Efficacy of *Raphanus sativus* in the treatment of paracetamol-induced hepatotoxicity in albino rats

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ABSTRACT

In the present study, the efficacy of a methanol extract of *Raphanus sativus* root (RSME) is tested in albino rats that developed hepatic damage due to administration of paracetamol (100 mg/kg body weight) for 30 days. Twenty rats were divided into three experimental groups (E1, E2, E3) and one control group (EC). Two doses of RSME (80 and 120 mg/kg body weight) were administered orally to E1 and E2, respectively, and a mixture of RSME (120 mg/kg) and paracetamol (100 mg/kg) was administered to E3 for 21 days. Group EC and another group of normal rats (EN) that served as controls were administered distilled water. At the end of the experiment rats were bled to assay thiobarbituric acid reactive substances (TBARS), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate aspartate transaminase (SGPT), reduced glutathione (GSH) and catalase. Results indicated that RSME reduced the levels of TBARS, SGOT and SGPT, and increased the level of GSH and the catalase activity in E1 and E2 as compared to the EC group. Group E3 showed decreases in TBARS, SGOT and SGPT levels, but the results were not statistically significant compared with the EN group. There was also a marked depletion in GSH level and catalase activity in this group. RSME reduced lipid peroxidation induced by paracetamol and brought the levels of SGOT and SGPT to normal, indicating liver recovery. It also brought about repletion of GSH levels and recovery of catalase activity. Results for group E3 indicated that RSME was not able to reverse the effects of paracetamol if administration continued.

KEY WORDS: Catalase. Glutathione. Lipid peroxidation. Raphanus sativus. Thiobarbituric acid reactive substance.

Introduction

The body's antioxidant system is usually able to combat the oxidative stress caused by free radicals generated by normal metabolic processes; however, modern life results in a variety of physical and mental stresses that generate free radicals and hence cell injury following lipid peroxidation.[1] Analgesics, sedatives and other drugs aid in oxidative stresses.[2] Paracetamol, also known as acetaminophen, is one such drug, which is usually taken for mild pain and fever. It is rapidly absorbed from the circulation and is primarily metabolised by the liver through three pathways.

The majority of metabolism is by glucuronidation and sulphation; however, a minor pathway involving P450 enzymes accounts for 5–10% of paracetamol metabolism in therapeutic use, but it is mainly metabolised by CYP2E1.[3,4] This pathway produces the intermediate toxic metabolite N-acetyl-P-benzoquinonimine, which requires glutathione for further metabolism to non-toxic metabolites. After glutathione is exhausted, the toxic metabolite binds to sulphydryl-containing protein in liver cells and causes lipid peroxidation, disrupting the cell membrane and eventually resulting in cell death.[5,6]

Any organ (e.g., liver, kidney, heart, pancreas) that has P450 enzymes suffers toxicity. Therefore, cases of hepatotoxicity due to paracetamol overdose occur each year and account for many deaths. Toxicity also results from prolonged use of the therapeutic dose, and therefore there is a need for a dietary supplement to help strengthen the antioxidant system of the body to combat the toxic effects of drugs such as paracetamol.

Raphanus sativus is an annual herb that is consumed as a vegetable and belongs to the family Brassicaceae. It decreases blood glucose levels in diabetic rats[7] and improves the histopathology of the colonic mucosa in rats fed a high fat diet.[8] Its antioxidant effects have been reported in alimentary hyperlipidaemic rats.[9]

Previous study indicates its inhibitory effect on lipid peroxidation in normal rats, and thus the present study aims to assess its effects on paracetamol-induced hepatotoxicity in albino rats.

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Materials and methods

Fresh *R. sativus* roots were obtained from local farmers and their authenticity was confirmed by the botany section of the Biology Department, University of Botswana. Methanol extract was prepared after chopping the roots into thin slices and drying. Dried roots were crushed to powder and soaked in 70% methanol for three days. The extract was then filtered and dried in a Buchi-type rotary vaporiser. The yield was 6% of the dried weight.

Male albino Wistar rats (200–250 g) were used for all experiments. Animals had free access to water and were fed on a commercial diet. All drugs were dissolved in water and were administered orally with the help of a tube.

All chemicals used were of analytical grade and obtained from Sigma Chemicals.

Thiobarbituric acid reactive substances

Lipid peroxidation in plasma was estimated in terms of thiobarbituric acid reactive substances (TBARS) by the method of Shushmskumari *et al*.,[10] with slight modification. Briefly, 0.1 mL plasma was treated with 2 mL TCA-TBA-HCl reagent (equal parts) and incubated in a boiling water bath for 10 min. The mixture was cooled and mixed with 2 mL freshly prepared 1 mol/L NaOH. Absorbance was measured at 535 nm.

Reduced glutathione

Reduced glutathione (GSH) was measured by the method of Ellman.[11] Briefly, 0.25 mL of blood haemolysate was mixed with 0.5 mL precipitating buffer (5% TCA in 1 mmol/L EDTA) and centrifuged. Supernatant was collected and mixed with 2.5 mL 0.1 mol/L phosphate buffer (pH 8.0). Colour was developed by adding 100 µL DTNB (0.01%) and read at 412 nm. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated using a kit obtained from Sigma, and the manufacturer's guidelines were followed.

Catalase activity

Catalase activity was assayed by the method described by Bisswanger.[12] Briefly, 0.2 mL plasma was added to 0.98 mL H_2O_2 solution (10 mmol/L). Decrease in the absorption at 240 nm was followed. Catalase activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071 mmol/cm) and the activity was expressed as micromoles of H_2O_2 oxidised per minute.

Statistical analysis

Data analysis was performed using the Sigma Stat (3.1 version) program. Data were subjected to descriptive statistics. Differences among the groups were analysed using two-way ANOVA, followed by Holm-Sidak test for comparisons.

Experimental design

To select the dosage of paracetamol and the duration to induce toxicity, different amounts were administered orally (25, 50, 75 and 100 mg/kg body weight) to four groups of rats. The rats were bled once a week to estimate the activity of SGOT and SGPT to assess the extent of toxicity caused by the different doses. On the basis of the results, it was decided that 100 mg paracetamol/kg body weight should be administered for 30 days to induce hepatotoxicity.

To assess the effect of extract of *R. sativus* root (RSME) on paracetamol-induced hepatotoxicity, 25 rats were used and divided into five groups, each containing five rats. The groups were normal controls (EN), the paracetamol control group (EC), and three experimental groups (E1, E2 and E3). The experiment was conducted in two phases. In the first phase, rats in all the groups were bled on day 1 to estimate TBARS, SGOT, SGPT, GSH and catalase. Then, the EN group was administered distilled water (1 mL), while the remaining groups were administered paracetamol (100 mg/kg body weight) for 30 days. On day 31 all the rats were bled to estimate the above parameters.

On day 31 the administration pattern was changed and it became day 1 of second phase of the experiment. The EN group continued on distilled water, while the EC group was administered distilled water. The E1 and E2 groups received RSME (80 mg/kg and 120 mg/kg body weight, respectively). The E3 group was administered a mixture of paracetamol (100 mg/kg) and RSME (120 mg/kg). The volume of extract administration was 1 mL in all groups. All the rats were bled on day 7, 14 and 21 to estimate the above-mentioned parameters.

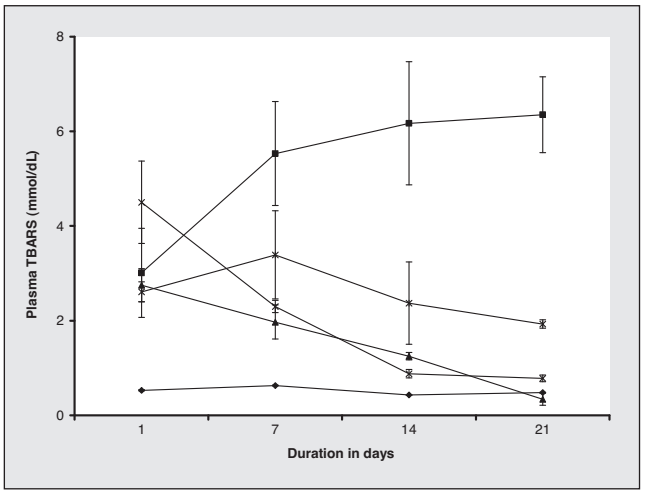

Fig. 1. Effect of RSME on TBARS in rats with developed paracetamol-induced hepatotoxicity. EN *vs*. E1, E2, E3 and EC on day 1 ($P<0.01$); EN *vs*. EC on days 7, 14 and 21 ($P<0.005$); EC *vs*. E1 on days 7 and 14 ($P<0.01$) and on day 21 ($P<0.001$); EC *vs*. E2 on day 21 ($P<0.001$).

Results

Results of paracetamol administration are presented in Table 1. The results show significant elevation in the levels of TBARS ($P<0.001$), SGOT ($P<0.001$) and SGPT ($P<0.001$) in the EC, E1, E2 and E3 groups, compared with levels on

Table 1. Effects of paracetamol administration plasma TBARS, SGOT, SGPT, GSH and catalase.

		TBARS mmol/dL	SGOT U/L	SGPT U/L	GSH mg/dL	Catalase U/dL
Day 1	EN	0.79 ± 0.01	28.39 ± 3.12	41.30 ± 4.61	48.91 ± 6.35	63.71 ± 5.80
	E	0.93 ± 0.03	30.47 ± 3.71	49.58 ± 4.81	50.61 ± 4.93	57.30 ± 4.76
Day 31	EN	0.83 ± 0.02	31.31 ± 4.15	43.73 ± 5.30	43.75 ±7.85	59.80 ± 3.07
	E	2.36 ± 0.09	95.73 ± 5.31	123.81 ± 7.30	18.73 ± 3.09	59.80 ± 3.07

EN: normal control group, administered distilled water only.

E: experimental and paracetamol control groups (E1, E2, E3 and EC).

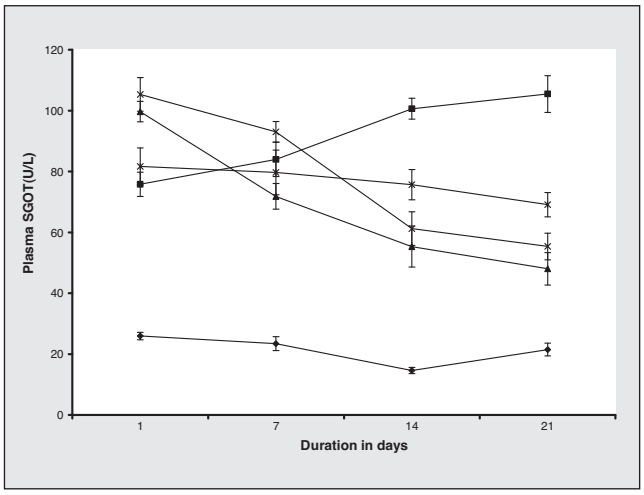

Fig. 2. Effect of RSME on plasma SGOT in rats with developed paracetamol-induced hepatotoxicity. EN *vs*. EC, E1, E2 and E3 ($P<0.01$); EC *vs*. EN on days 14 and 21 ($P<0.0001$); EC *vs*. E1 on days 14 and 21 ($P<0.05$); EC *vs*. E3 on day 21 (NS)

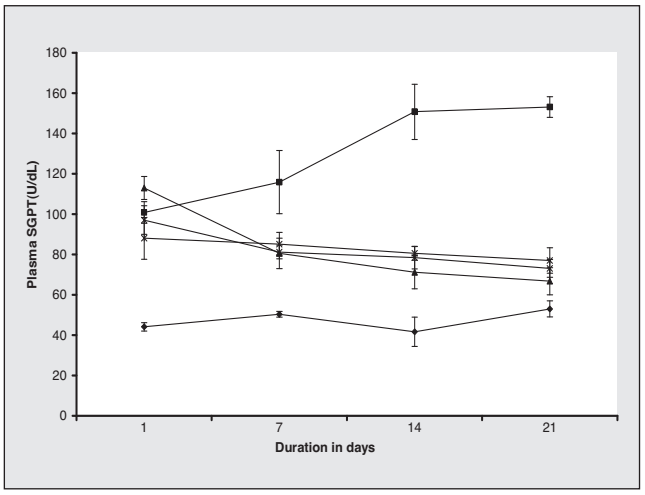

Fig. 3. Effect of RSME on SGPT in rats with developed paracetamol-induced hepatotoxicity. EN *vs*. EC, E1, E2 and E3 on day 1 ($P<0.05$); EC *vs*. E1 on days 14 and 21 ($P<0.05$) *vs*. E3 on day 21 (NS)

day 1 and also with levels in group EN on day 31. A significant decrease in the levels of GSH ($P<0.001$) and catalase activity ($P=0.002$) was noted in these groups. Rats in these groups also showed loss in body weight (10–14 % loss), marked decrease in food uptake and sluggishness.

Results of administration of RSME are presented in Figures 1, 2 and 3. Figure 1 shows the effect on plasma TBARS levels. Elevation is seen in the EC and E3 groups, while group E1 shows very low levels. Figure 2 shows a marked elevation in the activity of SGOT in all groups on day 1 except group EN. The EC group showed a continuous increase in the levels of SGOT while EN maintained the normal range up to day 21.

Figure 3 shows similar trends for SGPT. On day 1, values in all experimental groups and the EC group differed from the value in EN ($P<0.001$). Observations for 21 days showed that EN maintained its normal range, EC showed the increasing trend while groups E1 to E3 showed decreasing trends in the activity of SGPT.

Effects of RSME on plasma GSH are presented in Figure 4. It shows a marked depletion in the levels of plasma GSH in all groups except EN group on day 1. A continuous decrease was seen in the EC group. All experimental groups showed a recovery trend.

Results of RSME administration are presented in Figure 5. The results show that there is significant reduction in the activity of catalase in all groups when compared with the EN group on day 1. Activity in the EN group was maintained throughout the experimental period, while the EC group showed further reduction in catalase activity. Groups E1 and E2 showed improvement in catalase activity.

Discussion

Paracetamol is most commonly used as an analgesic and antipyretic drug. Although it is considered to be a safe drug, paracetamol overdosing or prolonged usage,

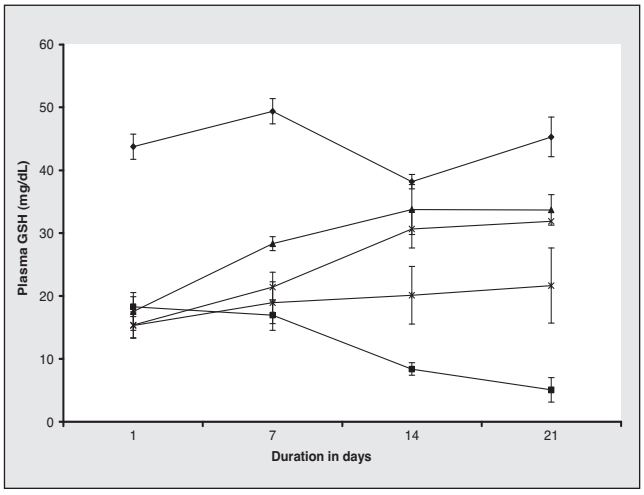

Fig. 4. Effect of RSME on plasma GSH in rats with developed paracetamol-induced hepatotoxicity. EN *vs*. EC, E1, E2 and E3 ($P<0.025$); EC *vs*. E1 and E2 on days 21 ($P<0.005$); EC *vs*. E3 on day 21 (NS)

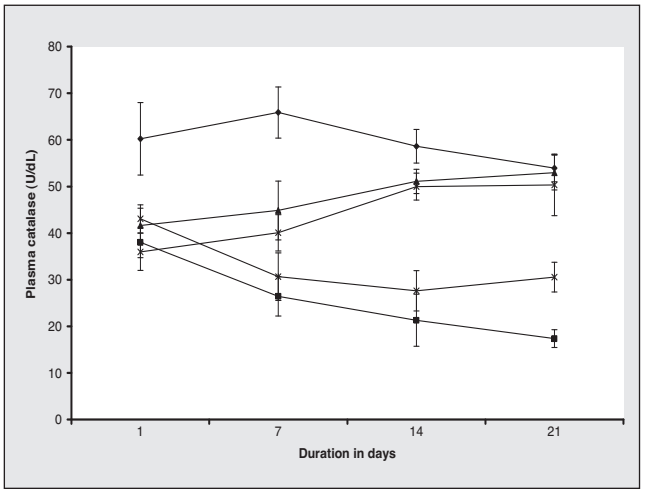

Fig. 5. Effect of RSME on plasma catalase in rats with developed paracetamol-induced hepatotoxicity. EN *vs*. EC, E1, E2 and E3 on day 1 ($P<0.05$); EN *vs*. ES on days 21 ($P<0.01$).

even at therapeutic levels, can cause liver failure and eventual death.[13] Metabolism of paracetamol through the cytochrome P450 pathway produces reactive N-acetyl-P-benzoquinoneimine. This metabolite binds to sulphydryl-containing proteins in liver cells and causes lipid peroxidation.[14]

In the present study, significantly high levels of TBARS in all the groups clearly indicates a high rate of lipid peroxidation, and administration of RSME significantly reduced TBARS levels in some of the groups studied. Very high levels of TBARS in the paracetamol control group indicate that lipid peroxidation, once generated by paracetamol, continues unless the trend is reversed by antioxidants such as RSME.

Repeated dosing of paracetamol to rats is reported to decrease their sensitivity to its hepatotoxic effects,[15] and this could be the cause of the decrease in the rate of lipid peroxidation seen in the present study after seven days. However, as this decrease was not significant, it suggests that the antioxidant system did not protect against paracetamol-induced lipid peroxidation

The enzymes SGOT and SGPT are localised in hepatic cells, and damage to these cells results in release of these enzymes to the circulation. Thus, high levels in the circulation are markers of hepatic cell damage.[22] In the present study, levels of these enzymes were significantly elevated in all groups except EN, indicating the presence of hepatic cell damage due to lipid peroxidation during the first phase of the experiment. Subsequently, decreasing levels of these enzymes in the second phase of the study indicates the restorative effect of RSME, which could be due to restoration of glutathione level.

Glutathione is required for the metabolism of toxic N-acetyl-P-benzoquinonimine to a non-toxic metabolite.[20] When the supply of glutathione is exhausted, this radical can no longer be scavenged and it causes lipid peroxidation.[15,20] Low levels of GSH in the test groups in the present study reflect the cause of hepatic cell damage. Termination of paracetamol administration and continuous administration of RSME to groups E1 and E2 resulted in restoration of glutathione levels.

Sulphur-containing amino acids are the precursors of glutathione, and their dietary intake is effective in elevating GSH levels.[16,21] Small cysteine-rich proteins have been isolated from radish,[17,18] and it appears that the cysteine present in the radish root contributes to the synthesis of GSH by providing cysteine precursors, and hence it participates in the recovery process.

Catalase is an antioxidant enzyme that helps to neutralise the toxic effects of paracetamol in the liver[19] The present study showed that catalase activity was very low at the start of the second phase of the experiment. This low activity may have been due to the enzyme's involvement in detoxification. However, activity returned to normal as the experiment progressed, and RSME appeared to provide maximal efficacy in relation to catalase activity at 80 mg/kg.

The present study clearly indicates that RSME can have an effect in protecting against paracetamol-induced hepatotoxicity through its antioxidant properties and by inhibiting lipid peroxidation and elevating glutathione levels. However, administration of RSME and paracetamol to rats already demonstrating hepatotoxicity was not able to reverse the harmful effects of the drug. □

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