Jurnal Sains Kesihatan Malaysia **2**(2) 2004: 85-95

Protective Effect of the Ethanol Extract of *Zingiber officinale* Roscoe on Paracetamol Induced Hepatotoxicity in Rats

NORINA ABDULLAH, NUR ZAKIAH MOHD SAAT, HAZLIN ABU HASAN, SITI BALKIS BUDIN & SAZLINA KAMARALZAMAN

ABSTRAK

Kesan perlindungan hepar ke atas hepatotoksisiti akut aruhan Parasetamol (1000 mg/kg) oleh ekstrak etanol rizom Zingiber officinale Roscoe telah dikaji ke atas sampel plasma dan tisu hepar yang diperolehi dari tikus jantan Sprague-Dawley. Ekstrak etanol diberi secara oral kepada tikus pada dos 200 mg/kg atau 300 mg/kg pada 0, 4 dan 8 jam selepas Parasetamol diberikan secara oral. Sampel darah dan hepar tikus diambil bagi analisa biokimia 24 jam selepas aruhan hepatotoksisiti untuk mengkaji paras superoksida dismutase (SOD), malondialdehid (MDA) serta aspartate transaminase (AST). Keputusan dibandingkan dengan kumpulan tikus yang diberi antidot N-acetylcysteine (NAC) (500 mg/kg) pada 0, 4 dan 8 jam selepas dos Parasetamol. Hasil yang diperolehi menunjukkan pada dos 200 mg/kg ekstrak berjaya menurunkan paras SOD plasma secara signifikan ($p < 0.05$) dan dos 300 mg/kg berjaya menurunkan paras SOD plasma, paras MDA hepar, AST serum serta meningkatkan paras protein plasma secara signifikan ($p < 0.05$). Kesimpulannya ekstrak etanol Z. officinale mempunyai kesan perlindungan terhadap hepatotoksisiti aruhan Parasetamol pada kedua tahap dos dan kesan perlindungan ini bertambah baik pada dos yang lebih tinggi.

Kata kunci: hepatotoksisiti, Zingiber officinale, Parasetamol, superoksida dismutase, malondialdehid, aspartate transaminase

ABSTRACT

The protective effect of the ethanol extract of the rhizome of Zingiber officinale Roscoe on acute hepatotoxicity induced by paracetamol (1000 mg/kg) was studied in plasma and hepatic tissue samples obtained from male Sprague-Dawley rats. The ethanol extract was given in oral doses of 200 mg/kg and 300 mg/kg to the rats at 0, 4 and 8 hrs after paracetamol was given orally. The plasma and liver of the rats were subjected to biochemical analysis 24 hrs after hepatotoxicity was induced to determine the levels of superoxide dismutase(SOD), malonaldehyde (MDA) and aspartate transaminase (AST). The results were compared to the rats which were given the antidote N-acetylcysteine (NAC) (500 mg/kg) at 0, 4 and 8 hrs after the paracetamol dose. The results showed that at 200 mg/kg the extract reduced the plasma levels of SOD significantly ($p < 0.05$) while at a higher dose of 300 mg/kg it reduced plasma SOD, hepatic MDA, serum AST and increased the levels of plasma proteins significantly ($p < 0.05$). In conclusion, the ethanol extract of Z. officinale showed protective effect against paracetamol induced hepatotoxicity at both dose levels and the protective effect was better at the higher dose.

Key words: hepatotoxicity, Zingiber officinale, paracetamol, superoxide dismutase, malondialdehyde, aspartate transaminase

INTRODUCTION

Zingiber officinale Roscoe or commonly known as ginger belongs to the family Zingeberaceae. The rhizome of Z. *officinale* is commonly used in cooking and in traditional medicine. It has been used to treat headaches, rheumatism, burns, peptic ulcer, dyspepsia, depression and impotence (Tyler & Robbers 1999). The oleoresin from the rhizome contained 6-gingerol and its homologs which have been shown to possess anti-inflammatory, antipyretic, antihepatotoxic, analgesic and cardiotonic properties (Surh 1999).

Several studies had been carried out to determine the medicinal properties of *Z. officinale*. According to Mustafa et al. (1993), Z. *officinale* was found to inhibit the activity of cyclooxygenase and lipoxygenase and hence decrease the pain in rheumatism and headaches. Topic et al. (2002) also

found that *Z. officinale* could inhibit lipid peroxidation by maintaining the levels of antioxidants in the serum of rats treated with malathion. This is in concordance with an earlier study which found that 6-gingerol in *Z. officinale* was a potent scavenger for the peroxyl radical which is the main product of lipid peroxidation (Aeschbach et al. 1994). Yamahara et al. (1988) found that 6-gingerol decreased the size of gastric lesions by 54.5%. Other studies had also found the antiemetic, anticholesterolaemic and antiplatelet effects of *Z. officinale* (Mowrey & Clayson 1982; Tanabe et al. 1993; Guh et al. 1995).

In hepatotoxicity, levels of protein in the plasma and hepatocytes decrease as a result of the disruption in the ability of hepatocytes to synthesise protein. Malondialdehyde (MDA) is elevated as a result of lipid peroxidation (Taourel 1992) while superoxide dismutase (SOD), an endogenous antioxidant is increased in situations where oxidative stress or assaults from xenobiotics or toxins occur as in paracetamol toxicity. SOD functions to destroy reactive oxygen species or free radicals. Aspartate transaminase (AST), like alanyl transferase (ALT) and gamma glutamyl transferase (GGT), is an enzyme which is elevated in hepatocellular damage and is also a good marker of hepatic damage (Alberti & Price 1981). In a study by Lin et al. (1995), the protective effect of *Curcuma xanthorriza* was observed against paracetamol and carbon tetrachloride induced hepatotoxicity in rats. *C. xanthorriza* was able to reduce the levels of the hepatic enzymes alanine transferase (ALT) and aspartate transferase (AST). Donatus et al. (1990) also found that low doses of curcumin, the active ingredient in *C. xanthorriza* was able to prevent paracetamol induced lipid peroxidation from occurring in rats. There has been no studies on the hepatoprotective effect of *Zingiber officinale*. This study was carried out to determine the protective effect of the ethanol extract of the rhizome of Z. *officinale* against hepatotoxicity induced by paracetamol in rats. This was achieved by measuring the protein, SOD and MDA levels in the rat plasma and hepatocytes by biochemical analyses.

MATERIALS AND METHODS

PLANT MATERIALS

The rhizomes of *Zingiber officinale* were obtained from Kuala Selangor, Selangor and were verified by a botanist. The rhizomes were chopped and air dried at room temperature for three days prior to pulverization using the electric blender (National MX-895 M). The pulverized rhizomes were then extracted in 99.8% ethanol for 3 days following which the extract was filtered. The filtered extract was then condensed using a rotavapor (Buchi R-200) until a crude extract was formed. The crude extract was then dissolved in ethylene glycol using an autovortex mixer and the ethanol stock mixture of 100 mg/ml was prepared.

Commercial paracetamol (500 mg) soluble tablets were obtained from Guardian Pharmacy, Bangsar. While ethylene glycol, sodium hydroxide, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium iodide, trichloroacetic acid, disodium hydrogen phosphate and anhydrous sodium dihydrogen phosphate were obtained from Merck, Germany. Bovine serum albumin, nitro blue tetrazolium, tiobarbituric acid, L-Methionine, riboflavin, EDTA, Tritin-X100, a-ketoglutaric acid and 2,4-dinitrophenylhydrazine were obtained from Sigma, USA. Ethanol was obtained from BDH Laboratory Supplies, UK; hydrochloric acid and potassium tartarate were obtained from Ajax Chemicals, Australia. N-acetylcysteine (NAC) was obtained from Euroscience Sdn Bhd. The paracetamol solution (250 mg/ml) was prepared by dissolving 75 tablets of soluble paracetamol (500 mg/tablet) in 150 ml of distilled water. The NAC solution was prepared by dissolving 5.5 g of NAC in 55 ml of distilled water.

PREPARATION OF ANIMAL SAMPLES

Thirty 8-week old male Sprague-Dawley rats, each weighing between 200-250g were obtained from the Animal Unit, Universiti Kebangsaan Malaysia (UKM). They were fed on commercial rat chow and water *ad libitum* and placed in a controlled environment in the animal house at the Institute of Medical Research, Kuala Lumpur. Prior to carrying out the study, approval was obtained from the UKM Ethics Committee on Animal Studies.

The rats were divided into 5 groups consisting of 6 rats per group. The first group which was used as a blank control were only fed orally with distilled water at 10 ml/kg followed by three doses of oral ethylene glycol (1 ml/kg) immediately after the distilled water was given, at 4 and 8 hrs later. The rats in the second group were given oral paracetamol (1000 mg/kg) to induce hepatotoxicity (Price &

Jollow 1986) followed by three doses of oral ethylene glycol (1 ml/kg) immediately after paracetamol was given and at 4 and 8 hrs post-paracetamol dose. Ethylene glycol was used as a vehicle for solubilising the *Z. officinale* crude extract to enable it to be taken into an oral syringe for feeding to the rats. Ethylene glycol had been used for this purpose in studies conducted by Woong (2002) and Asurah (2001) on the protective effects of *Curcuma xanthorrhiza*.

The rats in the third and fourth test groups were given oral paracetamol (1000 mg/kg) followed by the ethanol extract of *Zingiber officinale* at concentrations of 200 mg/kg and 300 mg/kg respectively by the oral route. The ethanol extract of Z. *officinale* was given in three equal doses immediately after the paracetamol dose, 4 and 8 hrs after the paracetamol dose. The fifth group was a positive control group whereby the rats were given oral paracetamol (1000 mg/kg) followed by the antidote NAC (1000 mg/kg) (Amimoto et al 1995) which was given at time intervals similar to the ethanol extract of *Z. officinale* given to the rats in groups 3 and 4. They were then fed oral ethylene glycol (1ml/kg) at the same time as the NAC (Woong, 2002). The rats were sacrificed 24 hrs later and blood samples were obtained through cardiac puncture and from the orbital sinus. The rats were also dissected immediately to obtain their liver samples for biochemical and histological tests.

SAMPLE ANALYSIS

The blood samples were processed to obtain the plasma and serum. The Biuret method was used to obtain the total protein measurement (Smith et al. 1985). Bovine serum albumin (BSA) was used in the preparation of the standard protein solution. Copper sulphate in the alkaline solution of the Biuret reagent which binds to the peptide bonds of proteins was detected by the ultraviolet (UV) spectrophotometer at the wavelength of 540 nm. A standard curve of UV readings plotted against the different concentrations of standard total protein was used to determine the concentrations of total protein in the plasma and hepatic tissues of the samples. Plasma from the rats were used to test for MDA, SOD and total protein. MDA levels in the samples were detected via the calorimeter technique using the Stock & Dormandy method (Stock et al. 1972), where spectrophotometer readings were obtained at the wavelength of 532 nm. Plasma samples were assayed for SOD using standardized methods and readings were obtained using the spectrophotometer at the wavelength of 560 nm. The method of Reitman and Frankel (1957) was used to determine the levels of AST enzyme in the serum of the rat samples. The levels of AST in the samples were obtained by reading from the AST standard curve which was plotted using spectrophotometer readings at 540 nm.

STATISTICAL ANALYSIS

The results from the biochemical tests were analysed by analysis of variance (ANOVA) using the SPSS version 11 statistical programme. The results were described as mean values ± the mean of standard errors with a confidence interval of $p < 0.05$.

RESULTS

The results in Fig.1 demonstrated that rats which were given 300 mg/kg of *Zingiber officinale* and 1000 mg/kg of NAC after treatment with 1000 mg/kg paracetamol showed significantly ($p < 0.05$) higher levels of plasma proteins compared to rats given paracetamol (1000 mg/kg) alone. In the hepatic tissues, rats treated with either 200 mg/kg and 300 mg/kg did not show significantly elevated protein levels compared to rats treated with paracetamol alone ($p > 0.05$) (Figure 1). However, rats treated with paracetamol and NAC showed significantly higher levels ($p < 0.05$) of protein in hepatic tissues compared to rats treated with paracetamol alone and paracetamol with *Z. officinale* (300 mg/kg).

MDA levels in the plasma of rats treated with paracetamol (1000 mg/kg) alone were significantly elevated ($p < 0.05$) compared to rats in the blank group, however no significant differences were noted in MDA levels in the plasma of rats treated with 200 mg/kg, 300 mg/kg or NAC compared to the blank group (Figure 2). This suggested that all three treatments were able to decrease MDA levels in plasma significantly to the baseline. Rats treated with paracetamol followed by NAC showed a significant decrease in plasma MDA levels compared to rats treated with paracetamol only. However, although *officinale* treated rats also showed lower plasma MDA levels compared to rats challenged with paracetamol only, these differences were not significant ($p > 0.05$).

In hepatic tissues (Figure 2), rats treated with 300 mg/kg of *Z. officinale* or NAC showed significantly decreased ($p < 0.05$) levels of MDA compared to rats treated with paracetamol alone. However, this effect was not seen in hepatic tissues of rats treated with 200 mg/kg of *Z. officinale*. The same trend was seen with respect to plasma levels of SOD (Figure 3), whereby all three groups of rats which were treated with 200 mg/kg, 300 mg/kg and NAC showed significant decreases ($p < 0.05$) in SOD compared to rats treated with paracetamol alone. There was no significant difference ($p > 0.05$) in tissue SOD levels seen in rats treated with paracetamol alone compared to controls (Fig. 3). Neither were there any significant differences ($p > 0.05$) in tissue levels of SOD in any of the treatment modalities compared to paracetamol treated rats or controls. There was also no significant differences ($p > 0.05$) between the different doses of *Z. officinale* or between NAC with *Z. officinale* in terms of effectiveness in decreasing hepatic tissue SOD levels.

Z. officinale at 300 mg/kg demonstrated significant decreases ($p < 0.05$) in serum AST levels compared to rats treated with paracetamol alone (Figure 4). There were no significant decreases ($p > 0.05$) in serum AST demonstrated by treatments with 200 mg/kg of *Z. officinale* or NAC. A dose-related increase in the efficacy of *Z. officinale* was clearly demonstrated in this experiment. However, surprisingly, with respect to serum AST levels, 300 mg/kg of *Z. officinale* managed to show better results compared to NAC as levels of serum AST were significantly ($p < 0.05$) lower with 300 mg/kg of *Z. officinale* than NAC (1000 mg/kg).

DISCUSSION

This study demonstrated that *Zingiber officinale* showed a dose-dependent efficacy in significantly increasing plasma proteins in rats with paracetamol-induced acute hepatotoxicity, where 300 mg/kg of Z. *officinale* showed significant enhancement of plasma proteins compared to 200 mg/kg of Z. *officinale*. However, rats treated with NAC (1000 mg/kg) demonstrated better results in that both the plasma and hepatic tissue protein levels were significantly increased. This is expected as NAC is the established antidote for paracetamol toxicity. However, the mechanism as to how *Z. officinale* preserves the levels of proteins is still unknown. One possible mechanism is that gingerol, the active ingredient in *Z. officinale* increases the levels of glutathione which bind to the toxic metabolites of paracetamol such as N-acetyl-p-benzoquinone imine (NAPQI) and increases its rate of excretion from the body. Alternatively, *Z. officinale* might inhibit the activity of the cytochrome P450 enzyme system which decreases the formation of NAPQI from ingested paracetamol. This mechanism had previously been suggested by Jagethia et al. (2003) and Ahmed et al. (2000) who found that *Z. officinale* was able to increase levels of glutathione and decrease lipid peroxidation.

Zingiber officinale (300 mg/kg) also was shown to decrease the levels of MDA in the hepatic tissues although this was not shown in the plasma. This suggested that *Z. officinale* at 300 mg/kg was able to inhibit a limited amount of lipid peroxidation although not to the same level of effectiveness compared to NAC which was successful in decreasing the levels of MDA in both plasma and hepatic tissues. *Z. officinale* (300 mg/kg) was also shown to be superior to *Z. officinale* (200 mg/kg) in decreasing the levels of MDA in hepatocytes suggesting a concentration-dependent effect.

Similarly, *Z. officinale* at 200 mg/kg and 300 mg/kg was able to decrease SOD levels in the plasma of rats with hepatotoxicity significantly although this was not achieved to a significant level in the hepatic tissues. The same result was obtained with NAC (1000 mg/kg). However, NAC showed distinct superiority over 200 mg/kg and 300 mg/kg of *Z. officinale* treatment when it demonstrated significantly ($p < 0.05$) lower SOD levels compared to the other two treatments. This suggested that *Z. officinale* had some antioxidant properties which decreased the need for endogenous SOD to act as an antioxidant. This is supported from other studies carried out by Reddy et al. (1992), Krishnakantha et al. (1993) and Ahmed et al. (2000) found that *Z. officinale* had antioxidant properties.

With reference to the AST enzyme levels *Z. officinale* (300 mg/kg) was able to significantly decrease it more effectively compared to NAC (1000 mg/kg). According to Sener et al. (2003), 150 mg/kg NAC delivered intraperitoneally was able to decrease serum AST levels significantly after paracetamol toxicity, although the levels of AST did not reach levels as in the control group.

CONCLUSION

In conclusion, *Zingiber officinale* at 300 mg/kg showed better protective and antioxidant effects in hepatocytes after acute hepatotoxicity induced by paracetamol compared to a lower dose of 200 mg/kg. In most cases, at the higher dose *Z. officinale* demonstrated antihepatotoxicity efficacy comparable to NAC, the antidote to paracetamol toxicity. In the future, more concentrations of the extracts need to be used to determine a dose-response relationship. Further studies should also include the effects of the extract on other parameters of hepatic damage and the determination of its possible role in the treatment of acute paracetamol poisoning or other inflammatory hepatic diseases.

ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia for the funding of this project. They would also like to thank Asmah Hamid, Sharifa Abdul Aziz, Ahmad Rohi, Khairana Husain and the staff of the faculty for their support and cooperation.

REFERENCES

Aeschbach, R., Loeliger, J., Scott, B.C., Murcia, A., Buttler, J. 1994. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chem. Toxicol.* 32 (1): 31-36.

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 and Chem. Toxicol.* 38: 443-450.

Alberti, K.G.M.M. & Price, C.P. 1981. The diagnostic value of isoenzyme measurement. *Recent Advances in Clinical Biochemistry*. New York: Churchill Livingstone.

Amimoto, I., Matsura, T., Koyama, S.Y., Nakanishi, T., Yamada, K., Kajiyama, G. 1995. Acetaminophen-induced hepatic injury in mice: the role of lipid peroxidation and effects of pretreatment with coenzyme Q10 and alpha-tocopherol. *Free Radic. Biol. Med.* 19: 169-176.

Asurah, T. 2001. Tesis tahun akhir: Kesan anti ulser etanol temu lawak (*Curcuma xanthorriza*) terhadap ulser gaster tikus. UKM.

Donatus, I. A., Santoso, B., Hadjar M. I., Sardjoko & Vermeulen, N.P.E. 1990. Preventive effect of curcumin on paracetamol-induced hepatotoxicity in mice. *European J. Pharmacol*. 183(6): 2451-2453.

Guh, J.H., Ko, F.N., Jong, T.T. & Teng, C.M. 1995. Antiplatelet effect of gingerol isolated from *Zingiber officinale*. *J. Pharm. Pharmacol.* 47(4): 329-332.

Jagetia, G.C., Baliga, M.S., Venkatesh, P., Ulloor, J.N. 2003. *Radiat. Res*. 160(5): 584-592.

Krishnakantha, T.P. & Lokesh, B.R. 1993. Scavenging of superoxide anions by spice principles. *Indian J. Biochem. Biophys.* 30(2): 133-134.

Lin, S.C., Lin, C.C., Lin, Y.H., Supriyatna, S. & Teng, C.W. 1995. Protective and therapeutic effects of *Curcuma xanthorrhiza* on hepatotoxin-induced liver damage. *Am. J. Chin. Med.* 23(3-4): 243-54.

Mowrey, D.B. & Clayson, D.E. 1982. Motion sickness, ginger and psychophysics. *Lancet*. 8273: 655-657.

Mustafa, T., Srivastava, K.C. & Jensen, K.B. 1993. Pharmacology of ginger, *Zingiber officinale*, *J. Drug Dev.* 6: 25-39.

Price, V.F. & Jollow, D.J. 1986. Strain differences in susceptibility of normal and diabetic rats to acetaminophen hepatotoxicity. *Biochem. Pharmacol.* 35 (4): 687-695.

Reddy, A.C. & Lokesh, B.R. 1992. Studies on spice priciples as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol. Cell Biochem.* 111: 117-124.

Reitman, S. & Frankel, S. 1957. Colorimetric method for the determination of serum transaminase activity. *Am. J. of Clin. Pathol.* 28: 56-63.

Sener, G., Sehirli, A.O. & Ayanoglu-Dulger, G. 2003. Protective effects of melatonin, Vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. *J. Pineal. Res*.35: 61-68.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. & Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76-85.

Stocks, J., Offerman, E.L., Modell, C.B. & T.L. Dormandy, T.L. 1972. The susceptibility to autoxidation of human red cell lipids in healthy and disease. *Br. J. Haematol.* 23: 713-719.

Surh, Y.J. 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medical phenolic substances. *Mutation Res.* 428: 305-327.

Tanabe, M., Chen, K.I. & Kano, Y. 1993. Cholesterol biosynthesis inhibitory component from *Zingiber officinale Roscoe*. *Chem. and Pharm. Bull*. 41(4): 710-713.

Taourel, D.B. & Guerin, M.C. 1992. Is malonaldehyde a valuable indicator of lipid peroxidation? *Biochemical Pharmacology.* 44: 985-988.

Toda, S., Miyake, T., Arichi, H., Tanizawa, H. & Takino, Y. 1985. Natural antioxidants III, antioxidative compounds isolated from rhizomes of *Curcuma longa L.Chem. and Pharm. Bull.* 33: 1725-1728.

Topic, B., Tani, E., Tsiakitzis, K., Kourounakis, P.N., Dere, E., Hasenohrl, R.U., Hacker, R., Mattern, C.M., Huston, J.P. 2002. Enhanced maze performance and reduced oxidative stress by combined extracts of *Zingiber officinale* and gingko biloba in the aged rat. *Neurobiol. of Aging*. 23: 135-143.

Tyler, V.E. & Robbers, J.E. 1999. Tyler's herbs of choice: the therapeutic use of Phytomedicals. 2nd Ed. New York: The Haworth Herbal Press.

Woong, M.F. 2002. Tesis: Kesan perlindungan ekstrak etanol temulawak (*Curcuma xanthorriza*) terhadap hepatotoksisiti tikus aruhan Parasetamol. UKM.

Yamahara, J., Mochizuki, M., Rong, H.Q., Matsuda, H. & Fujimura, H. 1988. The anti-ulcer effect in rats of ginger constituents. *J. Ethnopharmacol.* 23: 299-304.

Norina Abdullah
Department of Pharmacy
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur

Hazlin Abu Hassan
Nur Zakiah Mohd Saat
Siti Balqis Budin
Sazlina Kamaralzaman
Department of Biomedical Sciences
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur