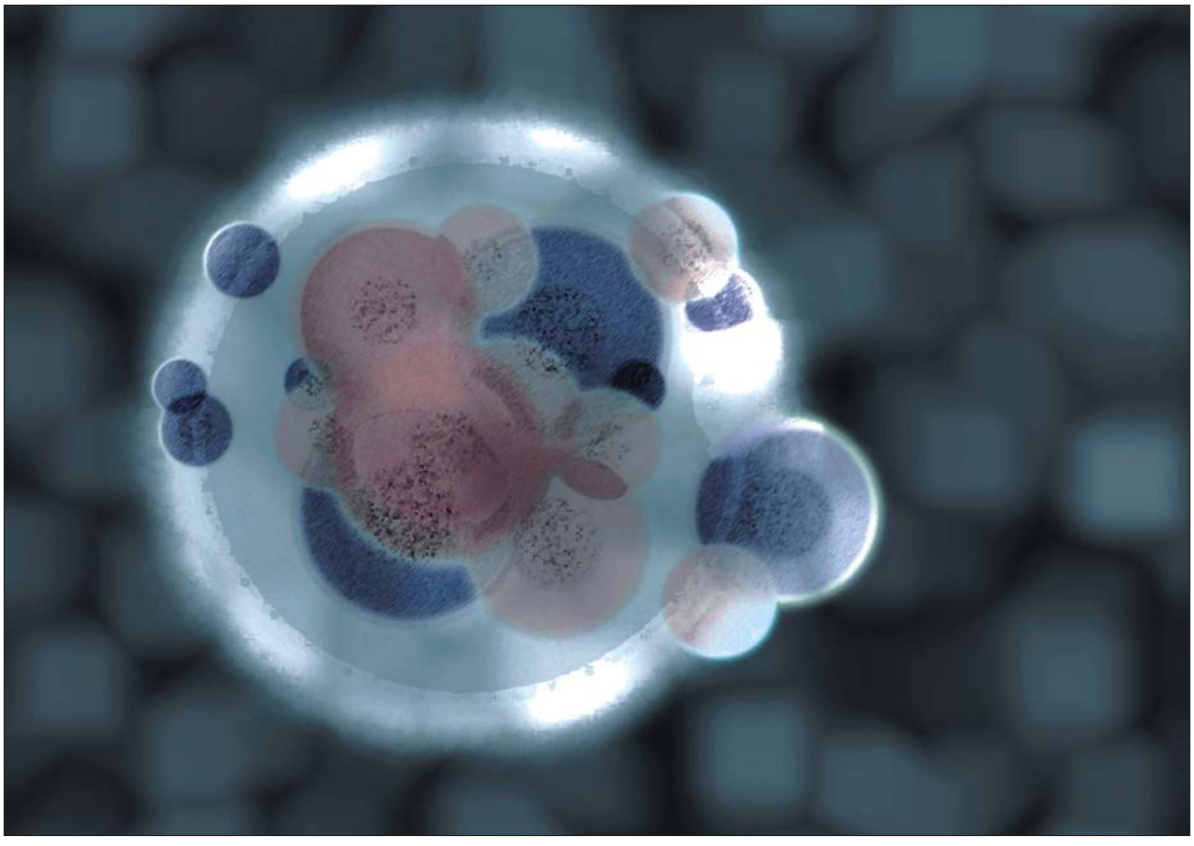

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 4 (4): 463-469, 2004
ISSN 1727-3048
© 2004 Asian Network for Scientific Information

Evaluation of Hepatoprotective Effects of *Raphanas sativus* L.

[1]Rifat-uz-Zaman and Mobasher Ahmad
[1]Department of Pharmacy, University of Sargodha, Sargodha, Pakistan
Faculty of Pharmacy, Punjab University, Lahore, Pakistan

Abstract: The hepatoprotective study of *Raphanas sativus* fruit powder (RS), it's aqueous (RS-A) and ethanolic (RS-E) extracts was performed on rabbits. The fruit powder (0.5-1.5 g kg^{-1} of body weight) tested in cadmium chloride (Cd) treated rabbits. The effects of aqueous and ethanolic extracts of RS (equivalent to 1.5 g kg^{-1} of body weight) were also noted (with Cd). Both RS-A and RS-E significantly decreased the total bilirubin level and activity of enzymes i.e. ALT and AP in serum of Cd-treated rabbits. RS-E declined the serum ALT activity more effectively than the RS or RS-A in the treated animals. The lowering effect of the RS was more marked as compared to its extracts on the serum AP and total bilirubin level in the treated rabbits. Anti-inflammatory/anti-apoptosis activity of RS may be responsible for this hepatoprotective activity.

Key words: *Raphanas sativus*, hepatoprotective, hepatitis, ALT (GPT), AP, bilirubin

INTRODUCTION

Raphanas sativus (Syn: Mulli) [Brassicaceae (Cruciferae)] has long been used for the treatment of gastrointestinal (GIT) disorders in the folkloric system of medicines[1]. It is wildly growing plant all over the world. Many varieties are cultivated differing greatly in size, shape and color of the root on commercial scale[2]. The RS has a hot, sharp, bitter taste. It has been found to be stomachic, binding and anthelmintic and useful in the diseases of heart. RS has been used in inflammations, hiccough, leprosy and cholera. The juice relieves earaches and flowers have been reported to be bechic and cholagogue[3]. The seeds have been used as carminative, tonic, peptic and corrective while seeds and leaves diuretic, laxative and lithontriptic. All parts of this plant have been found to be effective in the cases of gravel and urinary tract diseases[3]. RS has been reported to possess anti-inflammatory, anti-fungal, anti-viral and anti-tumor activities[4,5]. The presence of alkaloids, coumarins, saponins, flavonoids and anthocyanins has been reported in the seed and fruit[6]. RS has been considered useful in the GI-disorders in the traditional medicine. The present study was therefore, carried out to validate its folkloric use. Moreover, both acute and chronic hepatitis have been caused major health problems in Pakistan and hepatitis rate in Pakistan is alarmingly high i.e. approximately 10% (reliable statistics are not available in Pakistan)[7]. Hence, the present study was also planned to search for the effective cure of hepatitis, to serve the ailing humanity and to make use of the natural herbal wealth (plant source of drugs) of the country. So, to contribute to the improvement of economy of the country by involving the industrial use of natural drugs and saving the foreign exchange presently being spent on the import of different synthetic drugs.

MATERIALS AND METHODS

Animals: Adult healthy albino male rabbits weighing 1.0-1.5 kg each were used in this study. The animals were housed under conditions of 23±12°C temperature, 55±15% humidity and 12 h light (7.00-19.00)[8]. They were fed according to a strict schedule with green fodder (*Medicago sativa*). Normal tap water was allowed *ad libitum*[9-11]. The animals were divided randomly into different groups, 6 rats each. The animals were used in this study according to the guidelines of National Policy.

Chemicals: All chemicals used were of analytical grade and obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). The toxic drug, Cadmium chloride was provided by E. Merck (Darmstadt, FRG).

Plant drug and the extracts: RS was purchased from local market (Bahawalpur), dried and powdered in an electric grinder. The finely powdered drug (1.0 kg) was macerated in about 2.0 L of distilled water and ethanol separately and extracted at room temperature 3 times. The combined

Corresponding Author: Rifat-uz-Zaman, Department of Pharmacy, University of Sargodha, Sargodha, Pakistan
E mail: zamaniub@yahoo.com

filtrates were evaporated by a rotary evaporator. Both aqueous and ethanol extracts were stored at -20°C till used[12,13].

Treatments: The hepatocytes necrosis and apoptosis was induced by i.v. administration of Cd[14,15]. Its toxic dose was also determined (data not shown).

Finely powdered plant drug in the doses of 0.5, 1.0 and 1.5 g kg^{-1} and its extracts (equivalent to 1.5 g kg^{-1} of powder) were given to the treated groups of animals by oral route t.d.s for two weeks in the gelatine shells[13]. The treated control group received placebo drug according to the same schedule. The animals were administration, a single dose of Cd (2.45 mg kg^{-1}, i.v.) on 15th day[14,15]. The blood samples were drawn at 0, 2, 4, 8, 24 and 48 h intervals post administration of toxic agent for the biochemical evaluations.

Determination of serum total bilirubin level: Two hundred μL fresh serum was added in each test tube labeled blank (B) and sample (S). In the S-tube 2.4 ml of 0.13 ml L^{-1} caffeine benzoate solution and 0.2 ml of diazo reagent were added. While 2.4 ml of distilled water and 0.3 ml of sulfanilic reagent (29 mmol L^{-1} sulfanilic acid in 0.17 mol L^{-1} HCl) was added in the B-tube. The contents were mixed and the absorbance of sample was read against blank at 530 nm[16].

Determination of serum ALT activity: ALT (GPT) activity in serum was measured by the method of Reitman and Frankle[17]. 0.5 ml of ALT substrate in each S and B labeled test tubes was added at 37°C. One hundred μL serum in S-tube and 100 μL distilled water in B-tube were added. Then 0.5 ml 2, 4-DNPH reagent was mixed to contents of each tube following 1.0 h incubation. After further 10 min incubation at 37°C 5.0 ml of sodium hydroxide 0.4 mol L^{-1} was added to each tube. The contents were mixed and the absorbance of sample against blank was read at 505 nm.

Determination of serum AP activity: Serum AP activity was determined according to the method of Rick[18]. The rate of change of absorbance/minute was determined following the addition of 2.5 ml of p-Nitro phenyl phosphate 10 mmol L^{-1} buffered with Diethanolamine 1.0 mmol L^{-1}, pH 9.8 and Magnesium chloride 0.5 mmol L^{-1} to the 50 μl of serum. AP activity was obtained by multiplying with a factor (f = 2754).

RESULTS AND DISCUSSION

Cadmium chloride (Cd) was given to a group of treated control rabbits in a dose of 2.45 mg kg^{-1}, i.v. This treatment elevated highly significantly ($P<0.001$) the serum total bilirubin level in comparison with the 0 h values of treated control groups of rabbits. Mean maximum effect of the treatment was attained after 4 h intervals which was subsided 48 h post administration of Cd (Table 1). *Raphanus sativus* fruit powder (RS) was administered in three different doses (0.5, 1.0 and 1.5 g kg^{-1} of body wt.) in addition to Cd (2.45 mg kg^{-1}, i.v.) in three different groups of treated rabbits. RS inhibited the serum levels of total bilirubin induced by Cd in a dose-dependent manner in the treated animals. The inhibitory effects were found more with the dose of 1.5 g kg^{-1}. Mean maximum effects of the treatments were attained after 4 h intervals (Table 1). Aqueous extract of *Raphanus sativus* fruit (RS-A) and ethanol extract of *Raphanus sativus* fruit (RS-E) equivalent to 1.5 g kg^{-1} of the powder produced the similar high significant effects in the Cd-treated rabbits (Fig. 1). The rise in serum total bilirubin is a common indicator of jaundice/hepatic damage, that's why Miller *et al.*[19] have been used serum bilirubin as indices of hepatotoxicity[20,21]. The treated control rabbits showed high significant ($P<0.001$) elevation in the serum ALT level induced by Cd (2.45 mg kg^{-1}, i.v.) in comparison with their 0 h values. The mean maximum level was attained at 4.00 h while the effect was completely abolished at 48 h post treatment of Cd (Table 2). RS attenuated the effects on serum levels of ALT, induced by Cd in a dose-dependent manner. The maximum inhibitory effects were found with the dose of 1.5 g kg^{-1}. Mean maximum effects of the treatments were established after 4 h intervals (Table 2). RS-A and RS-E (equivalent to 1.5 g kg^{-1} of powder) caused similar highly significant effects in the Cd-treated rabbits (Fig. 2).

A high significant ($P<0.001$) elevation in the serum AP level was also noted in the treated control group of rabbits caused by Cd (2.45 mg kg^{-1}, i.v.) in comparison with the 0 h values. Mean maximum elevation was found after 4 h of administration of Cd which returned to the normal values after 48 h interval (Table 3). RS successfully blocked the effects of Cd in a dose-dependent manner on serum levels of AP. The maximum inhibitory effects were produced with the dose of 1.5 g kg^{-1} and mean maximum effects of the treatment

Table 1: Effects of different doses of *Raphanus sativus* fruit powder on serum total bilirubin at different time intervals in cadmium chloride-treated rabbits

Group No.	Treatments	Serum total bilirubin concentration at different time intervals (h) 0	2	4	8	24	48
01	Cadmium chloride (2.45 mg kg^{-1}, i.v.)	7.18±0.43	13.18±1.28**	19.22±1.61**	17.25±1.87**	13.25±2.05**	7.19±0.44
02	*Raphanus sativus* fruit (0.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	6.62±0.42	9.00±1.06*	9.37±0.93*	9.17±0.60**	7.02±0.31	6.28±0.32
03	*Raphanus sativus* fruit (1.0 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	7.55±0.47	8.31±0.80	8.14±0.55	8.05±0.51	10.43±1.47	6.56±0.59
04	*Raphanus sativus* fruit (1.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	6.51±0.35	6.54±0.36	6.76±0.30	6.76±0.31	6.69±0.33	6.47±0.35

Table 2: Effects of different doses of *Raphanus sativus* fruit powder on serum ALT at different time intervals in cadmium chloride-treated rabbits

Group No.	Treatments	Serum ALT concentration/activity at different time intervals (h) 0	2	4	8	24	48
01	Cadmium chloride (2.45 mg kg^{-1}, i.v.)	12.83±1.450	25.50±2.61**	47.50±2.81**	36.17±2.8**	27.00±2.24**	13.67±1.200
02	*Raphanus sativus* fruit (0.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	12.00±1.710	22.50±1.69**	29.00±0.86**	34.00±1.39**	29.17±2.50**	12.17±1.620
03	*Raphanus sativus* fruit (1.0 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	12.00±0.073	20.33±0.48**	24.00±0.76**	38.17±0.81**	31.67±0.50**	11.83±0.634
04	*Raphanus sativus* fruit (1.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	12.17±1.710	14.50±1.69	15.67±0.86	21.50±1.39**	22.50±2.50**	10.33±1.620

Cadmium chloride: significant from 0 h (Normal) reading* P<0.05; ** P<0.001
Test drugs: significant from treated control (Cadmium chloride) * P<0.05; ** P<0.001
All the other values are N.S. from treated control (Cadmium chloride)
Mean±S.E.M = Mean values±Standard error of means of six experiments

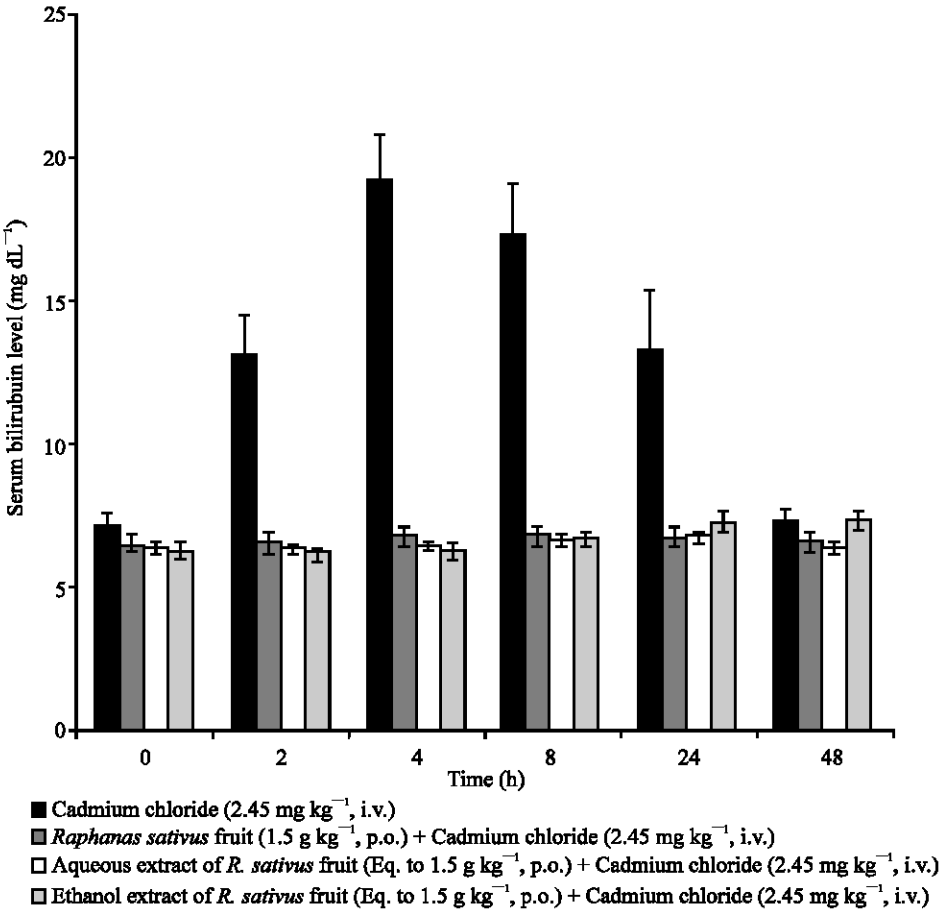

Fig. 1: Comparative effects of cadmium chloride, *Raphanus sativus* its aqueous and ethanol extracts at different time intervals on serum total bilirubin in treated rabbits

Table 3: Effects of different doses of *Raphanus sativus* fruit powder on serum AP at different time intervals in Cadmium chloride-treated rabbits

Group No.	Treatments	Serum AP activity at different time intervals (h) 0	2	4	8	24	48
01	Cadmium chloride (2.45 mg kg^{-1}, i.v.)	46.33±2.42	62.33±2.25**	74.00±2.13**	65.17±2.52**	50.33±4.62*	46.67±2.70
02	*Raphanus sativus* fruit (0.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	56.00±1.30*	64.67±2.00**	74.67±3.20**	144.50±4.50**	165.00±5.60**	63.50±2.60*
03	*Raphanus sativus* fruit (1.0 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	56.20±1.70*	61.80±1.20**	70.70±1.40**	127.00±1.60**	116.20±2.90**	58.50±2.10
04	*Raphanus sativus* fruit (1.5 g kg^{-1}, p.o.)+ Cadmium chloride (2.45 mg kg^{-1}, i.v.)	53.20±2.20	54.80±2.20	56.20±2.80	61.00±2.70*	57.50±3.80	52.50±2.00

Cadmium chloride: significant from 0.00 h (Normal) reading* P<0.05; ** P<0.001
Test drugs: significant from treated control (Cadmium chloride) * P<0.05; ** P<0.001
All the other values are N.S. from treated control (Cadmium chloride)
Mean±S.E.M = Mean values±Standard error of means of six experiments

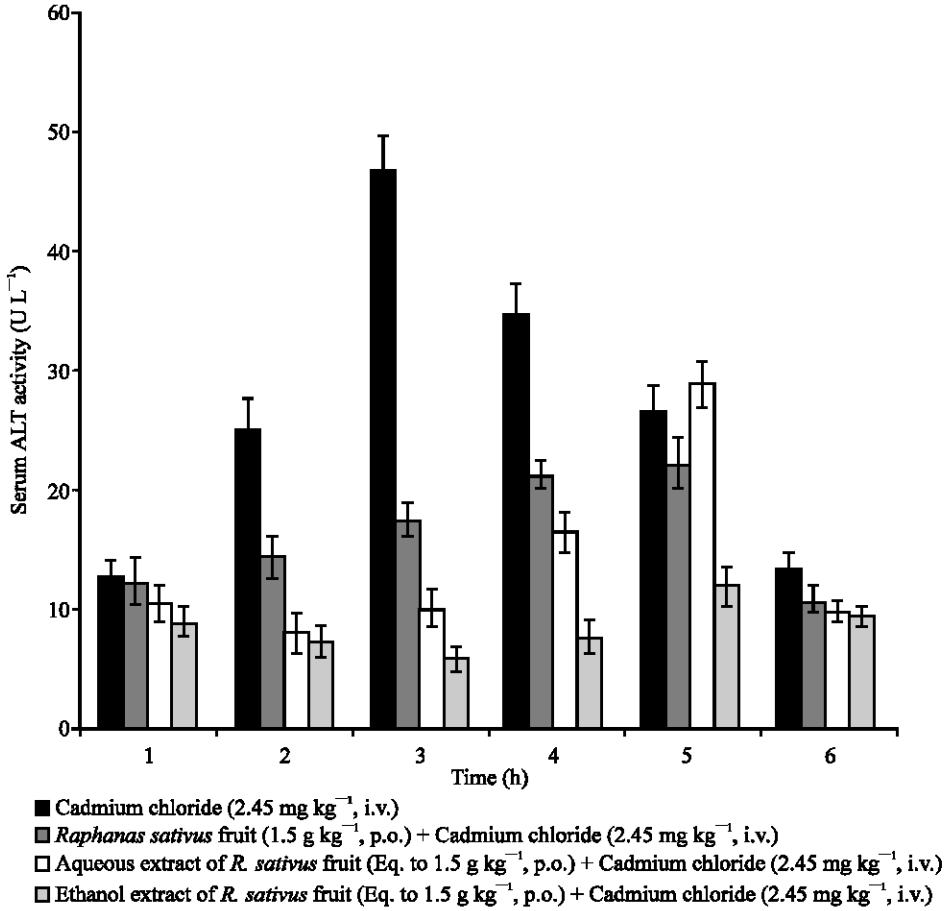

Fig. 2: Comparative effects of cadmium chloride, *Raphanus sativus* its aqueous and ethanol extracts at different time intervals on serum ALT in treated rabbits

was found after 4 h intervals (Table 3). The similar highly significant inhibitory effects were caused by RS-A and RS-E (equivalent to 1.5 g kg^{-1} of powder) in the Cd-treated rabbits (Fig. 3).

The heavy metal Cd causes hepatotoxicity upon acute administration[9]. Kupffer cells, the resident macrophages of the liver, have been suggested to play a role in Cd-induced hepatotoxicity[14,15]. Previous reports suggested a major role for inflammation in acute Cd-induced hepatotoxicity. It is produced multifocal hepatocellular necrosis and increased plasma ALT activity. The hepatotoxicity involves the inhibition of Kupffer cell function which results in a decreased inflammatory response and an altered progression of hepatic injury. It has further been indicated that Kupffer cell function is critical to cadmium induced hepatocellular necrosis[22-25].

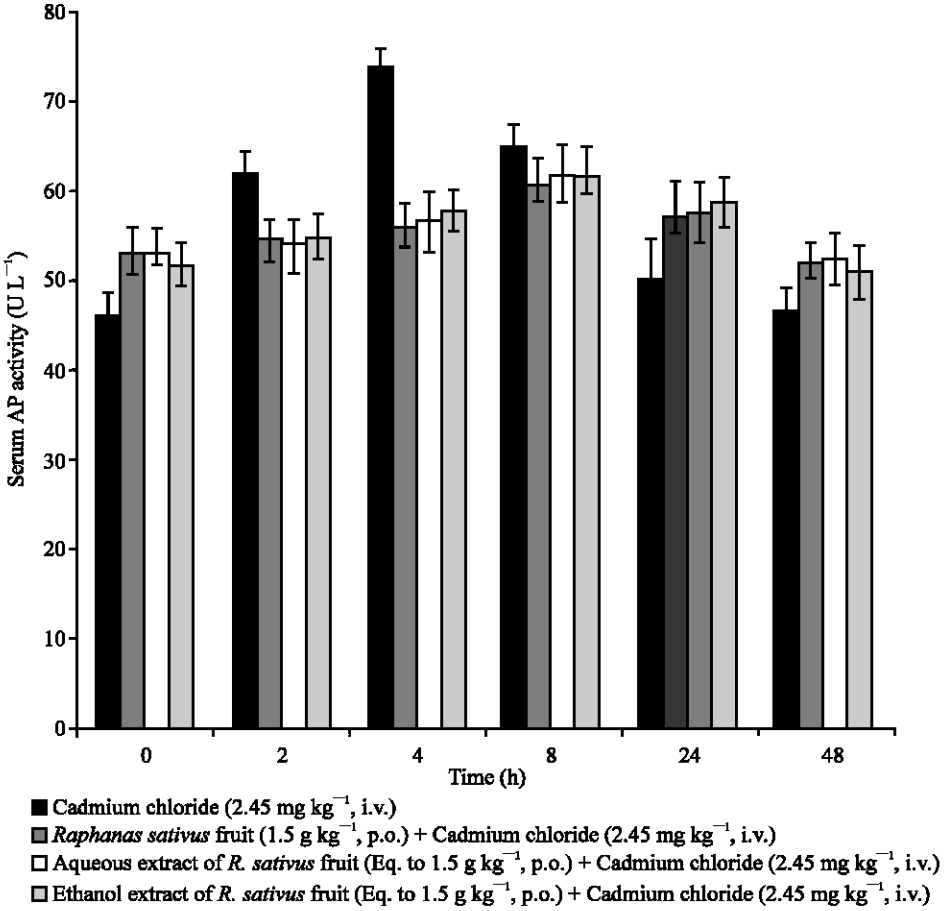

Fig. 3: Comparative effects of cadmium chloride, *Raphanus sativus* its aqueous and ethanol extracts at different time intervals on serum AP in treated rabbits

According to the de la Fuente *et al.*[26] and Chuang *et al.*[27] Cd induces apoptosis of lymphoid cells and they suggested that this phenomenon may contribute to its immunotoxic effect *in vivo*[28-30]. The organ dysfunction is usually attributed to cell death caused by over production of free radicals derived from inflammation where expression and release of proinflammatory tumour necrosis factor-alpha (TNF-α) rapidly increases and the formation of free radicals e.g., superoxide anion [O_2^-] and nitric oxide [NO^-] are inevitably overproduced[31].

Therefore, the higher level of serum enzymes i.e., ALT and AP activities and serum total bilirubin are the characteristics of hepatic damage[20,21]. It seems the RS, RS-A and RS-E interfere with the inflammatory, necrotizing effects or apoptosis induced by Cd because the release of enzymes i.e., ALT and AP, from hepatocytes and serum elevation of bilirubin were inhibited successfully in the experimental animals.

Data further suggested that RS, RS-A and RS-E have protected the tissues i.e., liver, against damage or necrosis induced by toxic agent i.e., Cadmium chloride and the findings are in accordance with Vitoria[32] and Takaya *et al.*[33]. The recommended dose is 1.5 g kg^{-1} of the body weight for RS and RS-A and RS-E (equivalent to the 1.5 g kg^{-1} of the body weight of powdered). Further investigation may be done to evaluate the *R. sativus* as hepatoprotective tool.

REFERENCES

1. Sipos, P., K. Hasgymasi, A. Lugasi, E. Feher and A. Blazovics, 2002. Effects of black radish root (*Raphanus sativus* L. var niger) on the colon mucosa in rats fed a fat rich diet. Phytother. Res., 16: 677-9.
2. Murty, S., S.S. VA and S.N. Subrabmanyam, 1989. A Text Book of Economic Botany. Wiley Eastern Ltd. New Dehli, India, pp: 649-50.
3. Kirtikar, R.K. and D.B. Basu, 1987. Indian Medicinal Plants. 2nd Edn. International Book Distributors, 9/B, Rajpur Road, 1st floor, Dehadun 28001, India, pp: 178-80.

4. Fant, F., W. Vranken, W. Broekaert and F. Borremans, 1998. Determination of the thee-dimensional solution structure of *Raphanus sativus* antifungal protein 1 by 1H NMR. J. Mol. Biol., 29: 279: 257-70.
5. Terras, F., H. Schoofs, K. Thevissen, R.W. Osborn, J. Vanderleyden, B. Cammue and W.F. Broekaert, 1993. Synergistic enhancement of the antifungal activity of wheat and barley thionins by radish and oilseed rape 2S albumins and by barley trypsin inhibitors. Plant Physiol., 103: 1311-1319.
6. Tawfik, N.I., B.A.H. El-Tawil, A.H. El-Refai, A.A. Khalaf and A.M. Khalil, 1978. Constituents of local plants. Part 1. Chemical investigations on some cultivated Saudi Arabian plants. Qual. Plant Foods Hum. Nutr., 28: 203-10.
7. CDC., 1990. Protection against viral hepatitis: recommendations of the Immunization Practice Advisory Committee (ACIP). MMWR., 39: 8-22.
8. Sorba, G., A. Gasco, G. Coruzzi, M. Adami, C. Pozzoli, G. Morini and G. Bertaccini, 1997. Mixed anti-secretary and gastroprotective activities of a new H_2-antagonist containing a nitric oxide-donor furoxan moiety. Arzneim-Forsch/Drug Res., 47: 849-54.
9. Ishizuka, Y., T. Kamisai and M. Sato, 1996. Anti-gastric acid secretary mechanism of 1, 6-Dihydro-2-[2-(2-methylpropoxy) anilino]-6-oxo-5-pyrimidinecarboxylic acid. Arzneim-Forsch/Drug Res., 46: 919-22.
10. Sckiguchi, H., K. Hamada, H. Aijima, F. Taga, H. Uchida and K. Nishino, 1993. Healing-promoting action of the new histamine H_2-receptor n-ethyl-[3-[3-(piperidinomethyl) phenoxy] propyl] urea with dual action on chonic gastric and duodenal ulcers induced by acetic acid in rats. Arzneium-Forsch/Drug Res., 43: 139-43.
11. Xiao-Bo, S., M. Tsukasa and Y. Haruki, 1992. Purification of anti-ulcer polysaccharide from the leaves of *Panax ginseng*. Planta Medica, 58: 445-448.
12. Akhtar, M.S. and M. Munir, 1989. Evaluation of the gastric anti-ulcerogenic effects of *Solanum nigram*, *Brassica oleracea* and *Ocimum basilicum* in rats. J. Ethnopharmacol., 27: 163-76.
13. Rifat-uz-Zaman, 2004. Study of cardioprotective activity of *Raphanus sativus* L. in the rabbits. Pak. J. Biol. Sci., 7: 843-847.
14. Basinger, M.A., M.J. Mark, D.C. Warren, M. Ernest, Jr. Walker and S. Melinda, 1987. Chelating-agent suppression of cadmium-induced hepatotoxicity. Toxicol. Environ. Helth., 22: 261-72.
15. Harstad, E.B. and C.D. Klaassen, 2002. Gadolinium chloride pretreatment prevents cadmium chloride-induced liver damage in both wild-type and MT-null mice. Toxicol. Appl. Pharmacol., 180: 178-85.
16. Henry, R.J., 1964. Method for Determination of Total Bilirubin. Principles and Techniques. In: Clinical Chemistry. Harper and Row. Pub., Inc., New York.
17. Reitman, S. and F. Frankel, 1957. Method for determination of transaminases. Am. J. Clin. Pathol., pp: 28-58.
18. Rick, W., 1977. Method for Determination of Alkaline Phosphatase. *Klinsche* Chimie and Mikroskopie Sringer Verlag, Berlin, 5: 242.
19. Miller, M.G., F.P. Veronica and J.D. David, 1986. Poisonous susceptibility of the fasted hamster to acetaminophen hepatotoxicity. Biochem. Pharmacol., 35: 817-26.
20. Dobbs, N.A., C.J. Twelves, W. Gregory, C. Cruickshanka, M.A. Richards and R.D. Rubens, 2003. Epirubicin in patients with liver dysfunction. Development and evaluation of a novel dose modification scheme. Eur. J. Cancer., 39: 580-6.
21. Xu, Q., Z. Lu and X. Zhang, 2002. A novel role of alkaline phosphatase in protection from immunological liver injury in mice. Liver, 22: 8-14.
22. Sauer, J.M., M.P. Waalkes, S.B. Hooser, A.T. Baines, R.K. Kuester and I.G. Sipes, 1997. Tolerance induced by all-trans-retinol to the hepatotoxic effects of cadmium in rats: role of metallothionein expression. Toxicol. Appl. Pharmacol., 143: 110-9.
23. Sauer, J.M., M.P. Waalkes, S.B. Hooser, R.K. Kuester, C.A. McQueen and I.G. Sipes, 1997. Suppression of Kupffer cell function prevents cadmium induced hepatocellular necrosis in the male Sprague-Dawley rat. Toxicology, 121: 155-64.
24. Greabu, M. and R. Olinescu, 2002. The formation of oxidative stress condition in the experimental chemically induced hepatotoxicity. Rocz. Akad. Med. Bialymst, 47: 86-94.
25. Marinho, R., R. Pinto, M.L. Santos and M. de, 2002. Evidence for prostaglandin-producing supressor cells in HCV patients with normal ALT. Dig. Dis. Sci., 47: 556-61.
26. de la Fuente, H., D. Portales-Perez, L. Baranda, F. Diaz-Barriga, V. Saavedra-Alamis, E. Layseca and R. Gonzalez-Amaro, 2002. Effect of arsenic, cadmium and lead on the induction of apoptosis of normal human mononuclear cells. Clin. Exp. Immunol., 129: 69-77.
27. Chuang, S.M., I.C. Wang, Y.S. Hwua and J.L. Yang, 2003. Short-term depletion of catalase suppresses cadmium-elicited c-Jun N-terminal kinase activation and apoptosis: role of protein phosphatases. Carcinogenesis, 24: 7-15.
28. Ferre, N., J. Camps, M. Cabre, A. Paul and J. Joven, 2001. Hepatic paraoxonase activity alterations and free radical production in rats with experimental cirrhosis. Metabolism, 50: 997-00.

29. Hoglen, N.C., B.P. Hirakawa, C.D. Fisher, S. Weeks, A. Srinivasan, A.M. Wong, K.L. Valentino, K.J. Tomaselli, X. Bai, D.S. Karanewsky and P.C. Contreras, 2001. Characterization of the caspase inhibitor IDN-1965 in a model of apoptosis-associated liver injury. J. Pharmacol. Exp. Ther., 297: 811-8.
30. Xue, F., T. Takahara, Y. Yata, M. Minemura, C.Y. Morioka, S. Takahara, E. Yamato, K. Dono and A. Watanabe, 2002. Attenuated acute liver injury in mice by naked hepatocytes growth factor gene transfer into skeletal muscle with electroporation. Gut, 50: 558-62.
31. Liaw, W.J., C. Tzao, J.Y. Wu, S.J. Chen, J.H. Wang and C.C. Wu, 2003. Inhibition by terbutaline of nitric oxide and superoxide anion levels of endotoxin-induced organs injury in the anesthetized rat. Shock, 19: 281-8.
32. Vitoria, A.P., P.J. Lea and R.A. Azevedo, 2001. Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry, 57: 701-10.
33. Takaya, Y., Y. Kondo, T. Furukawa and M. Niwa, 2003. Antioxidant constituents of radish sprout (Kaiware-daikon), *Raphanus sativus* L. J. Agric. Food Chem., 51: 8061-8066.