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EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF *RAPHANUS SATIVUS* (FAMILY: *BRASSICACEAE*) ROOT AGAINST ACETAMINOPHEN INDUCED HEPATOTOXICITY IN EXPERIMENTAL RATS

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ABSTRACT

The present study was conducted to evaluate hepatoprotective potential of ethanol extract from *Raphanus sativus* root (ERS). Hepatoprotective potential of ERS was tested at doses of 100, 200 and 400 mg/kg (p.o.), against acetaminophen (paracetamol) induced hepatotoxicity in rats. The ERS at different doses and silymarin treated animal groups showed significant decrease in levels of different biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), and alkaline phosphatase (ALP), that were elevated by acetaminophen intoxication. The levels of total bilirubin, bilirubin direct, bilirubin indirect, total protein, albumin and serum globulin along were also restored to normal by ERS and silymarin treatment. ERS at 400 mg/kg showed significant ($P<0.001$) result and comparable to silymarin treated group of rats. Histological studies supported the biochemical findings and treatment with ERS at dose level of 100, 200 and 400 mg/kg, p.o. was found to be effective in restoring acetaminophen-induced hepatotoxicity in rats.

Keywords: *Raphanus sativus*; Acetaminophen induced hepatotoxicity; Hepatoprotective activity

1. INTRODUCTION

Liver is a vital organ that plays a role in controlling critical biochemical and physiological activities including homeostasis, growth, energy and nutrient supply, detoxification of drugs and other xenobiotics, and also combating infections. Therefore, it is very susceptible to being damaged by hepatotoxic agents **(Mahmood *et al.*, 2014)**. The hepatotoxic agents damage liver cells by inducing lipid per oxidation and other oxidative liver damages in liver. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. As such liver is highly affected primarily by toxic agents such as CCl4, Paracetamol, D-galactosamine, alcohol, rifampicin and thioacetamide through different mechanisms and the other agent causing hepatotoxicity include statins, isoniazid, and various anti-microbial agents **(Naveen *et al.*, 2016)**.

The toxic liver injury can reproduce virtually any known pattern of injury, including necrosis, steatosis, fibrosis, cholestasis, and vascular injury. Liver injury during cancer chemotherapy may not always reflect hepatotoxic anticancer drugs; the clinician must also consider reactions to antibiotics, analgesics, antiemetics, or other medications. Preexisting medical problems, tumor, immunosuppression, hepatitis viruses and other infections, and nutritional deficiencies or total parenteral nutrition all may affect a host's susceptibility to liver injury. Attributing liver injury to a toxic reaction is therefore difficult **(King *et al.*, 2001)**.

Liver diseases are serious ailments, classified as acute or chronic hepatitis (inflammatory liver deseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxident agents of natural origin have attracted special interest because they can protect human body from free radicals **(Sreejith *et al.*, 2014)**. Liver damage or failure is always associated with hepatocytes necrosis and elevated levels of biochemical parameters like SGOT, SGPT, ALP and Total bilirubin levels. The death rate increases by each year due to heapatic disorders. In fact, drug-induced liver toxicity is the leading cause of acute liver failure according to FDA **(Nallamilli *et al.*, 2013).** Symptoms vary depending on the degree of exposure and hence extent of the liver damage or injury. In which the liver is a metabolically active organ responsible for biotransformation and clearance of

xenobiotics from the body. It is an important target of drugs and pathogens that may initiate liver cell damage and compromise its overall function. The general strategy for prevention of liver damage includes reduction of reactive metabolites by using antioxidants. Natural polyphenolic compounds such as resveratrol, quercetin, curcumin and silymarin possess antioxidant properties and anti-inflammatory effects and have been the subject of considerable research as liver protectants. Treatment of drug induced hepato-toxicity is mainly supportive and discontinuation of offending drug is the first step **(Rathee *et al.*, 2017).**

Liver diseases are a problem worldwide, and the conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Thus, interest and toward medicinal plants as new sources of hepatoprotective agents. A number of plant-based traditional medicines or formulations containing herbal extracts are sold in the market for liver disorders **(Kamisan *et al.*, 2013).**

Hepatoprotective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Many extracts of plants are also used for the treatment of liver disorders. **(Eesha BR *et al.*, 2011).**

Raphanus sativus L. belongs to the family Brassicaceae (Cruciferae, an older name). It is known as Radish in English, Daikon in Japanese, and "Laifu" or "Luobo" in Chinese. It is an annual herb, consumed as vegetable. Commonly known as Mooli. With its high adaptive ability, high yield, and abundant nutritional value, *Raphanus sativus* L. has long been grown as a food crop worldwide, especially in India, China, Japan, Korea, and Southeast Asia **(Wang *et al.*, 2005)**. The leaf, seed, and root of *Raphanus sativus* L. are claimed to have various medicinal uses **(Guti´errez *et al.*, 2004)**.

Radishes are annual or biennial brassicaceous crops grown for their swollen tap roots which can be globular, tapering, or cylindrical. The root skin colour ranges from white through pink, red, purple, yellow, and green to black, but the flesh is usually white. Smaller types have a few leaves about 13 cm (5 in) long with round roots up to 2.5 cm (1 in) in diameter or more slender, long roots up to 7 cm (3 in) long. Both of these are normally eaten raw in salads. A longer root form, including oriental radishes, daikon or mooli, and winter radishes, grows up to 60 cm (24 in) long with foliage about 60 cm (24 in) high with a spread of 45 cm (18 in) **(Brickell**

***et al*., 1992)**. The flesh of radishes harvested timely is crisp and sweet, but becomes bitter and tough if the vegetable is left in the ground too long **(Vegetable gardeing 2004)**. Leaves are arranged in a rosette. They have a lyrate shape, meaning they are divided pinnately with an enlarged terminal lobe and smaller lateral lobes. The white flowers are borne on a racemes inflorescence **(Gopalakrishnan *et al*., 2007)**. The fruits are small pods which can be eaten when young **(Brickell *et al*., 1992)**.

The different successive extracts so obtained were subjected to preliminary phytochemical screening by applying different qualitative testes for phytoconstituents. The different extracts contained alkaloids, carbohydrates, phenolic compounds, flavonoids, amino acids and volatile oil.

The aqueous, methanolic and hydrophobic radish extracts or specific phytochemicals that are present in radishes including glucosinolates and isothiocyanates. Studies have shown that radishes contain other phytochemicals that have been associated with beneficial health effects including phenolic acids and anthocyanins.

Radish sprouts contained significantly greater concentrations of glucosinolates (3.8-fold) and isothiocyanates (8.2-fold) than the mature radish taproot and also contained significantly greater concentrations of phenolics (on average 6.9-fold). The anthocyanin concentrations of the mature radish taproot were significantly greater than in the sprouts of red, pink, and purple varieties. The primary anthocyanidins present in the red and pink radish varieties were pelargonidin and delphinidin, while the primary anthocyanidin in the purple radish variety was cyaniding **(Yamasaki *et al*., 2009 & Beevi *et al*., 2010)**.

Radishes have long been grown as a food crop, but they also have various medicinal actions. The plant is used in the treatment of intestinal parasites, though the part of the plant used is not specified. The root is best harvested before the plant flowers. Its use is not recommended if the stomach or intestines are inflamed. The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints. The juice of the fresh leaves is diuretic and laxative. The seed is carminative, diuretic, expectorant, laxative and stomachic. It is taken internally in the treatment of, abdominal bloating, wind, acid regurgitation, diarrhea and bronchitis. The root is antiscorbutic, antispasmodic, astringent, cholagogue, and diuretic. It is crushed and used as a poultice for burns, bruises and smelly feet. Radishes are also an excellent food remedy for stone, gravel and

scorbutic conditions. The plant contains raphanin, which is antibacterial and antifungal. It inhibits the growth of *Staphylococcus aureus, E. coli, streptococci, Pneumococci* etc. Radish preparations are useful in liver and gall bladder troubles. The roots are said to be useful in urinary complaints, piles and in gastrodynia. The roots stimulate the appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile. Consuming radish generally results in improved digestion, but some people are sensitive to its acridity and robust action. The plant also shows anti-tumor activity **(Visentin *et al*., 1992, Nakamura *et al*., 2001, Sgherri *et al*., 2003 & Burits *et al*., 2000)**.

2. MATERIALS AND METHODS

2.1 Plant materials

The root of *Raphanus sativus* was collected from Dalpatpur in Moradabad, Uttar Pradesh, India. The taxonomic identity of the plant was confirmed by the Dr. Ashok Kumar, Assistant Professor, Department of Botany, IFTM University Moradabad (U.P.), India.

2.2 Preparation of extract

Crude extract was prepared by the method of **(Salah Abbes JB *et al*., 2008)** and **(Wang L *et al*., 2004)**. *Raphanus sativus* root were washed to remove soil and other contaminants. The root of *Raphanus sativus* were crushed in a juicer. The plant material was then filtered through a cloth mesh followed by second filtration through Whatman Qualitative Grade-1 filter papers. The filtrate was collected and kept at 4 ^{0}C for use **(Jung KY *et al*., 2000)**.

2.3 Preliminary phytochemical studies

Extract obtained from ethanol were subjected to various chemical tests for determination of phyto constituents presents in them **(*Evans et al.*, 2002)**.

2.4 Animals

All experiments were performed on Wistar albino rats weighing 160–180 g obtained from Animal Facility, IFTM University, Moradabad. Animals were housed individually in polypropylene cages and kept in a room maintained at an average temperature of 22°C ± 3°C and humidity 55.6%, with 12 h darkness (lights on from 06:30 to 18:30 h) and fed with standard pellet diet before a week of start of experiment. The rats were allowed to have free access to food and tap water. All experiments were performed in accordance with the protocol approved by the IAEC affiliated to CPCSEA, New Delhi.

2.5 Experimental Design

The hepatoprotective potential of successive *Raphanus sativus* root extracts were assessed

in rats intoxicated with acute dose of Acetaminophen **(*Araya* et al., 1987)**. Wistar albino rats were randomized and divided into 6 groups of six animals in each. Group 1 served as normal control and fed orally with 1% CMC 5 ml/kg body weight daily for seven days. Group 2 rats were similarly treated as group 1. Animals of group 3 were fed with standard drug silymarin 100 mg/kg; p.o. daily for seven days. Group 4 to 6 were treated with successive extracts of *Raphanus sativus*. On the day, Acetaminophen suspension was administered orally, at a dose of 2 g/kg body weight to all groups animals except the rats in group 1 (normal control), 30 min after the respective treatment with extracts of *Raphanus sativus*, silymarin and vehicle, to induce hepatic injury. Detailed experimental protocol shown in **Table 1**.

Table 1: Experimental design for hepatoprotective activity of ERS in Acetaminophen induced hepatotoxicity

Groups	Treatment	Dose
1	Normal Control	---
2	Acetaminophen (Toxic control)	2 g/kg, p.o.
3	Acetaminophen + Standard silymarin (Standard Control)	100 mg/kg, p.o.
4	Acetaminophen + ERS (Low Dose)	100 mg/kg, p.o.
5	Acetaminophen + ERS (Medium Dose)	200 mg/kg, p.o.
6	Acetaminophen + ERS (High Dose)	400 mg/kg, p.o.

ERS - *Raphanus sativus Extract*

2.6 Collection of blood and liver

After 48 hours of Acetaminophen administration, blood was collected from all groups of rats from retro-orbital plexus. Serum was separated by centrifugation at 4000 rpm at 4ºC for 15 min and analysed for various biochemical parameters. Afterwards, the rats were sacrificed under light ether anaesthesia. The livers from all the animals were collected, washed and used for histological studies.

2.7 Biochemical parameter estimation

Serum biochemical parameters like serum aspartate transaminase (AST), serum alanine transaminase (ALT), alkaline phosphatase (ALP), total protein (TP), direct bilirubin (DB) and total bilirubin (TB) were determined by using microplate spectrophotometer (BIO-Tek, USA.) using biochemical kits obtained from Ranbaxy Diagnostic Ltd. Baddi, HP, India and Roche Diagnostics India Pvt. Ltd. Mumbai, India.

2.8 Histopathological studies

Liver tissue was collected and fixed in 10% buffered formalin for histopathological analysis. Liver tissue sections were taken with the help of microtome and stained with haematoxylin and eosin. The sections were observed under photomicroscope.

2.9 *In-vitro* antioxidant studies

2.9.1 Free radical scavenging activity

Scavenging effect on DPPH

Principle

The DPPH (1, 1-Diphenyl- 2-picryl hydrazyl) is stable free radicals are reduced to a corresponding1, 1-Diphenyl-2-Picryl Hydrazine when it reacts with hydrogen donors. The DPPH radical is purple and upon reaction with hydrogen donor's it becomes colorless and formation of the non radical form of DPPH. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm. It is a light sensitive compound so protect from light **(Macdonald-Wicks *et al*., 2006; Lewis, 2012)**.

Preparation of test sample

100mg of dried ethanol extract was dissolved in 100ml of methanol to make a stock solution of 1mg/ml. Aliquots from this stock solution was further diluted with methanol as per the concentration required.

Preparation of DPPH (0.1mM) solution

39.43mg DPPH was dissolved in 10ml methanol. From this solution 1ml was taken and diluted with methanol up to 10ml. Then 5ml was taken from this prepared solution and diluted with methanol up to 50ml.

Procedure

To 2ml of various concentration of extract, 2ml solution of DPPH (0.1mM) was added. An equal amount methanol and DPPH was served as control. After 20 min incubation in dark, absorbance was measured at 517nm. The experiment was performed in triplicate. The percentage scavenging was calculated by the formula **(Shirwaikar *et al*., 2006).**

$$\textbf{\% Scavenging} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

2.9.2 Estimation of Total Flavonoids

One gm of drug was macerated with 100ml methanol (hot decoction) for 1 hours followed by filtration. Taken 1ml of extract and placed in 10ml volumetric flask then added 3ml methanol and 0.3 ml $NaNO_2$ and then added 3ml of $AlCl_3$ after 5 min. Added 2ml of 1M NaOH after 6min.and total volume was made up to 10ml with methanol. The solution was mixed well in volumetric flask and absorbance was measured against a blank at 510nm and the total flavonoids content was calculated using following equation **(Zhinsen *et al*., 1999)**.

Calculation:-

Linear regression equation (y = mx + c)

Y = 0.013X + 0.000

Where,

Y = Absorbance, X = Concentration

2.9.3 Estimation of Total Phenolic Content

Preparation of standard curve

10mg of standard Gallic acid was weighed accurately and dissolved in 100ml methanol in volumetric flask to prepare a stock solution of 100µg/ml. From the above stock solution 0.5 to 3.0 ml of aliquots were pipette out into 25ml volumetric flask and 10ml of water and 1.5 ml Folin Ciocalteu's reagent were added to it. The mixture was kept for 5min. and then added 4ml of 20% sodium carbonate solution and volume was making up with distilled water. The mixture was kept for 30min and the absorbance was recorded at 765nm in UV spectrophotometer.

Calibration curve of Gallic acid (50-300µg) was plotted between concentration and absorbance **(Anandjiwala *et al*., 2007)**.

Calculation:-

Linear regression equation (y = mx + c)

Y = 0.015X - 0.007

Where,

Y = Absorbance, X = Concentration

Preparation of test solution

One gram of drug was dissolved in 100ml methanol to prepare a solution of 10mg/ml. From the above test solution 1ml of solution was pipette out into a 25ml volumetric flask then added 10ml of water and 1.5 ml Folin Ciocalteu's reagent were added to it. The mixture was kept for 5min. and then added 4ml of 20% sodium carbonate solution and volume make up with distilled water. The mixture was kept for 30 min and the absorbance was recorded at 765nm in UV spectrophotometer. The amount of total phenolics content was calculated using a standard curve of Gallic acid **(Anandjiwala *et al*., 2007)**.

3. RESULT

Effect of ERS on serum marker enzymes and bilirubin levels during Acetaminophen induced hepatotoxicity are presented in table 2. Levels of serum marker enzymes of hepatic injury like ALP, AST and ALT as well as serum bilirubin and protein were elevated significantly in Acetaminophen alone treated control group animals when compared with normal animals and these elevated levels were decreased to almost normals levels by ERS administration in a dose dependent manner **(Table 2)**.

3.1 *In-vitro* Anti-Oxidant Activity

3.1.1 DPPH Radical Scavenging Activity

ERS possess a concentration–response relationship in DPPH radical scavenging activity, an increase in concentration is synonymous of an increase in scavenging activity.

Ascorbic acid a potent free radical scavenger was used as positive control and found that ascorbic acid produced more potent effect

with IC_{50} value 2.71µg/ml, and produced 39.00, 54.21, 66.04, 78.22 and 90.50 scavenging effect in 2, 4, 8, 16 and 48µg/ml concentrations respectively.

ERS showed significant effect. IC_{50} value of ERS was 2.4µg/ml. At 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µ/ml concentration, ERS produced 26.30, 32.60, 40.30, 50.60, 62.30, 74.60, and 84.30 scavenging effect **(Figure 1, 2)**.

3.1.2 Estimation of Total Flavonoids

The total flavonoids content is reported as quercetin equivalents by reference to standard curve (Y= 0.015X + 0.002 and R^2=0.998) **(Figure 3)**.

3.1.3 Estimation of total phenolic content

Preparation of standard curve

The total Phenolic content is reported as Gallic acid equivalents by reference to standard curve (Y= 0.015X - 0.002 and R^2=0.999) **(Figure 4)**.

Table 2: Effects of ERS on rat serum parameters in Acetaminophen induced hepatotoxicity model.

Group	ALP (IU/L)	AST (IU/L)	ALT(IU/L)	TB(mg/dl)	DB(mg/dl)	TP(mg/dl)
Normal control	90.0 ± 8.5	68.6 ± 3.5	67.3 ± 4.0	1.0 ± 0.1	0.39 ± 0.03	7.5 ± 0.2
Acetaminophen (Control)	190.8±16.5[a]	280.5 ± 6[a]	213.9± 8.7[a]	5.1 ± 0.05[a]	1.00 ± 0.1[a]	5.1 ± 0.2[a]
Silymarin	95.1 ± 14.6[b]	77.0 ± 4.0[b]	78.9 ± 4.0[b]	1.1 ± 0.03[b]	0.43± 0.03[b]	7.6 ± 0.1[b]
Acetaminophen + ERS (100 mg)	175.6±12.7[d]	179.3± 3.6[d]	174.5± 9.7[d]	3.5 ± 0.2[d]	0.75± 0.09[d]	5.9 ± 0.2[d]
Acetaminophen + ERS (200 mg)	137.1 ± 9.0[c]	124.9± 4.4[c]	124.4± 5.4[c]	2.7 ± 0.1[c]	0.67± 0.09[c]	6.4 ± 0.1[c]
Acetaminophen + ERS (400 mg)	102±8.7[b]	80.9±5.2[b]	72.3±4.5[b]	1.9±0.1[b]	0.55±0.09[b]	6.9±0.1[b]

Values are the mean ± SEM, n=6. Statistical analysis was performed with ANOVA followed by Dunnett test; [a]*P*<0.001 when compared with control; [b]*P*<0.001 when compared with toxicant; [c]*P*<0.01 when compared with toxicant; [d]*P*<0.05 when compared with toxicant. ERS = *Raphanus sativus* extract, ALT = Alanine transaminase, AST = Aspartate transaminase, ALP = Alkaline phosphatase, DB = Direct bilirubin, TB = Total bilirubin, TP = Total protein

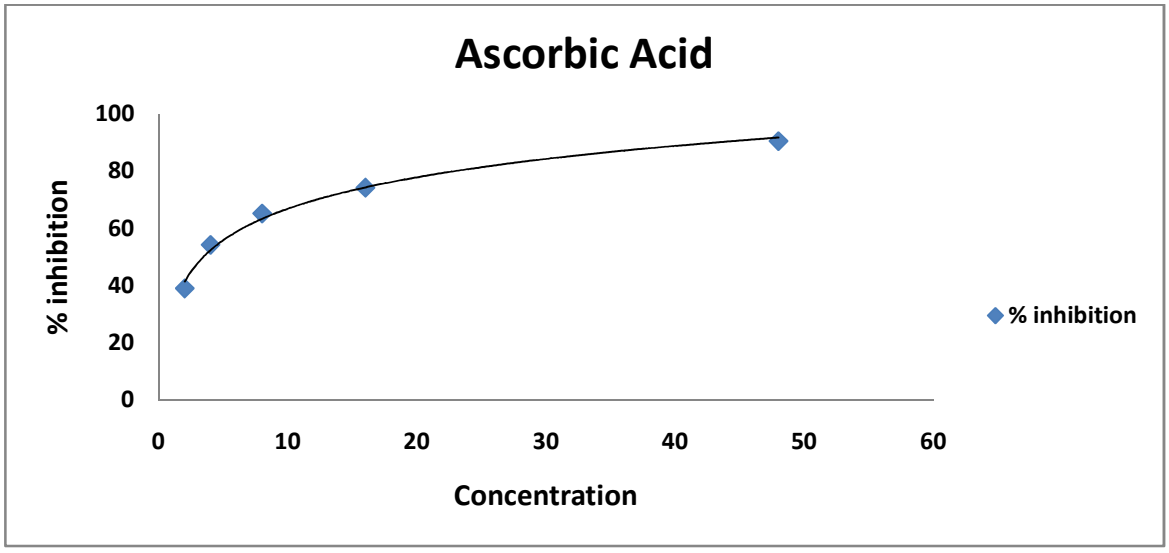

Figure 1: Calibration curve of Ascorbic acid

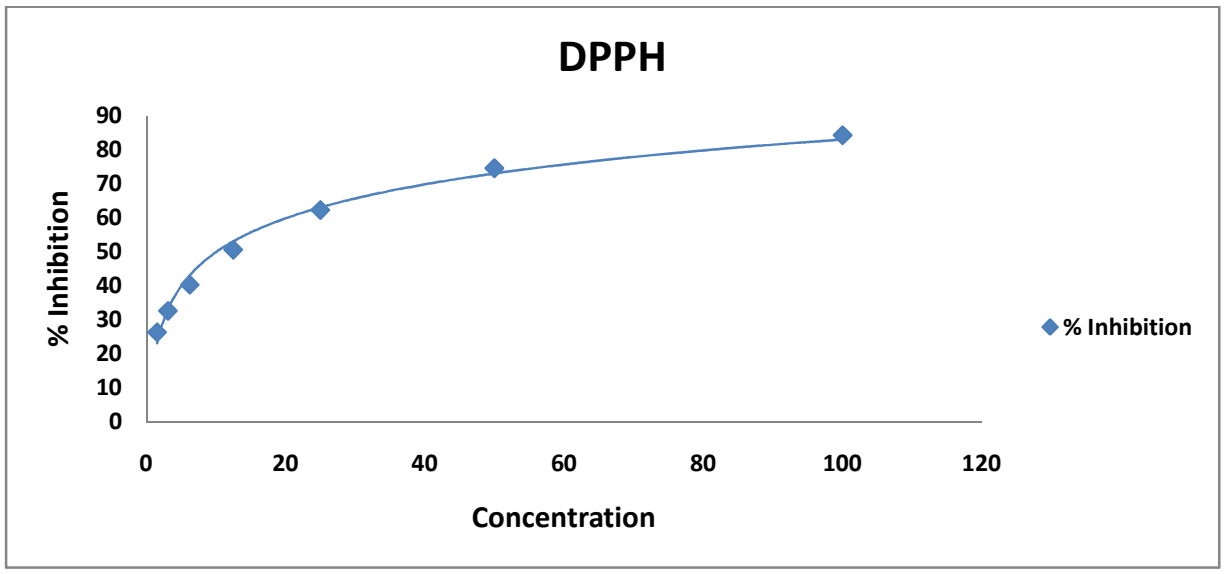

Figure 2: Calibration curve of DPPH

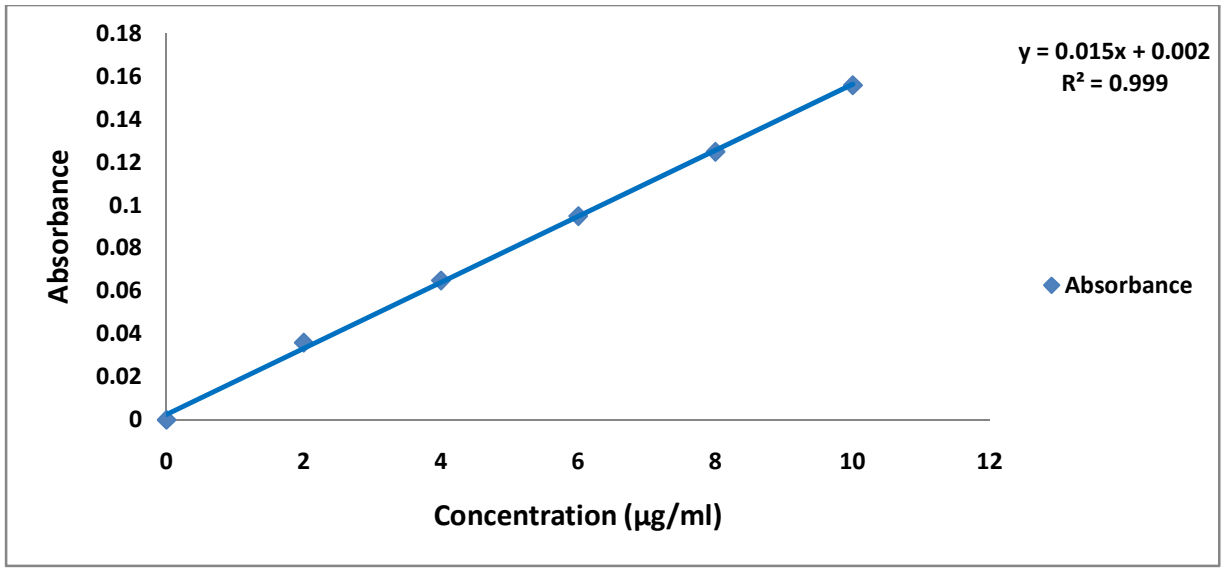

Figure 3: Calibration curve of quercetin

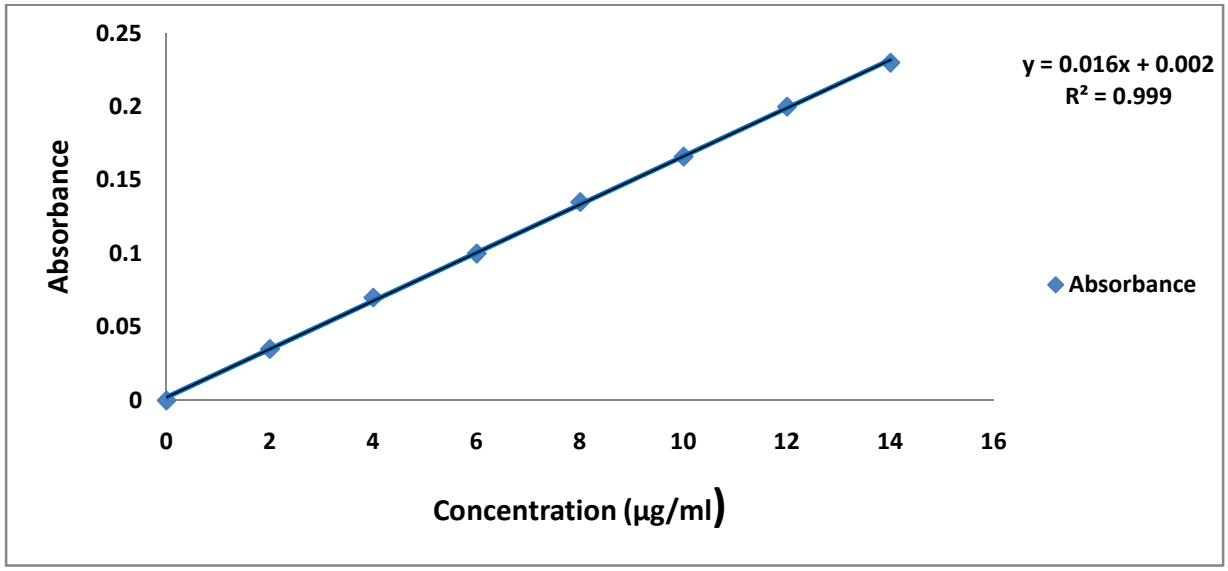

Figure 4: Calibration curve of Gallic acid

4. DISCUSSION

Acetaminophen is one of the most commonly used hepatotoxins in the experimental study of liver diseases and proves highly useful as an experimental model for the study of acute hepatic injury (***Clawson*, 1989**). Acetaminophen induced liver damage is mainly attributed to the toxic metabolites, mainly trichloromethyl radical, for the initiation of Acetaminophen dependant lipid peroxidation. These free radicals may again react with oxygen to form trichloromethyl peroxyl radicals which may attack lipids onthe membrane of endoplasmic reticulum to elicit lipid peroxidation, finally resulting in cell necrosis and consequent cell death (***Recknagel et al.*, 1989**). Marked increase in release of hepatic enzymes into the blood stream is often associated with massive elevation of serum enzymes. In the present study this is evidenced by an elevation in the serum marker enzymes namely SGOT, SGPT, SALP, GGTP and total bilirubin. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology which has been disturbed by the hepatotoxins. Because liver performs many vital functions in the human body, damage of liver causes unbearable problems. Thus study about hepatoprotective compounds is of importance.

In this study, Acetaminophen administration to rats leads to a marked elevation in the levels of serum enzymes like SGOT, SGPT, SALP and GGTP and total bilirubin level. This might be due to release of these enzymes from the cytoplasm, into the blood

stream rapidly after rupture of the plasma membrane and cellular damage **(*Sallie et al.*, 1991)**. Treatments with ethanol extract of *Raphanus sativus* root significantly reduced the levels of these marker enzymes in Acetaminophen treated rats. This implies that the extract tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes **(*Thabrew & Joice*, 1987)**.

Effective control of SALP, bilirubin and total protein levels point toward an early improvement in the secretary mechanism of the hepatic cells. Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver. So, the result of present investigation indicates that the ethanol extract of *Raphanus sativus* root possess good hepatoprotective activity. Serum total bilirubin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of SALP, total bilirubin and total protein are due to increased synthesis, in presence of increasing biliary pressure **(*Moss & Butterworth*, 1974)**.

GGTP is more frequently elevated than either of the transaminases or alkaline phosphatase. It provides a more specific test for hepatic involvement than does aspartate transaminase determination, since GGTP is virtually absent from skeletal and cardiac muscle, where as aspartate transaminase elevation from these non-hepatic sources may occur as a result of myopathy or cardiomyopathy. Significant increased activity of GGTP indicates a severe damage to tissue membranes during carbon tetrachloride toxicity because GGTP is a membrane bound enzyme **(*Chander et al.*, 1994)**. Administration of ethanol extract of *Raphanus sativus* root attenuated the Acetaminophen induced rise in GGTP, thus revealing membrane stabilizing activity of *Raphanus sativus*.

The protein level reduced due to the Acetaminophen induced hepatotoxicity was raised by ERS suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The hepatoprotective effects exhibited by ERS 100, 200 and 400 mg/kg were statistically similar to that observed with standard silymarin at 100 mg/kg dose level.

Further it is evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of Acetaminophen or by inhibition of lipid peroxidation induced byAcetaminophen **(*Mehta et al.,* 1999)**. Phytoconstituents like flavonoids **(*Baek et al.,* 1996)**, triterpenoids **(*Xiong et al.,* 2003)**, saponins **(*Tran et al.,* 2001)** and alkaloids **(*Vijyan et al.,* 2003)** are known to possess hepatoprotective activity. Phytochemical investigations of ERS root revealed the presence of phenol compounds, flavonoids, diterpenoids, sterols and tannins. The present study revealed that the extract is found to possess significant protective effect against hepatotoxicity induced by acetaminophen which may be attributed to the individual or combined action of phyto constituents present in it. The component(s) of the extract responsible for this effect however was not investigated. Further investigations are needed for identification of the active compounds responsible for hepatoprotective activity.

After the preliminary phytochemical quantitative estimation and screening of phytoconstituents, extracts from plant were injected to *in vitro* hepatoprotective and antioxidant evaluation with a vision to select maximum active extracts of these plants. The antioxidant action of extracts is usually linked with the existence of reductones, separate the free radical chain reaction and offering hydrogen atom to free radical leads to neutralization of free radicals and estimated using the DPPH scavenging assay.

5. CONCLUSIONS

Our result shows Ethanol extract of *Raphanus sativus* roots possess hepatoprotective activity and it may be due to presence of antioxidants present in it, partially.

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