G+M
	---------- 4 Weeks ----------			
MDA (nmol/g)	33.76± 0.29[a]	32.50 ± 0.19[a]	49.12± 0.27[c]	34.78± 0.21[d]
GSH (nmol/g)	34.40± 0.44[a]	38.78 ± 0.31[b]	11.92± 0.25[c]	33.22± 0.19[d]
GSH-PX (U/mg protein)	0.296± 0.01[a]	0.397 ± 0.03[b]	0.044± 0.01[c]	0.320± 0.01[d]
	---------- 6 Weeks ----------			
MDA	34.63± 0.22[a]	32.90 ± 0.33 [b]	50.02± 0.35[c]	37.20± 0.49[d]
GSH	35.70± 0.55[a]	40.16 ± 0.24 [b]	12.04± 0.18[c]	35.24± 0.26[d]
GSH-PX	0.276± 0.01[a]	0.551 ± 0.01 [b]	0.037± 0.01[c]	0.295± 0.02[d]

Means (± SE).with different superscripts (a, b, c, d) within the same raw were significantly different at P<0.05.

Table 3 Glutathione peroxidase mRNA expression in control, ginger, malathion and ginger protected groups after 6 weeks

Groups	Glutathione peroxidase (R.U)
Control (C)	22.4 ± 0.19 [a]
Ginger (G)	1200 ± 0.17 [b]
Malathion (M)	5.6 ± 0.27 [c]
G+M	386.9 ± 0.29 [d]

Means (± SE).with different superscripts (a, b, c, d) within the same raw were significantly different at P<0.05.

.

Table 4 Biochemical parameters in in control, ginger, malathion and ginger protected groups after 2, 4 and 6 weeks (Mean± SE).

Parameters	Treatment groups			
	Control	Ginger	Malathion	M+G
	---------- 2 Weeks ----------			
ALT (U/L)	21.44 ± 0.80 [a]	22.02 ±1.14 [a]	29.06 ±0.37 [b]	21.70 ±0.73 [ac]
AST (U/L)	47.66 ±0.92 [a]	45.76 ± 1.14 [a]	60.02 ±0.26 [b]	45.52 ±0.95 [ac]
Total protein(g/dl)	5.5 ±0.38 [a]	5.7 ±0.26 [a]	4.6 ±0.40 [b]	5.74 ±0.11 [ac]
Albumin(g/dl)	2.4 ±0.24 [a]	2.4 ±0.42 [a]	3.00 ±0.28 [a]	2.96 ±0.30 [a]
	---------- 4 Weeks ----------			
ALT	21.94 ±0.69 [a]	21.82 ±0.74 [a]	49.62 ±0.56 [b]	26.04 ±0.95 [c]
AST	45.60 ±0.98 [a]	43.34 ±0.53 [a]	61.18 ±0.29 [b]	49.50 ±0.42 [c]
Total protein	6.00 ±0.16 [a]	6.12 ±0.26 [a]	4.04 ±0.24 [b]	6.04 ±0.29 [ac]
Albumin	3.02 ±0.16 [a]	3.02 ±0.16 [a]	2.66 ±0.11 [b]	3.04 ±0.13 [ac]
	---------- 6 weeks ----------			
ALT	20.08 ±0.09 [a]	20.16 ±0.07 [a]	50.28 ±0.22 [b]	23.72 ±1.08 [c]
AST	45.24 ±1.00 [a]	45.44 ±0.84 [a]	61.02 ±0.36 [b]	47.18 ±0.74 [ac]
Total protein	6.48 ±0.26 [a]	6.68 ±0.16 [a]	3.94 ±0.21 [b]	6.26 ±0.19 [ac]
Albumin	3.14 ±0.18 [a]	3.22 ±0.24 [a]	2.84 ±0.17 [b]	3.00 ±0.19 [ac]

Means (± SE).with different superscripts (a, b, c, d) within the same raw were significantly different at $P<0.05$.

4. DISCUSSION

The liver is an important organ which is actively involved in many metabolic functions and is the frequent target for a number of toxicants. Hepatic damage is associated with distortion of these metabolic functions. Organophosphate compounds are generally applied as relatively nontoxic sulphar- (thion) derivatives. This process is believed to take place primarily in liver.

The results of the present study in the liver homogenate in malathion intoxicated rats showed significant increase in MDA associated with significant decrease in GSH and GSH-PX activity. MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid. Therefore, the hepatic content of MDA is used as an indicator of liver tissue damage involving a series of chain reactions [19]. These results was reinforced by previous studies [5] that reported a significant decrease in GSH and GSH-PX concomitant with significant increase in MDA in liver of rats intoxicated with malathion. Malathion induces oxidative stress, leading to generation of oxygen free radicals during its metabolism and alterations in antioxidant enzymes [2].

Our results in the GSH-PX disagree with the work of *Banerjee et al.* [10] who found increased in the activity of GSH-PX in human erythrocytes upon malathion poisoning this may be due to generation of free radicals enhance the production of GSH-PX as an adaptive mechanism to the oxidative stress produced.

Malathion is detoxified via conjugation reactions with GSH. Conjugation of GSH to this pesticide or its metabolite in vivo could be a major pathway of detoxification [4]. It was observed that ginger administration resulted in significant reduction in liver MDA with significant elevation in both GSH and GSH-PX, compared with control group. Protection with ginger before malathion administration showed no significant changes in MDA, GSH and GSH-PX enzyme compared with malathion group. These results are supported by earlier works [9, 7, 20] reported that rats supplemented with ginger showed decreased levels of thiobarbituric acid reactive substances

.

and hydroperoxides, but increased level of GSH in liver.

These findings may be attributed to the ability of ginger to scavenge reactive oxygen species, inhibit their generation, and chelate transition metal ions that can participate in free radical transformation and the processes of lipid peroxidation as it contain more than 50 antioxidant substances isolated from there rhizomes. Furthermore, the ginger extracts contain substance called 6-gingerol, seems to be a good scavenger of peroxyl radicals, a major product of lipid peroxidation that damages intramembranous protein receptors.

Our results concerning GSH-PX mRNA expression showed significant increase in ginger group compared with control group. Meanwhile, malathion group showed significant reduction in its expression compared with control group. Protection with ginger before malathion administration showed significant elevation in the expression of GSH-PX enzyme than malathion group. This matches to the results of enzyme activity in serum attributed to ginger contain phenolic substances have antioxidant properties. These polyphenols have potential mechanisms of actions in cytoprotection against oxidative stress also these polyphenols increase the antioxidant enzymes gene expression, with consequent modulation of antioxidant enzyme activities that drive the intracellular response against oxidative stress [17].

Regarding to the results of total serum proteins and albumin showed significant decrease in malathion group than control group. These results were ascertained by [1, 8, 12] who found that there were significant decreases in both total protein and albumin in malathion toxicity which may be due to changes in protein and free amino acids metabolism and their synthesis in liver and also due to damaging effect of malathion on liver cells. It was found that protection with ginger before malathion administration significantly increase total protein and enhance the albumin synthesis. Our results are in agreement with previous reports [13-12] who recorded that there were significant increases in both total protein and albumin.

Concerning the liver transaminases, there were significant increases in both ALT and AST in malathion group compared with control one. These data are a sensitive index of hepatic damage. Normally the reactive metabolites are detoxified by combining with hepatic glutathione, which when become exhausted, these metabolites binds to liver macromolecules resulting in damage to hepatocyte in addition to damage that caused by free radical generation. Our results agree with reports recorded before [1, 12]. In contrast to these results, the protection by ginger before malathion administration showed significant decrease in both ALT and AST compared with malathion group. These results support the previous result [12-27], who studied the hepatoprotective action of ginger against malathion.

5. CONCLUSION

The results of this study suggested that ginger has a potent antioxidant effects and protects the liver against the oxidant damage caused by malathion.

5. REFERENCES

1. Abdelgadir, E.,Adam, S. 2010. Effect of various level of dietary malathion on waster Rats. *Science Alert*. **210**: 650-662.
2. Abdullah, N., Mohd Saat, N., Abu Hasan, H., Budin, S., Kamar Al-Zaman, S. 2004. Protective effect of the ethanol extract of (zingiber officinale Roscoe) on paracetamol induced hepatotoxicity in Rats. *Jurnal Sains kesihatan Malaysia* **2**: 85-95.
3. Ahmed, R.S., Seth, V., Pasha, S.T., Banerjee, B.D. 2000. Influence of dietary ginger (Zingiber officinale Rosc) on oxidative stress induced by malathion in rats. *Food Chem. Toxicol.* **38**: 443-450.

.

4. Ajith, T.A., Nivitha, V., Usha, S. 2007. Zingiber officinale roscoe alone and in combination with alpha-tocopherol protect the kidney against Cisplastin induced acute renal failure. *Food Chem. Toxicol.* **45**: 921-927.
5. Akhgari, M., Abdollahi, M., Kebryaeezadeh, A., Hosseini, R., Sabzeuari, O. 2011. Biochemical evidence for free radical-induced lipid peroxidation as a mechanisim for subchronic toxicity of malathion in blood and liver of rats. *Hum Exp Toxicol.* **22**: 205-211.
6. Almeida, M.G., Fanini, F., Davino, S.C., Aznar, A.E., Koch, O.R., Barros, S.B. 1997. Pro- and anti-oxidant parameters in rat liver after short-term exposure to hexachlorobenzene. *Hum. Exp. Toxicol.***16**: 257-261.
7. Al-Tahtawy, R.H.M., El-Bastawesy, A.M., Abdel Monem, M.G., Zekry. Z.K., Al- Merzabani, H. 2011. Antioxidant activity of the volatile oils of zingiber officinale (ginger). *Spatula DD.* **1**: 1-8.
8. Al-attar, A.M. 2010. Physiological and histopathological investigations on the effects of alpha lipoic acid in rats. *J. Biomed. Biotech.* **503:** 201-209.
9. Anderson FH, Zeng L, Rock NR, Yoshida EM (2000). An assessment of the clinical utility of serum ALT and AST in chronic hepatitis C. Hepatology Research, 18 (1): 63-71.Atta, A.H., Elkoly, T.A., Mouneir, S.M., Gehan Kamel, Alwabel, N.A., Shaimaa Zaher 2010. Hepatoprotective effect of methanol extract of zingiber officinale and cichorium intybus. *Indian J. Pharmaceut. sci.***72**: 564-570.
10. Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K. 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicology letters* **107**: 33-47.
11. El-Denshary, E.S. 1998. Pharmaco-preventive and pharmaco-therapeutic significance of free radicals scavengers. The annual scientific conference. April 1-3, Department of Pharmacology, Fac. of Pharmacy, Cairo Univ.
12. El Gharib, M.A., El-Masry, Th.A., Emara, A.M., Hashem, M.A. 2010. Potential hepatoprotective effects of vitamin E and Nigella sativa oil on hepatotoxicity induced by chronic exposure to malathion in human and male albino rats. *Toxicol. Exp. Chem.* **92**: 391-407.
13. Ezeonu, C., Egbuna, P.A.C., Ezeanyika, L.U.S., Nkwonta, C.G., Idoko, N.D. 2011. Antihepatotoxicity studies of crude extract of zingiber officinale on CCL4 induced toxicity and comparison of the extracts fraction D hepatoprotective capacity. *Res. J. Med. Sci.* **5**: 102-107.
14. Heeba Gehan, H. and Abd-Elghany Manal, L. 2010. Effect of combined administration of ginger (Zingiber officinal Roscoe) and atorvastatin on the liver of rats. *Int. J. Phytother. Phytopharm.* **1**: 73-79.
15. Ikediobi, C., Badisa, V., Ayuk, T., Latinwo, L., West, J. 2004. Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. *Int. J. Mol. Med.* **14**: 87-92.
16. Luca, G.C., Reis, B.F. 2004. Simultaneous photometric determination of albumin and total protein in animal blood plasma employing a multicommutated flow system to carried out on line dilution and reagents solutions handling. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **60**: 579-583
17. Masella, R., Di Benedetto, R., Vari, R. Filesi, C., Giovannini, C. 2005. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathion-related enzymes. *J. Nutr. Biochem.* **16:** 577-586.
18. Ohkawa, H., Ohishi, N., Yagi, K. 1999. Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351-358.
19. Olsvik, P.A., Kristensen, T., Waagbø, R., Rosseland, R., Tollefsen, B.O., Baeverfjord, G., Berntssen, M.H. 2005. mRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon (Salmo salar) exposed to hyperoxic water during smoltification. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **141**: 314-323
20. Rezg, R. Ornagui, B.M., El-Fazza, S., Gharbi, N. 2008. Biochemical evaluation of hepatic damage in subchronic exposure to malathion in rats: Effect on superoxide dismutase and catalase activities using native PAGE. *Comptes Rendus Biologies* **331**: 655-662.

21. Sharaky, A.A., Kamel, M.A., Eweda, S.M. 2009. Protective effect of ginger extract against bromobenzene- induced hepatotoxicity in male rats. *Food Chem. Toxicol*. **47**: 1584-1590.
22. Sheu, S.S., Nauduri, D., Anders, M.W. 2006. Targeting antioxidants to mitochondria: A new therapeutic direction. *Biochim. Biophys. Acta Molec. Basis Dis*. **1762**: 256 - 265.
23. Snedecor, G.W., Cochran, W.G. 1982. Statistical method.7th ed., Iowa state university press, Ame Iowa, USA.
24. Surh, Y.J., Lee, E., Lee, J.M. 1998. Chemo-protective properties of some pungent ingredients present in red pepper and ginger. *Mutat. Res.* **402**: 459 - 267.
25. Vijayala Kshmi, B., Chandrasekhar, M. 2008**.** Oxidative stress and there resultant products as the target molecules in clinical diagnose of age related disease. *Current Trends in Biotechnology and Pharmacy*. **2**: 239-250.
26. Wilke BC, Vidailhet M, Favier A, Guillemin C, Ducros V, Arnaud J, Richard MJ (1992). Selenium, glutathione peroxidase (GSH-Px) and lipid peroxidation products before and after selenium supplementation. *Clinica Chimica Acta* **207**: 137-142
27. Yassin, N.A.Z., El Rokh, E.M., El- Shenawy, S., Ehasn, N.A., Sayed, W.H., Hassanein, H.M.D.E., Ibrahim, B.M.M. 2010. Study of the hepatoprotective effect of ginger aqueous infusion in rat. *J. Chem. Pharm. Res.* **2**: 476-488.

.

.

مجلة بنها للعلوم الطبية البيطرية
BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

تأثير الزنجبيل كمضاد للأكسدة وكواقى للكبد فى الفئران

منى عبدالظاهر الشيمى[1]، أسامة على عبدالله[2]، خالد محمد فرارة[1]

[1] قسم الباثولوجيا الاكلينيكية – كلية الطب البيطرى – جامعة بنها، [2] قسم الباثولوجيا الاكلينيكية – كلية الطب البيطرى – جامعة قناة السويس

الملخص العربى

تم تصميم هذه الدراسة لتحديد قدرة الزنجبيل فى الحماية ضد التدمير الناتج عن المؤكسدات بعد استخدام المالاثيون. تم تقسيم الفئران إلي 4 مجموعات (15 فأر فى كل مجموعة) المجموعة الأولي كمجموعة ضابطة. المجموعة الثانية: تم إعطائها 400 ملجم زنجبيل / كجم من وزن الجسم. المجموعة الثالثة (مجموعة المالاثيون): تم إعطائها 100 ملجم مالاثيون / كجم من وزن الجسم. المجموعة الرابعة: تم إعطائها الزنجبيل قبل إعطاء المالاثيون بأسبوعين و لمدة أسبوعين. أظهرت النتائج عن وجود زيادة معنوية فى نشاط الجلوتاثيون المختزل وفى نشاط انزيم الجلوتاثيون بيروكسيداز وذلك فى نسيج الكبد فى المجموعة المحمية بالزنجبيل عند مقارنتها بمجموعة المالاثيون. إضافة إلى ذلك فقد وجد زيادة معنوية فى تكوين الحامض النووى الخاص بالجلوتاثيون بيروكسيداز فى نسيج الكبد. وكذلك كانت هناك زيادة معنوية فى نسبة الزلال و البروتين الكلى وعلى العكس فإن هناك نقص معنوى فى المالون داى الدهيد الموجود فى الكبد وكذلك نقص معنوى فى إنزيمات الكبد. نستخلص من هذه النتائج أن الزنجبيل له تأثير قوى كمضاد للأكسدة وكمانع لتسمم الكبد فى الفئران المعالجة بالمالاثيون.

(مجلة بنها للعلوم الطبية البيطرية: عدد 22 (2)، ديسمبر 2011: 9- 16)