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# HEPATOPROTECTIVE ROLE OF EXTRACTS OF *MOMORDICA CHARANTIA* L. IN ACETAMINOPHEN-INDUCED TOXICITY IN RABBITS

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## ABSTRACT

An analysis of different serum enzymes including ALT, AST, ALP and LDH was carried out to evaluate the hepatoprotective and hepatocurative effects of *Momordica charantia*. The study was divided in two phases. In first the phase, liver toxicity was induced in rabbits with administration of acetaminophen, and then Momordica extract was given and hepatocurative effects were observed. The results indicated significant decrease in the elevated concentrations of these enzymes in acetaminophen-intoxicated rabbits. In the second phase, the extract of Momordica was given to the rabbits orally for 10 and 15 days respectively. Then, the animals were administered with acetaminophen and hepatoprotective effects of Momordica were observed. The hepatoprotective effects of Momordica extract was found to be more effective after 15 days as there was less elevation of the serum enzymes after liver damage.

**Key words:** Serum Enzymes, Hepatoprotective, Hepatocurative, *Momordica charantia,* Acetaminophen.

## INTRODUCTION

The medicinal importance of *Momordica charantia*, commonly named as bitter melon, has been recognized globally. The *M. charantia* has been reported to have antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, antimycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties (Ng *et al.,* 1992, Raman and Lau 1996, Basch *et al.,* 2003). Traditionally it has also been used in treating peptic ulcers; interestingly recent experimental studies have exhibited its potential against *Helicobacter pylori*. There are different reports available on clinical use of *M. charantia* in diabetes and cancer patients (Grover *et al.,* 2001). Studies by Limtrakul and co-workers (2004) suggest that some components of bitter melon extract may be effective in slowing the growth and spread of some types of cancer, particularly breast cancer. Nerurkar *et al*., (2008) observed that in mice bitter melon juice may be involved in lowering of plasma lipid possibly by inhibiting the synthesis of triacylglyceride synthesis. Nerurkar *et al.* (2010) also found that bitter melon juice results in significant reduction of lipid content with a simultaneous reduction in mRNA expression of adipocyte transcription factors.

Being the major drug-metabolizing and drug-detoxifying organ in the body, liver is subjected to potential damage from an enormous array of pharmaceutical and environmental chemicals (Kumar *et al.,* 1997). Acetaminophen (Paracetamol) is an analgesic and antipyretic drug that may cause liver necrosis (Simpson *et al.,* 2000). Acute liver injury may involve the parenchyma, bile secretory functions or both. Some drugs including acetaminophen (Gill and Sterling, 2001) have been reported to produce hepatic injury that is cytotoxic in nature. Many plant materials have been used for curing various ailments since pre-historic times, though scientific basis of these materials was not known. In recent years, the application of plant materials for curing and prophylaxis has been scientifically studied. There are several plants which have been shown to have therapeutic value. Some reports have appeared on the hepatoprotective and hepatocurative application of *Momordica charantia* L. The clinical use of its extract has been reported in diabetic and cancer patients. The main objective of the present work was to investigate hepatoprotective properties of *M. charantia* in acetaminophen intoxicated rabbits.

## MATERIALS AND METHODS

**1. Preparation of extract:** The fruits of *M. charantia* were procured from the local market, washed with water and air-dried. The whole fruits were ground along with the seeds. The juicy extracts of the fruit were filtered by standard filtration techniques.

**2. Animals:** Fifteen male rabbits of species *Oryctolagus cunniculus* and weighing 1-2 kg were selected for the study. The animals were acclimatized for one week in the University Animal House in standard conditions and fed with standard diet with water *ad libitum*. They were divided into three groups of five animals each as follows.

| Groups | Treatment | |
|---|---|---|
| Group I | Hepatocurative Group | Acetaminophen for three days then treatment with Momordica extract |
| Group II | Hepatoprotective 10 Group (HP10) | Momordica extract for ten days then intoxication with acetaminophen |
| Group III | Hepatoprotective 15 Group (HP15) | Momordica extract for fifteen days then intoxication with acetaminophen |

**3.1. Hepatocurative studies:** In this study, five animals were first administered orally with acetaminophen (paracetamol) for liver damage. Then the animals were given the Momordica extract for the curative purposes. Acetaminophen (1500 mg per kg of body dissolved in 20 ml of distilled water) was given as recommended by Kamran (2002). The extract (5ml/kg body weight/day) was given in its concentrated form on 4$^{th}$ day of the study (Natural Standard, USA). The blood was analyzed at day 0, 3, 6, 11, 16, and 20 of the study for various serum enzymes including AST, ALT, LDH and ALP.

**3.2. Hepatoprotective 10 Group (HP10):** Oral dose of Momordica extract (5 ml/kg body weight/day) was given for 10 days. Administration of acetaminophen (1500 mg of paracetamol per kg of body weight in 20 ml of distilled water) was started at 11$^{th}$ day of the study, for six days. The blood was analyzed on 13$^{th}$ day and 16$^{th}$ day, for serum enzymes including AST, ALT, LDH, and ALP.

**3.3. Hepatoprotective 15 Group (HP15):** Oral dose of Momordica extract (5 ml/kg body weight/day) was given for 15 days. Administration of acetaminophen (1500 mg of paracetamol per kg of body weight in 20 ml of distilled water) was started at 16$^{th}$ day of the study, for six days. The blood was analyzed on 18$^{th}$ day and 21$^{st}$ day, for serum enzymes including AST, ALT, LDH, and ALP.

**4. Chemicals:** Acetaminophen (Paracetamol) was used for liver intoxication. Test Kits for measuring AST, ALT, ALP and LDH, by Audit diagnostics, Ireland and Biocon Diagnostik, Germany, were used.

**5. Statistical Analysis:** The quantitative data are represented as mean $\pm$ SEM. SPSS software for window version 13.0 was used for statistical analyses. The significance of differences among the groups was tested by one-way ANOVA (Sokal and Rohlf, 1995).

## RESULTS AND DISCUSSION

Liver is the major organ in metabolism, secretion and detoxification in the body. Hepatic damage is therefore associated with the deformities of the functions. The present research study was designed to investigate the possible hepatoprotective role of the extract of *Momordica charantia* on acetaminophen induced toxicity in rabbits. The toxic metabolite, N-acetyl p-benzoquinoneimine (NAPQI) and others, derived from acetaminophen has been reported as the main source of liver toxicity (Thummel *et al.,* 2000; McClain *et al.,* 1999).

Tables 1, 2 and 3 show the comparison of the mean concentration ($\pm$ SD) of aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase in hepatocurative and hepatoprotective treatments of Momordica extract in acetaminophen intoxicated rabbits.

During the hepatocurative treatment, blood sampling was done on 0 day, for control reading. The concentration of AST in untreated (control) animals was 74.08$\pm$ 0.97 U/l. After administration of acetaminophen there was significant elevation in serum AST levels (96.7 $\pm$ 0.49 U/l) at day 3, showing liver damage. The AST concentrations were found to be significantly decreasing after treatment with the extract. The values were 93 $\pm$ 0.32, 91 $\pm$ 0.39, 89$\pm$ 0.97, and 83 $\pm$ 1.31 U/l at 6$^{th}$, 11$^{th}$, 16$^{th}$, and 20$^{th}$ day of the treatment, respectively (Table 1). In hepatoprotective ($P_{10}$) treatment, the control (0 day) AST reading was 73.12$\pm$ 0.55 U/l followed by 71.11 $\pm$ 0.76* U/l after administration of the extract. There was a rise in AST values (77.29 $\pm$ 0.78 and 84.9 $\pm$ 0.66* U/l) after exposure with acetaminophen on 13$^{th}$ and 16$^{th}$ day of sampling, respectively (Table 2). Similarly, the control (0 day) AST value was 76.42$\pm$ 1.03 U/l in hepatoprotective ($P_{15}$) treatment. The AST concentration was found to be 73.21 $\pm$ 1.12* U/l after treatment with the extract. There was a rise in AST values (78.40 $\pm$ 0.76 and 81.04 $\pm$ 0.45 U/l) after administration with acetaminophen at 18$^{th}$ day and 21$^{st}$ day of the sampling, respectively (Table 3).

The concentration of ALT in untreated animals was 66.29$\pm$1.1 U/l in the hepatocurative studies. After acetaminophen administration there was a significant elevation in serum ALT level (112.8 $\pm$ 1.27 U/l), at day 3. Then, ALT conc. was found to be significantly decreasing with Momordica extract. The values were 92.9 $\pm$ 1.3, 84.8 $\pm$ 1.16, 82.6 $\pm$ 0.58, 81.4 $\pm$ 0.70 at 6$^{th}$, 11$^{th}$, 16$^{th}$, and 20$^{th}$ day of sampling, respectively (Table 1). The control (0 day) ALT reading was 39.3$\pm$ 1.2 U/l in hepatoprotective ($P_{10}$) group. The value of ALT was 34.2 $\pm$ 1.16 U/l with Momordica extract. Then, with acetaminophen administration, ALT values were found to be elevated (47.4 $\pm$ 0.59 and 54.7 $\pm$ 1.02 U/l) at 13$^{th}$ day and 16$^{th}$ day of sampling (Table 2). In hepatoprotective ($P_{15}$) group, control reading for ALT was 39.9$\pm$ 0.76 U/l. Then, with administration of Momordica extract ALT level was found to be 36.6 $\pm$ 1.5* U/l. The animals were given acetaminophen, and there was a rise observed in ALT levels (38.6 $\pm$ 1.03 and 49.2 $\pm$ 0.70 U/l at 18$^{th}$ day and 21$^{st}$ day sampling, data shown in Table 3. Alanine

aminotransferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream (Sethumadhavan *et al.,* 2007) which is indicated in our results as well.

The concentration of ALP in untreated animals was 72.03±0.21 U/l, during the hepatocurative treatment. Acetaminophen was given to the rabbits which resulted in the significant elevation in serum ALP levels i.e. 159.1 ±0.78 U/l, after 3 days. After treatment with Momordica extract, ALP levels were observed to be significantly decreasing. The values are 148.5±1.2, 135.7± 0.64, 102.8± 0.99, 82.6 ± 1.13 for 6th, 11th, 16th, and 20th day respectively (Table 1). During the hepatoprotective ($P_{10}$) treatment, the control ALP value was 69.71± 0.22 U/l. The value of ALP after Momordica extract was 64.08 ± 1.01 U/l. There was a rise observed in ALP values (97.99 ± 0.86 and 110.28 ± 0.44 U/l) at 13th day and 16th day sampling, with administration of acetaminophen (Table 2). ALP control reading was 71.82 ± 0.55 U/l hepatoprotective ($P_{15}$) group. With Momordica extract the ALP value was observed to be 66.11 ± 1.22 U/l. The administration of acetaminophen resulted in increase in ALP values, like 85.33 ± 1.03 and 94.13 ± 0.85 U/l at 18th day and 21st day of sampling (Table 3).

**Table 1: Variations in AST, ALT, ALP and LDH enzyme concentrations during the hepatocurative treatment.**

| Treatments | Parameters studied | | | |
|---|---|---|---|---|
| | AST (U/L) | ALT (U/L) | ALP (U/L) | LDH (U/L) |
| Control (0 day sampling) | 74.08± 0.97 | 66.29 ±1.1 | 72.03 ± 0.21 | 251.2 ± 1.12 |
| Liver Damage (3rd day Sampling) | 96.7 ± 0.49* | 112.8 ±1.27 | 159.1 ± 0.78 | 318.31 ± 0.98 |
| Momordica Extract (6th day sampling) | 93 ± 0.32* | 92.9 ± 1.3 | 148.5 ± 1.2 | 281.6 ± 0.66 |
| Momordica Extract (11th day sampling) | 91 ± 0.39* | 84.8 ± 1.16 | 135.7 ± 0.64 | 285.5± 0.12 |
| Momordica Extract (16th day sampling) | 89± 0.97* | 82.6 ± 0.58 | 102.8 ± 0.99 | 270.7± 0.018 |
| Momordica Extract (20th day sampling) | 83 ± 1.31* | 81.4 ± 0.70 | 82.6 ±1.31 | 266.2± 0.47 |

P<0.05*, n=5, Data expressed in Mean ± S.M.

**Table 2: Variations in AST, ALT, ALP and LDH enzyme concentrations during the hepatoprotective (P 10) treatment.**

| Treatments | Parameters studied | | | |
|---|---|---|---|---|
| | AST (U/L) | ALT(U/L) | ALP(U/L) | LDH(U/L) |
| Control (0 day) | 73.12± 0.55 | 39.3 ± 1.2 | 69.71 ± 0.22 | 249.75±1.24 |
| Momordica Extract (10th day sampling) | 71.11 ± 0.76 | 34.2 ± 1.16 | 64.08 ± 1.01 | 247.72±0.86 |
| Liver damage (13th day sampling) | 77.29 ± 0.78 | 47.4 ± 0.59 | 97.99 ± 0.86 | 286.2 ± 0.05 |
| Liver damage (16th day sampling) | 84.9 ± 0.66 | 54.7 ± 1.02 | 110.28 ± 0.54 | 307.7± 0.79 |

P<0.05*, n=5, Data expressed in Mean ± S.M.

**Table 3: Variations in AST, ALT, ALP and LDH enzyme concentrations during the hepatoprotective (P 15) treatment.**

| Treatments | Parameters studied | | | |
|---|---|---|---|---|
| | AST (U/L) | ALT(U/L) | ALP(U/L) | LDH(U/L) |
| Control (0 day sampling) | 76.42 ± 1.03 | 39.9 ± 0.76 | 71.82 ± 0.55 | 252.1± 0.82 |
| Momordica Extract (15th day sampling) | 73.21 ± 1.12* | 36.6 ± 1.5* | 66.1 ± 1.22* | 248.81±1.10* |
| Liver damage (18th day sampling) | 78.4 ± 0.76* | 38.6 ± 1.03* | 85.33±1.03* | 264.62±1.32* |
| Liver damage (21st day sampling) | 81.04 ± 0.45* | 49.2 ± 0.70* | 94.13 ± 0.85* | 284.8±0.28* |

P<0.05*, n=5, Data expressed in Mean ± S.M.

During the hepatocurative treatment, LDH control reading was 251.2± 1.12 U/l. There was significant elevation in serum LDH levels, i.e., 318.31 ± 0.98 U/l, after acetaminophen administration, at day 3. Then, with Momordica extract, LDH concentrations were found to be significantly decreasing with Momordica extract. The values were, 281.6 ± 0.66, 285.5 ± 0.12, 270.7± 0.08 and 266.2± 0.47, U/l for 6th, 11th, 16th, and 20th day of the study respectively (Table 1). In hepatoprotective ($P_{10}$) group, control LDH reading was 249.75± 1.24 U/l. Then, Momordica extract was given and the value of LDH was 247.72 ± 0.86 U/l. After that, the animals were given acetaminophen. There was a rise observed in LDH value (286.2 ± 0.05 and 307.7 ± 0.79

U/l) at 13th day and 16th day sampling (Table 2). During the hepatoprotective ($P_{15}$) treatment, the control LDH reading was 252.1± 0.82 U/l. Momordica extract was given and the value of LDH was 248.81 ± 1.10 U/l. After that, the animals were exposed to acetaminophen. There was a rise observed in LDH value (264.62 ± 0.78 and 284.8 ± 0.28 U/l) at 18th day and 21st day of the sampling respectively (Table 3). Alkaline phosphatase is a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ. The increase in the level of this enzyme has also been reported in cholestasis (Sanmugopriya and Venkataraman, 2006).

The hepatoprotective role of Momordica extract in our findings seems to be due to enhanced antioxidant enzymes as reported by Semiz and Sen (2007) in rats. Dandagi and co-workers (2008) explored the hepaotoprotective activity of various extracts of *Ferula asafetida, M. charantia* and *Nardosta jatamansi* against experimental hepatotoxicity and the results demonstrated that the extracts of *Momordica charantia* Linn. have significant hepatoprotective activity. The study by Thenmozhi and Subramanian (2010) also confirmed the antioxidant and hepatoprotective potential of *Momordica charantia* fruit extract in ammonium chloride-induced toxicity in rats. The hepatoprotective activity of *Momordica charantia* may be attributed due to the presence of flavonoids, ascorbic acid and other components such as saponins, tannins, triterpenes and alkaloids (Hossain *et al.,* 2011). Our findings are also in accordance with Chaudhri *et al.,* (2009) who reported that the serum level of these liver enzymes (ALT, AST, ALP) is elevated in $CCl_4$ intoxicated rats however hydro-alcoholic extract of *M. charantia* again reduced amounts of theses enzymes to normal level indicating its hepatpprotective effect. It is, therefore, suggested that *M. charantia* may be used as a cheap remedy for the liver diseases in developing countries but further studies should be carried out to assess its pharmacological aspects.

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