International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 6 (6): 896-902, 2010
ISSN 1811-7775

Evaluation of Antihepatotoxic Effect of Watercress Extract and its Fractions in Rats

[1,2]A.R. Ebadollahi Natanzi, [1]M.H. Ghahremani, [3]H.R. Monsef Esfehani,
[4]M.B. Minaei, [2]H. Nazarian and [1]O. Sabzevari
[1]Department of Toxicology and Pharmacology, Faculty of Pharmacy,
Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Medicinal Plants, Natural Resources, Agriculture Center for Higher Education,
Agriculture Research, Education and Extension Organization, Karaj, Iran
[3]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Histopathology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract: In this study, *Nasturtium officinale* R. Br. (watercress), an edible plant from cruciferous plants and its three fractions were screened for their hepatoprotective effects against acetaminophen induced toxicity in rats. The total parts of plant were extracted with alcohol-water and then sequentially fractionated. The total extract and its three fractions namely; petroleum ether; *n*-butyl alcohol; and aqueous at two doses (50 and 175 mg kg^{-1}) were administrated to animals every 24 h for five successive days. Rats treated as post treatment with the total extract or three fractions at the dose of 175 mg kg^{-1} did not show any significance change in activity of aminotransferse enzymes (AST and ALT) compared to control. Acetaminophen caused significant hepatocellular damage and increase in serum levels of AST, ALT and LDH in comparison to control group ($p<0.001$). Post treatment with the total extract (175 mg kg^{-1}) and aqueous fraction (50 mg kg^{-1}) significantly prevented acetaminophen induced rise in serum levels of AST, ALT and LDH. LD_{50} value of the petroleum fraction was more than 3823 mg kg^{-1}, while the other two fractions and total extract were non-toxic up to 5734 mg kg^{-1}. Histopathological changes of liver induced by acetaminophen were considerably reversed following treatment by aqueous fraction and total extract of *N. officinale*. The findings of this study showed that *N. officinale* may play a protective role against acetaminophen-induced hepatotoxicity through maintaining the normal liver functions which further validates the use of *N. officinale* in Iranian traditional medicine as a hepatoprotective agent.

Key words: *Nasturtium officinale*, hepatoprotection, sequential fractions, paracetamol, hepatotoxicity

INTRODUCTION

One of the main duties of the liver is to detoxify xenobiotics entered deliberately or unintentionally to the body through Phase I and II processes which makes them ready for elimination (William, 1971; Guengrich, 2000). The human body encounters with the plenty of compounds which are naturally lipophilic and should be excreted by liver organ. Acetaminophen is an analgesic antipyretic medicine (with therapeutic doses ≤4 g day^{-1} for an adult) is mostly metabolized by liver through glucuronidation and sulfation pathways (Moling *et al*., 2006). This compound has been shown to be metabolically transformed to a reactive intermediate namely *N*-acetyl parabenzoquinoneimine, which can cause liver damage if is not detoxified (Dahlin *et al*., 1984; Zhang *et al*., 2004). The toxic metabolite production will increase following exposure to high levels of the parent compound while alternative detoxification mechanisms are compromised (Chen *et al*., 2009). The compounds presently used in hepatotoxicity have inadequate effects and sometimes accompanied with some serious adverse effect. N-acetylcysteine (NAC) which is widely used as an antidote to acetaminophen overdose can cause some adverse effects (Kanter, 2006). Therefore, an extensive search is focused on an alternative remedy which can be used more efficiently and safely in liver damages. Fortunately, the use of medicinal plants for liver diseases is growing at present. Among the families of medicinal plants, there are some plants belonging to Asteraceae; Zingiberaceae, Euphorbiaceae, Scrophulariaceae; Maringaceae; Acanthaceae families and the recent researches carried out on them; have indicated that they could in different ways effectively protect liver from

Corresponding Author: Omid Sabzevari, Department of Toxicology and Pharmacology, Faculty of Pharmacy,
Tehran University of Medical Sciences, Tehran-14155/6451, Iran Tel/Fax: +9821 6649 4994

active metabolites of acetaminophen and free radicals produced by carbon-tetra chloride (Prakash *et al*., 2008; Fakurazi *et al*., 2008, 2009; Basu *et al*., 2009) The plant *Nasturtium officinale* R. Br. (Brassicaceae) with common name watercress grows in north east of Iran, where the altitude is around 1300-1400 m and is used by Iranian as a salad vegetable. Two species of *Nasturtium* genus can be found in Iran; *Nasturtium officinale* and *Nasturtium microphyllum* Boenn. ex Rchb. (Brassicaceae). Little knowledge is available about protective effect of this plant. However, the plants of brassicaceae family have substantially bioactive components such as glucosinolates which can be converted by enzymatic breakdown to more bioactive products, like isothycyanates which have been reported to have a chemo preventive role (Hayes *et al*., 2008). In most literatures the anti cancer effects for watercress have been reported (Lhoste *et al*., 2004; Steinbrecher *et al*., 2009; Hofman *et al*., 2009; Konsue and Ioannides, 2010). Although, some information about its protective effect such as antioxidant activity (Ozen, 2009) and anti hyperlipidimia (Bahramikia and Yazdanparast, 2008) have been published; the studies on hepatoprotective effect are so limited. In addition, there is not distinct; which fraction(s) of watercress have a protective role. In this study, we report hepatoprotective effect of the total extract and the aqueous fraction of *N. officinale* (watercress) through a series of screening tests in whole animals. The study aims on the fraction which can efficiently prevent the liver injury resulted from acetaminophen.

MATERIALS AND METHODS

Plant collection: The whole plants of *N. officinale* were collected in April 2007 from north eastern part of Iran; the Ahar region in East Azerbaijan province, in altitudes of 1300 m. A sample was authenticated by Dr. Nazarian the botanist of Agriculture centre for higher education and a voucher specimen was preserved at Herbarium of Higher Education Center of ministry of Agriculture, Karaj, Iran (HHECA No. 3550).

Chemicals: The solvents including *n*-butyl alcohol, petroleum ether, methanol, ethanol, tween 80 and acetaminophen were supplied by Merck Company (Darmstadt, Germany). Methyl cellulose was obtained from Sigma Chemicals Company (St. Louis, MO, USA). All chemicals used were of the highest purity grade.

Total extract: The whole parts of plant washed and cleaned with plenty of water, then heated in alcohol to inhibit the enzyme myrozinase which is responsible for glucosinlate hydrolysis (Harbon, 1998). The total part of plant was dried and 100 g of the powdered plant was macerated with 80% ethanol solution by 4-6 times extraction within three days. The resulting extract was filtrated and then evaporated by rotary instrument under reduced pressure. The dried powder resulted from this extract was 3.20 g.

Liquid sequential extracts: A 35 g sample of the extract was emulsified with 8 volumes of high quality distillated water (w/v). The sequential fractions were obtained by increasing solvents polarity: (A) petroleum ether fraction (6.78 g of dry weight corresponding to 20.22% sample), (B) *n*-butyl alcohol fraction (6.27 of g dry weight corresponding to 18.70% of sample) and (C) aqueous fraction (19.68 g dry weight corresponding to 58.69% of sample).

Animals: All animal experiments were conducted according to the policy of MOH Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes and the protocol was approved by the Ethics Committee of the Tehran University of Medicine Sciences (TUMS), Tehran, Iran. Male Wistar rats weighing 150-180 g prepared by Pasteur's Institute (Tehran, Iran) were used through this study. The animals were kept under 12/12 h light dark in Animal Care Center, Faculty of Pharmacy, TUMS. The rats were allowed free access to standard laboratory feed and water before experiment. Liver injury was produced in the 12 h fasted rats.

Acute toxicity: The up and down procedure (OCED, 2001), employed with sequential doses using Albino Wistar rats (n = 4) in each step. Rats received total extract of *N. officinale* plant and its three fractions intraperitoneally (i.p.). The starting dose was 175 mg kg^{-1} and the default dose spacing was 3.2 times the pervious.

***In vivo* hepatotoxicity studies:** Impact of total extract and the fractions on activity of aminoterasferase enzymes were studied using four concentrations of 175, 560, 1792 and 5734 mg kg^{-1} obtained from the acute toxicity study. The extract and fractions intraperitoneally administered as a single dose to male rats. After 24 h, the animals bloods were collected by heart puncture and blood serum separated by centrifuging at 2000 g for 20 min. The AST and ALT activities determined using Elitech Diagnostics kit (Sees, France). The maximum dose used for the petroleum ether fraction was 3823 mg kg^{-1}.

***In vivo* hepatoprotective studies:** The hepatoprotective effect of the total extract and fractions were studied intraperitoneally at two doses of 50 and 175 mg kg^{-1}. The rats were divided into 6 groups of 4 animals: Group 1-4 received total extract; petroleum ether, aqueous and n-butanol fractions, respectively at both dose levels. Group 5 defined as positive control and received a single dose of acetaminophen i.p. (750 mg kg^{-1}); Group 6 served as vehicle blank and received the vehicle only (Tween 5%; 5 mL kg^{-1}). The hepatic injury induced in all rats (except the blank group) by administration of acetaminophen (150 mg mL^{-1}, 5 mL kg^{-1}, i.p.; 750 mg kg^{-1}) which was suspended in 1% methylcellulose. One hour after induction of toxicity by acetaminophen, the total extract and fractions of *N. officinale* were administrated to animals of in groups 1-4. In positive control group (group 5), methylcellulose replaced for acetaminophen. Twenty four hour later all groups except groups 5 and 6 received total extract and fractions of *N. officinale*. Groups 5 and 6 received 5% tween 80. The procedure was continued for 5 days. 24 h after the last treatment, animals were anesthetized with ketamine (75 mg kg^{-1}, i.p.) and blood samples were collected in tubes and centrifuged at 2000 g for 15 min to obtain serum. Activities of aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehyrogenase (LDH) were spectrometrically measured using Elitech Diagnostic kit.

Light microscopy: The liver tissues were completely excised and the samples were taken from left lobules of liver (about 5 mm) and placed in 10% neutral buffered formalin. The samples were then cut into small pieces; the sections prepared and stained by eosin-hematoxylin and consequently examined for any histopathological changes. Histological studies were scored 0-3 according to kupffer cell status, fatty changes (micro and macro structures), necrosis, infiltration of mononuclear cells and sinusoids extension.

Statistical analysis: The results are expressed as Mean±SD and all comparisons were made by ANOVA which followed by Student-Newman-Keuls test. p-values less than 0.05 considered significant. Fisher's exact test was also used to evaluate significance level in histopathology examinations.

RESULTS

The acute toxicity studies of *N. officinale* total extract using the up and down procedure (Guideline OECD 425) in male rats (4 animals in each step) demonstrated that lethal dose of this extract is more than 5734 mg kg^{-1} (Table 1). The LD_{50} for other fractions, except the petroleum ether's, were similar to the total extract. LD_{50} for petroleum ether fraction estimated to be more than 3823 mg kg^{-1}.

The results of hepatotoxicity of total extract of *N. officinale* and its three fractions in male rats have been shown in Table 2. As shown, rats treated with the total extract or three fractions at the dose of 175 mg kg^{-1} did not show any significance change in activity of aminotransferse enzymes (AST and ALT) compared to control (Table 2). The first dose of total extract and two fractions (n-butyl alcohol and aqueous fractions) which induced hepatotoxicity in rats was 5734 mg kg^{-1} as noticed by a significant difference in AST and ALT values to the control group ($p<0.001$, Table 2). For the petroleum fraction the significance difference was observed at 3823 mg kg^{-1} dose level (Table 2). The results obtained from measurement of acetaminophen induced toxicity have been summarized in Table 3. As illustrated in this Table 3, rats received acetaminophen without any treatment, developed significant hepatocellular damage, indicating a significant increase in serum levels of AST, ALT and LDH in comparison to control group ($p<0.001$, Table 3). Effect of post-treatment with the total extract and fractions of *N. officinale* plant at 50 and 175 mg kg^{-1} doses, on serum activities of hepatospecific enzyme were also demonstrated in Table 3. The results showed that the total extract of *N. officinale* plant and the aqueous fraction have exhibited significant reduction ($p<0.001$) in acetaminophen-induced elevation of serum AST, ALT and LDH (Table 3).

Table 1: Histopathological effects of total extract of *Nasturtium officinale* plant and its three fractions on rat livers at acute toxicity examinations

Histopathological change	Control	TEN[1] 5734 (mg kg^{-1})	AFN[2] 5734 (mg kg^{-1})	PFN[3] 3823 (mg kg^{-1})	n-ButF[4] 5734 (mg kg^{-1})
Increased kupffer cells	+	++	+++	++	+++
Infiltration of mononuclear cells	-	*+++	-	*+++	*+++
Extended sinusoids	+	++	+++	+++	++
Macro vesicular fatty change	-	++	-	*+++	-
Micro vesicular fatty change	-	++	++	*+++	++
Disarranged hepatocytes	-	++	-	*+++	++
Necrosis	-	-	-	++	-

-: No effect; +: Minor effect; ++: Medium effect; +++: Major effect. [1]TEN; Total Extract of *Nasturtium officinale*, [2]AFN; Aqueous Fraction of *Nasturtium officinale*, [3]PFN; Petroleum Fraction of *Nasturtium officinale*, [4]n-ButF; n-Butyl Fraction of *Nasturtium officinale*.*F value <0.05; significantly different from control (5% Tween 80) using fisher's exact test

The results of histopathological studies and the appearance of liver sections after post treatment of total extract of *N. officinale* and the three fractions have been delineated in Fig. 1a-d. As illustrated in Fig. 1a-d histopathological changes including centrilubular necrosis, extensive portal inflammation, micro and macro vesicular structures were reduced using aqueous fraction and total extract of *N. officinale* compared to acetaminophen group (Fig. 1b-d and 1e-f, Table 3). At the dose of 175 mg kg^{-1}, total extract significantly reduced ALT, AST and LDH serum levels (27.59, 32.69 and 38.64%, respectively). The reduction of ALT and AST levels by total extract was less than that of the aqueous fraction (Table 3).

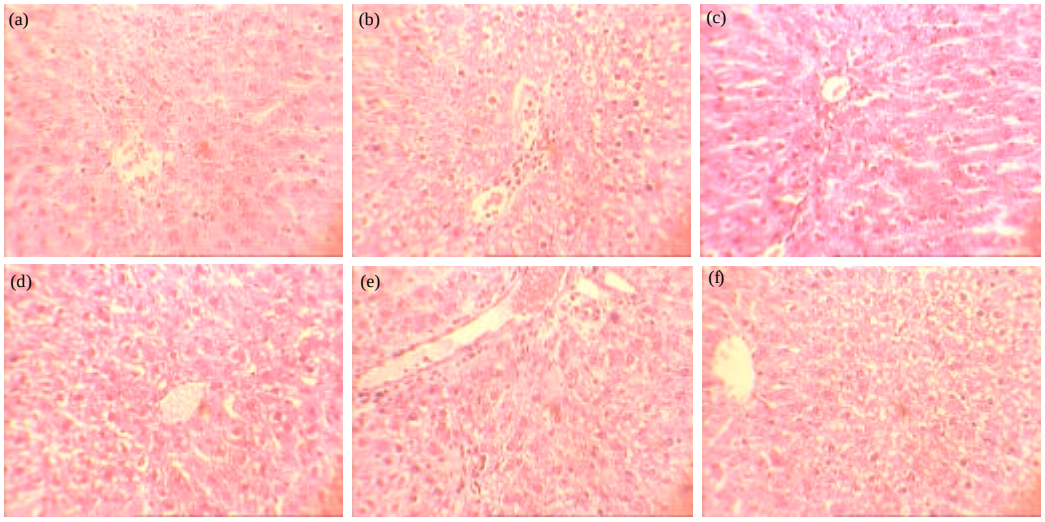

Fig. 1: The light micrographs of hepatic sections obtained from rats. (a) 5% Tween 80 treated (Normal) rats, (b) Acetaminophen (750 mg kg^{-1}) intoxicated rats (Positive control); (c) Acetaminophen plus 175 mg kg^{-1} of total extract of *Nasturtium officinale* plant; (d) Acetaminophen plus 50 mg kg^{-1} of aqueous fraction of alcoholic extract of *Nasturtium officinale* plant; (e) Acetaminophen plus 50 mg kg^{-1} of petroleum ether fraction of alcoholic extract of *Nasturtium officinale* plant; (f) Acetaminophen plus 50 mg kg^{-1} of *n*-butyl alcohol fraction of alcoholic extract of *Nasturtium officinale* plant. Note the necrosis, degenerative changes, foci of fatty changes; micro and macro vesicular next to port region; infiltration along with inflammation and hemorrhage all have been observed in group of Acetaminophen (positive control). Note also the marked protection by 50 mg kg^{-1} of aqueous fraction (d) obtained. No protection, especially against centrilobular necrosis is observed by 50 mg kg^{-1} of Petroleum ether fraction. Magnification: ×40

Table 2: Hepatotoxicity of total extract of *Nasturtium officinale* and its three fractions in male rats

Group	AST (IU L^{-1})	ALT (IU L^{-1})
Control	42.09±1.88	27.53±1.31
Total extract (175 mg kg^{-1})	47.97±1.22	31.62±0.84
Petroleum ether fraction (175 mg kg^{-1})	44.28±3.15	22.56±2.14
n-Butanol fraction (175 mg kg^{-1})	42.81±2.20	28.61±0.54
Aqueous fraction (175 mg kg^{-1})	40.78±1.22	28.01±1.97
Total extract (560 mg kg^{-1})	53.00±2.08*	35.12±1.45†
Petroleum ether fraction (560 mg kg^{-1})	50.18±2.79	26.83±2.59
n-Butanol fraction (560 mg kg^{-1})	46.45±1.92	30.88±1.56
Aqueous fraction (560 mg kg^{-1})	43.56±1.83	29.98±1.13
Total extract (1792 mg kg^{-1})	66.04±4.81**	43.93±3.35**
Petroleum ether fraction (1792 mg kg^{-1})	63.65±3.26**	36.34±3.07*
n-Butanol fraction (1792 mg kg^{-1})	56.26±3.06**	36.67±2.06*
Aqueous fraction (1792 mg kg^{-1})	60.04±2.33**	38.89±1.24**
Total extract (5734 mg kg^{-1})	77.94±1.16**	52.43±0.81**
Petroleum ether fraction (3832 mg kg^{-1})	78.37±3.52**	52.32±2.92**
n-Butanol fraction (5734 mg kg^{-1})	81.36±3.17**	53.56±2.13**
Aqueous fraction (5734 mg kg^{-1})	85.01±3.10**	53.80±4.01**

The results are the Mean±SD from 3-4 rats. †$p<0.05$ compared to control, *$p<0.01$ and **$p<0.001$ compared to control

Table 3: Effects of total extract and three fractions of *Nasturtium officinale* on biochemical parameters in acetaminophen-induced hepatotoxicity in male rats

Group	AST (IU L^{-1})	ALT (IU L^{-1})	LDH (IU L^{-1})
Control	62.39±2.04	32.65±1.70	400.48±44.72
Acetaminophen (750 mg kg^{-1})	93.79±3.73†	51.06±2.83†	629.36±16.91†
Acetaminophen + total extract (50 mg kg^{-1})	73.09±1.55**	40.38±2.08**	538.12±29.87*
Acetaminophen + petroleum ether fraction (50 mg kg^{-1})	126.97±8.48**	53.32±3.78	567.72±34.24
Acetaminophen + *n*-butanol fraction (50 mg kg^{-1})	82.53±1.31	42.99±0.93*	478.90±12.86**
Acetaminophen + aqueous fraction (50 mg kg^{-1})	56.13±3.66**	29.43±1.90**	445.36±24.37**
Acetaminophen + total extract (175 mg kg^{-1})	63.13±2.47**	36.97±1.76**	385.93±29.46**
Acetaminophen + petroleum ether fraction (175 mg kg^{-1})	192.65±10.08**	99.71±3.70**	648.70±30.43
Acetaminophen + *n*-butanol fraction (175 mg kg^{-1})	99.56±5.13	53.34±3.74	683.59±26.38
Acetaminophen + aqueous fraction (175 mg kg^{-1})	96.67±3.72	48.94±2.22	631.83±10.53

The results are the Mean±SD for 4 rats, *$p<0.01$ and **$p<0.001$ compared to acetaminophen, $^{\dagger}p<0.001$, compared to control

DISCUSSION

No lethality were observed for the total extract of *N. officinale* and *n*-butyl alcohol and aqueous fractions up to 5734 mg kg^{-1}; which means they are relatively non toxic (Hodgson and Cunny, 2004). The LD_{50} value for the petroleum ether fraction estimated to be more than 3823 mg kg^{-1}. Higher concentration of the extract was not possible to examine due to solubility limitation resulted from presence of lipophilic substances. At this dose, however, some significant histological changes including macro and micro vesicular structure of fatty changes along with disarrangement of hapatocytes were observed (Table 1). At the dose of 175 mg kg^{-1} no significant change in activity of AST and ALT was observed by the total extract of *N. officinale* and fractions, therefore, it can be concluded that they do not intrinsically induce toxicity.

Acetaminophen or CCl_4 induced hepatotoxicity is a commonly model used for liver damage study and screening the hepatoprotective activity of medicines (Handa *et al*., 1986). Structural damage and the function integrity of liver can be evaluated by a series of enzymes including AST, ALT and LDH (William, 1971; Kew, 2000; Beumer *et al*., 2005; Yemitan and Izegbu, 2006). The increased level of these enzymes activity in serum is a good indicator of liver integrity damage; since these are cytoplasmic enzymes in location and whenever the cellular damage occurs they are released into the circulation (Kew, 2000). Our findings showed that; high dose administration of acetaminophen (750 mg kg^{-1}) in animals causes severe hepatocellular injury as indicated by the significant elevation of ALT, AST and LDH ($p<0.001$, Table 3). At the dose of 50 mg kg^{-1}, aqueous fraction of *N. officinale* markedly reduced the elevation of serum ALT, AST and LDH levels (42.36, 40.15 and 29.24% respectively, $p<0.001$, Table 3). This shows that 5 days administration of the aqueous fraction can protect the animals against acetaminophen induced liver injury. Histological observation of the liver (Fig. 1d) showed preservation of damage in histostructural integrity of the liver cells (hepatocytes) which supports the biochemical data.

At 50 mg kg^{-1}, treatment of animals with the aqueous fraction demonstrated a better cellular protection than the total extract at both histopathological and biochemical evaluations. In this sense, reduction of the more specific hepatocellular damage marker (ALT) was 42.36 % for the aqueous fraction compared to 27.59 % for the total extract. The more effective protection observed by the aqueous fraction could be resulted from the presence of glucosinolate, flavonoids, alkaloids, terpens and other components (such as sinigrin like glucosinolates which was evidenced by TLC, data not shown). Induction of detoxifying enzymes by glucosinolates have been previously indicated (Wallig *et al*., 1998).

The hepatoprotective mechanism of *N. officinale* extract has not clearly defined yet, however, the antioxidant effects of this plant may play a role in the protective mechanisms (Ozen, 2009). Present finding showed that protective effect of watercress will be increased when it was fractionated based on polar solvents. It seems that; the significant reduction in kupffer cells number and also infiltration of mononuclear cells after post treatment of animals with aqueous fraction of watercress in comparison of acetaminophen groups showing this fact; the protective mechanisms of watercress is something besides of antioxidant activity that some literatures mentioned it and can be dependant some what to anti-inflammatory effect. Acetaminophen is mainly metabolized in the liver by glucuronidation and sulfation (Smith *et al*., 1986; Kessler *et al*., 2002). Hepatotoxicity of paracetamol has been attributed to the formation of a toxic reactive metabolite namely; *N*-acetyl-*p*-benzoquinoneimine (NAPQI), by Cytochrome P450 mixed function oxidaze system (Hinson, 1983; Khadr *et al*., 2007; Dahlin *et al*., 1984; Kitteringham *et al*., 1988; Jaeschke *et al*., 2002). This metabolite is usually formed when high doses of acetaminophen is used and cellular glutathione storage is depleted (Vermeulen *et al*., 1992; Reen *et al*., 2001). This highly reactive metabolite is capable of covalent binding to cellular macromolecules (proteins, DNA) to produce protein adducts (Vermeulen *et al*., 1992). In addition to the antioxidant activity of *N. officinale*, the plant extract

may inhibit the formation of the toxic metabolite or enhance the hepatic regeneration to maintain normal liver function in which the latter may play more important role.

CONCLUSION

The result of this study in general showed that, the aqueous fraction and the total extract of *N. officinale* plant at doses of 50 and 175 mg/kg/day, respectively, significantly diminish liver damage of acetaminophen. This study concluded that aqueous fraction of watercress had more protective effect which recommends focus of the future studies on this fraction. The probable mechanism by which watercress exert protective role is to normalize liver functions through stabilization of hepatocellular membranes or antioxidant activity. Further studies are required, however, to postulate hepatoprotective mechanisms of watercress which are currently under progress in our lab.

ACKNOWLEDGMENTS

This research was supported by a grant from the vice chancellor for research, Tehran University of Medical Sciences (TUMS), Tehran, Iran.

REFERENCES

Bahramikia, S. and R. Yazdanparast, 2008. Effect of hydro alcoholic extracts of Nasturtium officinale leaves on lipid profile in high fat diet rats. J. Ethnopharmacol., 115: 116-121.

Basu, S.K., M. Rupeshkumar and K. Kavitha, 2009. Hepatoprotective and antioxidant effect of *Andrographis echioides* N. against acetaminophen induced hepatotoxicity in rats. J. Biol. Sci., 9: 351-356.

Beumer, J.H., J.H.M. Schellens and J.H. Beijnen, 2005. Hepatotoxicity and metabolism of trabectedin: a literature review. Pharmacol. Res., 51: 391-398.

Chen, Y.H., F.Y. Lin, P.L. Liu, Y.T. Huang and J.H. Chiu *et al*., 2009. Antioxidative and hepatoprotective effects of Magnolol on acetaminophen-induced liver damage in rats. Arch. Pharm. Res., 32: 221-228.

Dahlin, D.C., G.T. Miwa, A.Y. Lu and S.D. Nelson, 1984. N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. Proc. Natl. Acad. Sci. USA., 81: 1327-1331.

Fakurazi, S., U. Nanthini and I. Hairuszah, 2008. Hepatoprotective and antioxidant action of *Moringa oleifera* Lam.against acetaminophen induced hepatotoxicity in rats. Int. J. Pharmacol., 4: 270-275.

Fakurazi, S., I. Hairuszah, J. Mohd-Lip, G. Shanthi and U. Nanthini *et al*., 2009. Hepatoprotective action of zerumbone against paracetamol induced hepatotoxicity. J. Med. Sci., 9: 161-164.

Guengrich, F.P., 2000. Metabolism of chemical carcinogen. Carcinogenesis, 21: 341-351.

Handa, S.S., A. Sharma and K.K. Chakraborti, 1986. Natural products and plants as liver protecting drugs. Fitoterapia, 57: 307-351.

Harbon, J.B., 1998. A Guide to Modern Techniques of Plant Analysis Recommended Techniques (A) Glucosinolate. 3rd Edn., Chapman and Hall, London, pp: 180-182.

Hayes, J.D., M.O. Kelleher and I.M. Eggleston, 2008. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. Eur. J. Nutr., 47: 73-88.

Hinson, J., 1983. Reactive metabolites of phenacetin and acetaminophen: A review. Environ. Health Perspect, 49: 71-79.

Hodgson E. and H. Cunny, 2004. A Text Book of Modern Toxicology. 3rd Edn., John Wiley and Sons, USA.

Hofman, T., A. Kuhnert, A. Schubert, C. Gill, I.R. Rowland, B.L. Pool-Zobel and M. Glei. 2009. Modulation of detoxification enzymes by watercress: *In vitro* and *in vivo* investigations in human peripheral blood cells. Eur. J. Nutr., 48: 483-491.

Jaeschke, H., G.J. Gores, A.I. Cederbaum, J.A. Hinson, P. Dominique and J.J. Lemasters, 2002. Mechanisms of hepatotoxicity. Toxicol. Sci., 65: 166-176.

Kanter, M.Z., 2006. Comparison of oral and i.v. acetylcysteine in the treatment of acetaminophen poisoning. Am. J. Health Syst. Pharm., 63: 1821-1827.

Kessler F.K., M. Kessler, D. Auyeung and J. Ritter, 2002. Glucurorinidation of acetaminophen catalyzed by multiple rat phenol UDP-glucuronosyl transferases. Drug Metabial. Dispos, 30: 324-330.

Kew, M.C., 2000. Serum aminotransferase concentration as evidence of hepatocellular damage. Lancet, 355: 591-592.

Khadr, M.E., K.A. Mahdy, K.A. El-Shamy, F.A. Morsy, S.R. El-Zayat and A.A. Abd-Allah, 2007. Antioxidant activity and hepatoprotective potential of black seed, honey and silymarin on experimental liver injuries induced by CCl_4 in rats. J. Applied Sci., 7: 3909-3917.

Kitteringham, N.R., C. Lambert, J.L. Maggs and J. Colbert, 1988. A comparative study of the formation of chemically reactive drug metabolites by human liver microsomes. Br. J. Clin. Pharmacol., 26: 13-21.

Konsue, N. and C. Ioannidis, 2010. Modulation of carcinogen-metabolising cytochromes P450 in human liver by the chemopreventive phytochemical phenethyl isothiocyanate, a constituent of cruciferous vegetables. Toxicology, 268: 184-190.

Lhoste E.F., K. Glouxk, I. De Wazier, S. Garrido and S. Lorry *et al*., 2004. The activities of several detoxification enzymes are deferentially induced by juices of garden cress and mustard in human Hep G2 cells. Chem. Biol. Interact, 150: 211-219.

Moling, O., E. Cairon, G. Rimenti, F. Rizzi, R. Pristira and P. Mian, 2006. Sever hepatotoxicity after therapeutic doses of acetaminophen. Clin. Ther., 28: 755-760.

OCED, 2001. The OECD Guideline for Testing of Chemical. The Organization of Economic Co-Operation Development, Paris, pp: 1-14.

Ozen, T., 2009. Investigation of antioxidant properties of Nasturtium officinale (watercress) leaf extracts. Acta Pol. Pharm., 66: 187-193.

Prakash, O., G.N. Singh, R.M. Singh, S.C. Mathur, M. Bajpai and S. Yadav, 2008. Protective effect of a herbal formula against carbontetrachloride induced hepatotoxicity. Int. J. Pharmacol., 4: 282-286.

Reen, R.K, M. Karan, K. Singh, V. Karan, R.K. Johri and J. Singh, 2001. Screening of various Swertia species extracts in primary monolayer cultures of rat hepatocytes against carbon tetrachloride and paracetamol-induced toxicity. J. Ethnopharmacol., 75: 239-247.

Smith, J.A., I.D. Hine, P. Beck and P.A. Routledge, 1986. Paracetamol toxicity: Is enzyme induction important? Hum. Toxicol., 5: 383-385.

Steinbrecher, A., K. Nimptsch, A. Husing, S. Rohrmann and J. Linseisen, 2009. Dietary glucosinolate intake and risk of prostate cancer in the EPIC-Heidelberg cohort study. Int. J. Cancer, 125: 2179-2186.

Vermeulen, N.P.E., J.G.M. Bessems and R. van de Streat, 1992. Molecular aspects of paracetamol induced hepatotoxicity and its mechanism based prevention. Drug Metab. Rev., 24: 367-407.

Wallig, M.A., S. Kingston, R. Stack and E. Jeffery, 1998. Induction of rat pancreatic glutathione s-transferase and quinone reductase activities by a mixture of glucosinolate breakdown derivatives found in Brussels sprouts. Food Chem. Toxicol., 36: 365-373.

William, R.T., 1971. Detoxification Mechamsms. John Willey, New York, USA.

Yemitan, O.K. and M.C. Izegbu, 2006. Protective effects of *Zingiber officinale* (Zingiberaceae) against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats. Phytother. Res., 20: 997-1002.

Zhang, Q., Z. Melnikov and D.E. Feierman, 2004. Characterization of the acetaminophen-induced degradation of cytochrome p450-3A4 and the proteolytic pathway. Basic Clin. Pharmacol. Toxicol., 94: 191-200.