Evaluation of pharmacological and toxic effects of ethanolic extract of radish pods in albino rabbits: A biochemical and histopathological study

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Abstract: Radish pods are known as vegetable eaten as a part of diet. Though the pharmacologic potential of radish has been well known but there are fewer reports regarding pharmacological and toxic effects of radish pods. On account of this reason, the current study was aimed to evaluate the pharmacological and toxic effects of ethanol extract of *Raphanus caudatus* (radish pods) in rabbits after 60 days of administration. The plant extract was administered in 250, 500 and 1000mg/kg doses and effect was observed on hepatic, renal, cardiac and lipid profile. The extract was found to be hepatoprotective, nephroprotective and cardioprotective. Also it showed hypocholestrolemic potential at 1000 mg/kg. However at higher doses the extract presented chronic gastritis. Conversely, no indication of histological alterations was seen in other vital organs such as liver, kidneys, heart. Thus there is critical requirement to identify toxic constituent/s inducing gastritis so that safety profile of the plant can be established for effective therapeutic use.

Keywords: Radish pods, pharmacological effects, toxic effects. albino rabbits.

INTRODUCTION

Herbal drugs are generally considered as safe (Younus and Siddiq 2017), however there are reports indicating potential adverse/ side effects. Thus there is dire need to evaluate the pharmacological and toxic effects of plants by scientific methods to establish their efficacy and safety.

Raphanus sativus commonly known as radish belongs to family Brassicaceae. It is a popular vegetable eaten throughout the world and in Pakistan and India known as Mungraa. The members of this family such as broccoli, cauliflower, turnip etc. are well recognized for their specific type of odor that is attributed to sulphur containing glucosinolate and isothiocyanate constituents (Ishida *et al*., 2014). Radish is rich source of flavonoids such as quercetin, apigenin, myricetin, kaempferol, and luteolin (Lugast and Hovari 2000). Moreover fatty acids (Hou *et al*., 2011; Uluata and Özdemir 2012), glycosides *Raphanus* (Jin *et al*., 2016), anthocyanins (Matera *et al*., 2015; Matera *et al*., 2012), alkaloids (Kim *et al* 2015) and proteins (Landon *et al*., 2000; Shin and Hwang 2001; Terras *et al*. 1992) are well reported bioactive principles of radish plant.

The present study was designed to evaluate pharmacological and toxic potential of ethanol extract of radish pods on different biochemical and histological aspects in albino rabbits.

In our study rabbits were selected as overall physiology of rabbits is comparable to humans. Rabbits are easy to handle and moreover adequate amount of blood samples can be collected at different stages of study.

MATERIALS AND METHODS

Fresh pods of Raphanus *sativus* L. were purchased from local market in Karachi, identified by Dr. Mohtasheem, Department of Pharmacognosy, University of Karachi. Specimen with voucher number: RSP-01-14/17 was deposited in Department of Pharmacognosy. Dried pods were grinded and used for extraction with ethanol using Soxhlet apparatus. Crude ethanol extract was used for further study.

Selection and dosing of animals

The present study was conducted on healthy albino adult rabbits weighing from 1000-1500 grams. The study was approved by University Board of Advanced Studies and Research. The rabbits were housed in cages individually for a week prior to study with free access to diet and water *ad libitum.* The animals were divided into four groups each containing 10 animals. Three groups were considered as test groups, administered with three different doses of extract (250, 500 and l000mg/kg) once a day according to body weight of animals through oral route for 60 days. One group served as control group which was kept on Normal saline according to their body weight (0.9% NaCl).

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Sample collection

At the end of dosing period (60 days), the blood samples of approximately 5-6 ml were collected from heart through cardiac puncture in gel tube. Following biochemical parameters were evaluated:

1. Hepatic parameters (TBR, DBR, SGPT, SGOT, γ-GT)
2. Renal parameters (Urea, Creatinine)
3. Cardiac parameters (CK, LDH)
4. Lipid profile (Cholesterol, triglyceride, HDL, LDL, VLDL)

All tests were performed by using commercial assay kits Merck (Germany). Spectrophotometer (Hitachi) was used to measure the absorbance of light

Histopathologic examination

After the study period, animals were sacrificed and organs (liver, kidneys, heart and stomach) were removed, sliced and washed in saline. The organs were fixed in 10% formaldehyde solution for microscopic histological study. Different processes of dehydration, clearing, infiltration and embedding and sectioning were carried out. Slides were prepared, observed and photographed to get permanent record.

STATISTICAL ANALYSIS

The data was expressed as Mean ± SEM (Standard error to mean) with post hoc one-way ANOVA. The significant cut off values was considered as $p<0.05$.

RESULTS

Tables 1, 2, 3 and 4 present the effects of ethanol extract of radish pods on hepatic, renal. Cardiac and lipid profile respectively of rabbits after 60 days of continuous dosing.

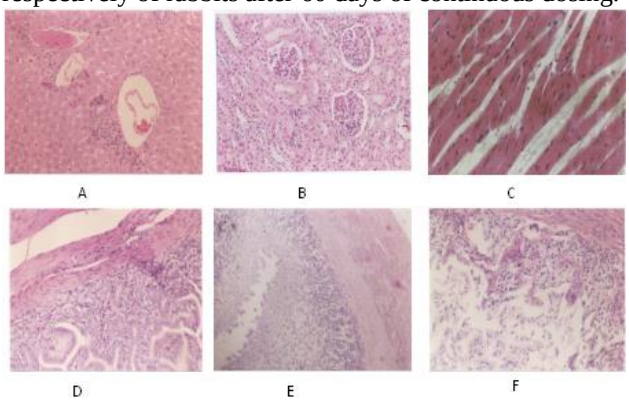

Fig. 1: Effect of ethanol extract of Radish pods on microscopic histological features of different organs

A) Hepatic Tissue showing no microscopic change B) Renal Tissue showing no microscopic change C) Cardiac Tissue showing no microscopic change D) Stomach Tissue showing no microscopic change E) Stomach Tissue showing inflammation of lamina propria with diminished epithelial layer F) Stomach Tissue showing chronic inflammation

The histopathological examination of liver, kidneys, hearts and stomach are presented in fig. 1. It is clear that ethanol extract of radish pods did not produce any morphological changes at any of tested dose in all organs. The only exception is stomach where gastritis was seen at higher doses of plant extract (figs. 1E & 1F).

DISCUSSION

It is well acknowledged that plants have enriched profile of bioactive constituents for example flavonoids, alkaloids, polysaccharides, coumarins, glycosides, lignans, saponins, polyines, thiophenes, proteins and polyphenolics. Ethanol extract of Raphanus caudatus is rich in secondary metabolites such as carbohydrates, flavonoids, alkaloids, terpenoids and tannins. Which indicates its therapeutic potential against different diseases. As a part of pharmacological and toxicological evaluation of Raphanus caudatus (radish pods), the hepatic, renal and cardiac safety profile of ethanol extract of radish pods was determined after 60 days dosing period.

Radish holds substantial hepato-protective action (Elshazly *et al*., 2016; Lee *et al*., 2012). An earlier study indicated liver protection effect by different extracts of radish leaves in carbon tetrachloride induced hepatotoxic rats (Syed *et al*., 2014). The hepatoprotective effects of radish pods' ethanol extract were also observed in current study and liver enzymes were not affected. Moreover microscopic histological examination of rabbit's liver also supports the hepato-protective potential of radish pods in 60 days use. This hepatoprotective effect may be linked with antioxidant property found in the radish pod ethanol extract.

Urea and creatinine are the important parameters to assess renal function. Elevated levels of these indicators are linked with dysfunction of kidneys. In current study, the pods extract did not affect urea and creatinine values at any tested dose. Moreover no histological changes seen in kidneys. The ethanol extract of Raphanus may possess nephro-protective activity because of occurrence of natural antioxidants such as vitamin E & C and its action to increase the level of renal antioxidant enzymes like catalase, glutathione-S-transferase as well as superoxide dismutase (Bojan *et al*., 2016). Different researches also reported renal protective role of radish (Rajagopal *et al*., 2013; Talele *et al*., 2012).

In current research, elevated levels of Creatinine kinase enzyme were noted in the animals kept on 1000 mg/kg dose of radish pods' ethanol extract. CK is an enzyme found in the cell membrane of myocardium. It is an important marker for assessing the function of cardiovascular system (Armstrong and Golan 2011). An increase in the serum levels of this particular enzyme

Table 1: Effect of ethanol extract of radish pods on hepatic parameters

Treatment Groups	TBR (mg/dl)	DBR (mg/dl)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	γ-GT (U/L)
Control	0.8±0.04	0.07±0.02	68±2.37	48±3.84	40±2.94	5±0.39
Radish pods-I (250 mg/kg)	0.11±0.02	0.08±0.01	58±6.93	32±4.83	36±2.02	5±0.54
Radish pods II (500 mg/kg)	0.19±0.01	0.1±0.01	70.2±27.31	30±3.55	33±0.91	5±0.45
Radish pods III (1000 mg/kg)	0.13±0.02	0.05±0.01	53±4.25	26±3.33	30±2.06	4±0.45

Table 2: Effect of ethanol extract of radish pods on renal parameters

Treatment Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	56.4±0.5	1.66±0.08
Radish pods- I (250 mg/kg)	57.5±0.41	1.48±0.09
Radish pods- II (500 mg/kg)	59.2±0.65	1.40±0.08
Radish pods- III (1000 mg/kg)	53±0.7	1.36±0.04

Table 3: Effect of ethanol extract of radish pods on cardiac parameters

Treatment Groups	CK (U/L)	LDH (U/L)
Control	243±11.24	537±8.44
Radish pods- I (250 mg/kg)	209±2.99	518±5.49
Radish pods- II (500 mg/kg)	261±10.74	502±5.42
Radish pods- III (1000 mg/kg)	409±5.15**	551±11.54

Table 4: Effect of ethanol extract of radish pods on lipid profile

Treatment Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	50.2±3.1	35.0±2.7	16.2±1.15	38.4±4.08	8.1±1.4
Radish pods- I (250 mg/kg)	40±2.4	40.1±3.1	17±1.64	38±2.52	10.2±3.0
Radish pods- II (500 mg/kg)	42.1±3.4	33.1±2.6	14.3±1.1	39.1±2.24	9.4±3.3
Radish pods- III (1000 mg/kg)	39.6±3.2*	27±1.9*	17.5±1.4	29.2±0.8*	6.5±1.7*

Values are mean ± SEM, n=10, One-way ANOVA, post hoc tukey HSD,*$p \leq 0.05$ and **$p \leq 0.005$

could be associated with myocardial injury (Thygesen *et al*. 2007). During cardiac puncture to take blood sample, there is chance of mild/moderate muscle injury to laboratory animals that may cause elevation in CK levels (O'brien *et al*., 1997).Though increase in CK levels is not supported with microscopic histological examination of rabbits' hearts, this effect warrants further investigation for confirmation.

LDH values were not affected by the ethanol extract of radish pods. At dose of 1000mg/kg, radish pods resulted in significant lowering of blood cholesterol, triglycerides, LDL and VLDL levels as compared to control. This infers that radish pods' extract might have cholesterol lowering effect at higher doses. According to Castro-Torres *et al*.,2014, radish plant exhibited hypocholesterolemic effect that was responsible for inhibition of gall stone formation (Castro-Torres *et al.* 2014). It is well identified that elevated levels of triglyceride, cholesterol, LDL and VLDL are associated with cardiovascular illnesses (Kris-Etherton *et al*. 2007). Hence hypolipidemic capability of radish pods may be useful in the prevention and treatment of cardiovascular disorders.

The radish pods extract exhibited chronic gastritis in rabbit model at higher doses. This toxic effect could be due to indefinite constituent/s at higher doses after chronic administration. There are some factors that may play role in the toxicity due to food consumption such as overconsumption, contamination and/or any unpredictable idiosyncratic reaction (Dolan *et al*. 2010; Santamaria 2006). Besides, there are chances of heavy metals retention in fruits and vegetables as a result of irrigation with effluent water. (Cherfi *et al*. 2016; Hu *et al*. 2013; Parveen *et al*. 2003).

CONCLUSION

The results of present study indicate that ethanol extract of radish pods possess hepatoprotective, nephroprotective and cardioprotective potential. Moreover it has hypocholestrolemic capability in long term. In contrast, the plant extract revealed chronic gastritis at higher chronic doses. Certainly this is an important issue and further studies are critically required for the identification of toxic constituent/s responsible for toxic effects of pods so that this vegetable can be safely used in daily di*et al*ong with establishment of its effective therapeutic use.

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