Research Article

Hepatoprotective Potential of Hydro alcoholic Extract of *Passiflora edulis* Sims Leaves Extract

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ABSTRACT

The hepatoprotective potential of hydro-alcoholic extract of *Passiflora edulis* leaves was evaluated against paracetamol and ethanol induced hepatotoxicity in rats. Two doses 200mg/kg and 400mg/kg, p.o of the extract were subjected for the evaluation of hepatoprotective potential against ethanol (2ml/100g) and PCM (2g/kg) induced liver injury. Silymarin (25mg/kg) was used as a standard drug. The parameters like SGPT, SGOT, ALP and Total Bilirubin (TB) were estimated to assess the liver functions. Liver antioxidant enzyme GSH was evaluated. In addition, histopathological study was also carried out. Both the doses of P. edulis extract showed dose dependent significant decrease of biochemical parameter such as ALP, SGOT, SGPT, TB and increased GSH level. Histopathology of liver showed reduced inflammation, centrilobular and bridging necrosis. It shows normal hepatocytes with significant reduction in areas of necrosis compared to toxic control. The result obtained was comparable with that of the standard drug Silymarin. The findings of the present study revealed that, hydro-alcoholic extract of *Passiflora edulis* might be beneficial against paracetamol and alcohol induced hepato toxicity.

Keywords: Ethanol, Hepatoprotective, Paracetamol, *Passiflora edulis*, Silymarin.

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INTRODUCTION

Maintaining a healthy liver is essential for overall health and happiness. It is, however, abused by poor drug habits, alcohol, and prescription or over-the-counter pharmaceuticals, which can lead to hepatitis, cirrhosis, and alcoholic liver disease. Hepatoprotection is defined as the ability to prevent liver damage[1]. Hepatocytes are brimming with drug-metabolizing enzymes, which can be split into two categories. Phase-I reactions are oxidative, reductive, and hydrolytic activities that generate the functional groups required for phase II reactions, which are usually conjugations. It appears that a lot of substances are converted to hazardous metabolite if the overall impact of these enzymes is to convert chemicals to more water-soluble forms that can be released easily by the body[2].

Conventional medications used in the treatment of liver illnesses are sometimes ineffective and can have major side effects; consequently, alternative drugs for the treatment of liver disease must be sought to replace currently used drugs with questionable efficacy and safety[3].Despite contemporary medicine's tremendous expansion, there are few synthetic medications accessible to treat hepatic diseases. Several herbs/herbal formulations, on the other hand, are said to have therapeutic effect in the treatment of hepatic disorders[4]. Herbal products are now associated with safety, in contrast to synthetics, which are seen to be harmful to humans and the environment[5].

Antioxidant action is found in natural plants, such as alkaloids, quercitin, tocopherol, flavonoids, coumarins, and other phenolic chemicals found throughout the plant kingdom[6]. Antioxidants may help to prevent tissue fibrosis by scavenging free radicals[7]. *Passiflora edulis* leaves contain flavonoids, cyanogenic chemicals, glycosides, vitamins, minerals, and terpenoid compounds; however their hepatoprotective potential has not been thoroughly evaluated. Hence, the current study was performed to look into the hepato protective potentials of *Passiflora edulis* leaves extract.

MATERIALS AND METHODS

Collection and authentication of plant material

The *Passiflora edulis* leaves were collected in the month of June-July from local market of Mangaluru district and authenticated by botanist.

Preparation of leaf extract

The leaves of *Passiflora edulis* were washed, cut and then shade dried. The dried leaves were then powdered to coarse powder. Extraction was carried out using a ratio of 1:10 for plant to solvent mass at room temperature using hydro-alcohol. The extracted material was then evaporated and concentrated using flash evaporated and stored at 4°C[8].

Experimental Animals

Healthy Wistar albino rats of either sex weighing 150-200 g were used. Animals used in the study were procured from registered breeder. The animal care and handling were carried out according to CPCSEA guidelines. Animals were acclimatized to the animal quarantine for one week prior to the experiment under controlled conditions of temperature (27 ± 2°C) and were housed in sterile polypropylene cages containing paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee, Srinivas College of Pharmacy, Mangalore, Karnataka (Approval No.: SCP/IAEC/F150/P150/2019).

Preliminary qualitative phytochemical analysis

Preliminary phytochemical analysis of extract was conducted to check the presence of various phytoconstituents[9,10].

Determination of Acute toxicity (LD_{50})[11]

The extract of the *Passiflora edulis* leaf in the dose of 2000 mg/kg body weight of animal was administered in 1ml/100g body weight of the animal. Acute toxicity study of the extract was done according to acute toxic classic method (OECD guideline 423, 2006) using albino female rats to determine the safe dose.

Paracetamol induced hepatotoxicity[12]

The Wistar albino rats (150-200g) of either sex was randomly divided into different groups of 6 animals each as follows.

Group I-Negative control (vehicle 1ml/kg; p.o)

Group II-Positive control (Paracetamol 2g/kg, p.o. and vehicle 1ml/kg; p.o)

Group III-Standard drug (Paracetamol 2g/kg, p.o. + Silymarin 25 mg/kg, p.o)

Group IV-Test drug (Paracetamol 2g/kg, p.o. + Extract of *P.edulis* leaf 200mg/kg, p.o.)

Group V-Test drug (Paracetamol 2g/kg, p.o. + Extract of *P.edulis* leaf 400mg/kg, p.o.)

All the drug preparations and *Passiflora edulis* extract was administered orally once daily for 11 days. All the groups except group I was intoxicated by oral administration of paracetamol (2 g/kg b/w) on 9th day of treatment. On 11th day, after 24h after the treatment, blood was collected through retro orbital puncture under light ether anaesthesia and serum was separated by centrifugation (2500 rpm for 20 min) and used for analysing various biochemical parameterslike SGOT, SGPT, ALP and bilirubin. Animals were sacrificed by euthanasia, the liver was dissected out and the part of which was placed in 10% formalin solution for histopathological studies and remaining portion was used for the assay of reduced glutathione enzyme (GSH).

Alcohol induced liver toxicity[12]

The Wistar albino rats (150-200g) of either sex was randomly divided into different groups of 6 animals each as follows.

Group I-Negative control (vehicle 1ml/kg; p.o)

Group II: Positive control (Ethanol (40% v/v, 2 ml/100g and vehicle 1ml/kg; p.o)

Group III: Standard drug (Ethanol (40% v/v, 2 ml/100g + Silymarin 25 mg/kg, p.o)

Group IV: Test drug (Ethanol (40% v/v, 2 ml/100g + Extract of *P.edulis* leaf 200mg/kg, p.o.)

Group V: Test drug (Ethanol (40% v/v, 2 ml/100g + Extract of *P.edulis* leaf 400mg/kg, p.o.)

All the four groups except group I was made hepatotoxic by oral administration of ethanol (40% v/v, 2ml / 100g body wt.) daily for 21 days. All the treatment was given orally once daily for entire 21 days. On 22nd day, after the treatment, blood was collected through retro orbital puncture under light ether anaesthesia and serum was separated by centrifugation (2500 rpm for 20 min) and used for analysing various biochemical parameterslike SGOT, SGPT, ALP and bilirubin. Animals were sacrificed by euthanasia, the liver was dissected out and the part of which was placed in 10% formalin solution for histopathological studies and remaining portion was used for the assay of reduced glutathione enzyme (GSH).

Statistical Analysis

The results were expressed as mean ± SEM and data were analyzed by one–way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical analysis of the extract revealed the presence of phytochemicals like flavonoids tannins, anthraquinone, saponin, coumarinand glycosides and steroid.

Evaluation of hepatoprotective activity of hydroalcoholic extract of *Passiflora edulis* leaf on paracetamol induced hepatic damage in rats

Administration of paracetamol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *Passiflora edulis* leaf extract exhibited marked protection against paracetamol induced hepatotoxicity.

The paracetamol induced group showed elevation in ALP, SGOT, SGPT and Total Bilirubin when compared to the control group. Histopathological study showed portal tract inflammation.

There was a significant (p<0.001) reduction in the ALP, SGOT, SGPT and Total Bilirubin levels after the treatment with Silymarin (100mg/kg). Histopathological study showed almost normal appearing hepatocytes.

There was a reduction (p<0.05) in the ALP, SGOT, SGPT and Total bilirubin levels after the treatment with 200 mg/kg *Passiflora edulis* leaf extract. Histopathological study showed mild portaltract inflammation.

There was a significant (p<0.01) reduction in the ALP, SGOT, SGPT and Total bilirubin levels after the treatment with 400mg/kg *Passiflora edulis* leaf extract. Histopathological study showed minimal portal tract inflammation.

Histopathological studies of the liver

The histopathological evaluation of PCM toxicity in all the groups was examined and shown in Fig.1. Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal. In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with *Passiflora edulis* extract (200mg/kg & 400mg/kg) found to reduce inflammation, centrilobular and bridging necrosis.

Table 1: Effect of Silymarin and extract of *Passiflora edulis* leaf on SGOT, SGPT, ALP and Total Bilirubin in PCM induced liver toxicity.

Groups	Treatment	ALP (U/l)	SGOT (U/l)	SGPT (U/l)	TB (mg/dl)
Vehicle Control	Distilled Water 1ml/Kg	140.5±0.5	102.5±0.61	84.5±0.92	0.63±0.08
Toxic Control	PCM 2g/Kg	363.3±4.17[a]	344.8±6.38[a]	207.7±6.81[a]	2.46±0.19[a]
Standard	Silymarin 25 mg/Kg	151.2±4.46***	120.5±2.22***	96.0±1.65***	0.83±0.07***
Low Dose	*Passiflora Edulis* 200mg/Kg	212.1±6.45*	197.5±2.62*	147.5±2.11*	1.46±0.07*
High dose	*Passiflora Edulis* 400 mg/kg	172.0±2.91**	157.0±2.64**	123.1±2.89**	0.97±0.14**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

It was observed that animals treated with PCM developed a hepatic damage, and decrease in GSH when compared to normal control. Animals treated with standard (Silymarin) showed extremely significant (P<0.001) increase in GSH.extract of *Passiflora edulis* leaf (200mg/kg) treated animals showed significant (P<0.05) increase in GSH.Extract of *Passiflora edulis* leaf (400mg/kg) treated animals showed moderately significant (P<0.01) increase in GSH.

Table 2: Effect of Silymarin and Hydro alcoholic extract of *Passiflora edulis* leaf on GSH in PCM induced liver toxicity.

Groups	Treatment	GSH (Abs at 412 nm)
Normal Control	Distilled water 1ml/kg	28.7±.6
Toxic Control	PCM 2g/kg	20.2±1.03 [a]
Standard	Silymarin 100mg/kg	25.3±0.58***
Low Dose	*Passiflora edulis* leaf (200 mg/kg)	21.2±0.96*
High Dose	*Passiflora edulis* leaf (400 mg/kg)	22.6±0.48**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, ap<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

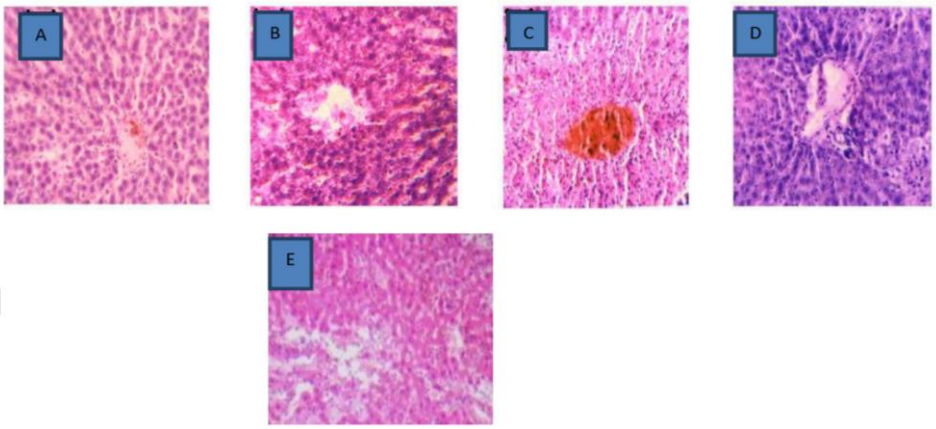

Figure 1: Effect of extract of *Passiflora edulis* leaf on liver histology in PCM induced liver toxicity.

A. Liver of normal rat; B. Liver of PCM induced rat; C. Liver of Silymarin treated rat; D. Liver of *Passiflora edulis* extract200 mg/kg treated rat; E Liver of *Passiflora edulis* extract 400mg kg treated rat.

Table 3: Effect of silymarin and Hydro alcoholic extract of *Passiflora edulis* leaf on SGOT, SGPT, ALP & TB in ethanol induced liver toxicity.

Groups	Treatment	ALP (U/l)	SGOT (U/l)	SGPT (U/l)	TB (mg/dl)
Normal control	Distilled Control Water 1ml/kg	105.9± 0.67	55.0± 1.02	44.1± 1.14	0.63± 0.06
Toxic Control	Ethanol 2ml/100g	125.0± 1.2	125.0± 1.2	112.0± 1.53	2.05± 0.07
Standard	Silymarin 25mg/kg	139.5± 2.6***	71.5± 1.0***	59.5± 1.3***	0.76± 0.05***
Low dose	Passiflora *edulis* Leaf 200 mg/kg	200.9± 1.35*	97.9± 1.3*	87.0± 1.2*	1.45± 0.05*
High dose	*Passiflora edulis* leaf 400 mg/kg	180.9± 1.1**	87.3± 1.1**	75.7± 0.8**	1.01±0.04**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

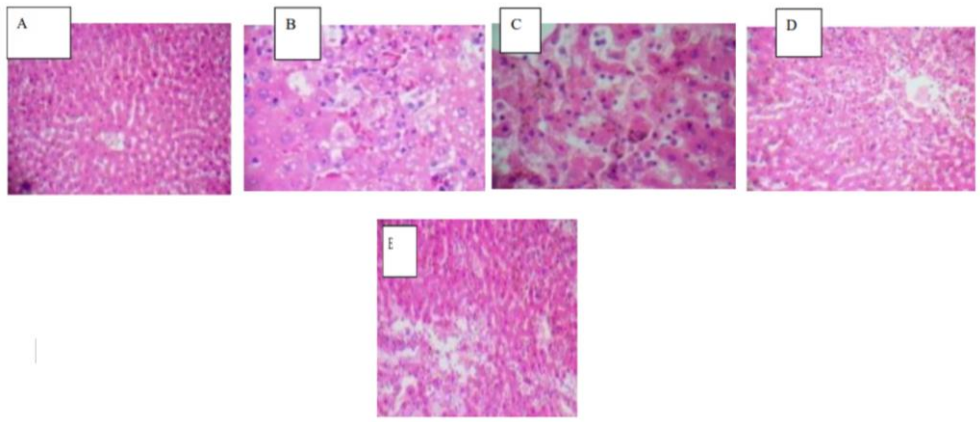

Figure 2: Effect of extract of *Passiflora edulis* leaf on liver histology in ethanol induced liver toxicity.

A. Liver of normal rat; B. Liver of ethanol induced rat; C. Liver of Silymarin treated rat; D. Liver of *Passiflora edulis* extract200 mg/kg treated rat; E Liver of *Passiflora edulis* extract 400mg kg treated rat.

Evaluation of hepatoprotective activity of *Passiflora edulis* leaf extract on ethanol induced hepatic damage in rats

Administration of ethanol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *Passiflora edulis* leaf extract exhibited marked protection against ethanol induced hepatotoxicity. The ethanol induced group showed elevation in ALP, SGOT, SGPT and Total Bilirubin when compared to the control group. Histopathological study showed portal tract inflammation.

There was a significant (p<0.001) reduction in the ALP, SGOT, SGPT and Total Bilirubin levels after the treatment with Silymarin (100mg/kg). Histopathological study showed almost normal appearing hepatocytes

There was a reduction (p<0.05) in the ALP, SGOT, SGPT and Total bilirubin levels after the treatment with 200 mg/kg *Passiflora edulis* leaf extract. Histopathological study showed mild portal tract inflammation.

There was a significant (p<0.01) reduction in the ALP, SGOT, SGPT and Total bilirubin levels after the treatment with 400mg/kg *Passiflora edulis* leaf extract. Histopathological study showed minimal portal tract inflammation.

It was observed that animals treated with ethanol developed a hepatic damage, and decrease in GSH when compared to normal control. Animals treated with standard (Silymarin) showed extremely significant (P<0.001) increase in GSH. Extract of *Passiflora edulis* leaf (200mg/kg) treated animals showed significant (P<0.05) increase in GSH. Extract of *Passiflora edulis* leaf (400mg/kg) treated animals showed moderately significant (P<0.01) increase in GSH.

Table 4: Effect of Silymarin & Aqueous extract of *Passiflora edulis* leaf on GSH in ethanol induced liver toxicity.

Groups	Treatment	GSH (at 412 nm)
Normal Control	Distilled water 1ml/kg	5.13± 0.12
Toxic Control	Ethanol 2ml/100g	2.03± 0.14a
Standard	Silymarin 25mg/kg	4.5± 0.12***
Low Dose	*Passiflora edulis* leaf (200 mg/kg)	2.8±0.08*
High Dose	*Passiflora edulis* leaf (400 mglkg)	3.8±0.05**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, [a]p<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Histopathological studies of the liver

The histopathological evaluation of Ethanol toxicity in all the groups was examined and shown in Fig. 2.

Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal. In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with *Passiflora edulis* (200 mg/kg & 400mg/kg) found to reduce inflammation, centrilobular and bridging necrosis.

DISCUSSION

The serum values of SGPT, SGOT, ALP, and Total billirubin are dramatically elevated after taking paracetamol. This is owing to its bioactivation to N-acetyl-p-benzoquine-imine, a poisonous electrophile. However, when hazardous dosages of paracetamol are administered, the sulfation and glucuronidation pathways become saturated, and cytochrome-450 enzymes oxidise a greater percentage of paracetamol molecules to extremely reactive N-acetyl-p-benzoquineimine (NAPQI). A semi Quinone radical, formed by one electron reduction of NAPQI, can covalently attach to macromolecules of the cellular membrane, causing lipid peroxidation and tissue damage[13].

Histopathological investigations that indicated portal tract inflammation corroborated paracetamol's hepatotoxic impact. The paracetamol-induced rise of SGPT, SGOT, ALP, and Total billirubin was prevented by pre-treatment with *Passiflora edulis* leaf extract for 9 days. These biochemical changes could be attributed to cytochrome P450 inhibition and/or glucuronidationpromotion[14]. Histopathological examinations revealed almost normal hepatocytes, confirming this theory.

In the presence of the alcohol dehydrogenase enzyme, ethanol is converted to acetaldehyde in the liver. The acetaldehyde dehydrogenase enzyme converts this acetaldehyde to acetate. These two enzymes are responsible for the reduction of (NAD) nicotinamide adenine dinucleotide to NADH. Long term exposure to alcohol causes the activation of kupffer cells that induce the generation of reactive oxygen species and finally precipitate to oxidative stress, this in turn promotes hepatocyte necrosis, apoptosis, lipid peroxidation, inflammation and fibrosis. During hepatic damage, cellular enzymes leaks into the serum, resulting in elevation in their concentrations. Ethanol also decreases the activity of Superoxide dismutase, Glutathione and Catalase, along with increase in the level of Lipid peroxidase in liver[15].

Histopathological studies of liver, treated with ethanol alone revealed the affected architecture of liver parenchyma with damaged hepatocytes. Treatment with Passiflora edulis leaf extract revealed significant improvement in architecture of liver parenchyma towards normal and regenerating hepatocytes indicating hepatoprotection[16].

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extracts. Preliminary phytochemical screening of *Passiflora edulis* leaf extract in our studies has shown presence of saponins, flavonoids and polyphenols. This indicates that the flavonoids present in the leaf extract could have the strong antioxidant and free radical scavenging activity in all used assay. Natural antioxidants from the plant extracts provide a measure of production of radical scavengers that slows the process of oxidative damage in liver. The present study revealed that the *Passiflora edulis* leaf extract have proved its synergistic antioxidant effects of bioactive constituents for observed hepatoprotective activity.

CONCLUSION

The present study was undertaken to assess the hepatoprotective activity of *Passiflora edulis* leaf extract. The extract found to have significant hepatoprotective activity in both; Paracetamol induced hepatic injury models. Our results show that the hepatoprotective effects of leaves extract may be due to both an increase in the activity of the antioxidant-defense system and an inhibition of lipid peroxidation. Biochemical and histopathological studies have revealed that leaf have comparable hepatoprotective activity with that of Silymarin. It leads to the conclusion that the *Passiflora edulis* leaf extract can be utilized for its hepatoprotective activity.

REFERENCES

1. Ernest Hodgson. A Textbook of Modern Toxicology. A John Wiley and Sons, Inc. Publication; 2004; 3:263-4.
2. Zimmerman HJ. Hepatotoxicity: The adverse effect of drugs and other chemicals on the liver. 1999; 2(2):21-38
3. Ali SA, Al-amin TH, Mohamed AH, Gameel AA. Hepatoprotective activity of aqueous and methanolic extracts of *Capparis deciduas* stems against carbon tetrachloride induced liver damage in rats. J Pharmacol and Toxicol. 2009;4(1):167-72.
4. Subramaniam A, Pushpangadan P. Development of phytomedicine for liver disease. Ind J Pharmacol.1999;31(2):166-75.
5. Rajesh MG, Latha MS. Preliminary evaluation of the antihepatoptoxicity activity of kamilari, a polyherbal formulation. J.Ethnopharmacol2003; 91(2):99-104.
6. Cetkovic GS, Djilas SM, Brunet CJM, Tumbas VT. Thin layer chromatography analysis and scavenging activity of *Marigold* extracts. International Bibilographic Information on Dietary Suppliments. 2003; 34(5):93-102.
7. Jose J K, Kuttan R, Hepatoprotective activity of *Emblica officinalis* and Chyavanprash J Ethnopharmacol. 2000;72(2):135-40.
8. Noriega P, Mafud DD, Souza BD, Soares-Scott M, Rivelli DP, Barros SB, et.al. Applying design of experiments (DOE) to flavonoid extraction from *Passiflora alata* and

P. edulis. Rev Bras Farmacog. 2012;22(5):1119-29.

9. Kokate CK, Purohit PA, Gokhale BS. Pharmacognosy. 22nd ed. Pune: NiraliPrakashan; 2003; 207-32
10. Trase EG, Evans CW. Pharmacognosy. 12thed. Eastbourne: English languag Book society; 1985; 344.
11. OECD Guidelines for testing of chemicals, Acute oral toxicities, Environmental Health and Safety Monograph series on Testing and adjustment No. 425, 2001.
12. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. Leaf extract. Indian. J. Exp. Biol. 2006;44(4):305-11.
13. Diadelis R, Jan NM, Commandeur ED et al. Vemeulen. Eur J Pharmacol 1995:293-301
14. Jeyakumar SM, Nalini N, Venugopal P Menon. Antioxidant activity of ginger *(Zingiber officinale Rose)* in rats fed a high fat diet. Med Sci Res. 1999; 27: 341-4.
15. Stewart S, Jones D and Day CP. Alcoholic liver disease: New insights into mechanisms and preventative strategies. Trends Mol Med. 2001; 7: 408-13.
16. Arun K, Balasubramanian U. Comparative study on hepatoprotective activity of *Phyllanthus amarus* and *Eclipta prostrate* against alcohol-induced in albino rats. Int J Environ Sci. 2011; 2(1):361-79.

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