Research Article

Hepatoprotective and antidyslipidemic effect of *Ferula asafoetida* in CCl_4 induced hepatotoxicity and CCl_4associated dyslipidemia in rats

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Abstract

Objective: The present study was conducted to evaluate hepatoprotective and antidyslipidemic activity of aqueous extract of *Ferula asafoetida* in CCl_4 induced hepatotoxicity and associated dyslipidemia and oxidative stress in rats. **Material and methods:** Hepatotoxicity was induced by administration of CCl_4(1mg/kg) in alternate days for a period of 14 days. The animals were divided into 6 groups and aqueous extract of *F. asafoetida* at dose level of 50mg/kg, 100 mg/kg and 200 mg/kg was administered respectively for 21 days. Liv-52 (1ml/kg) was used as the standard drug. At the end day of the experiment, the blood was collected by retro-orbital puncture and the hepatoprotective effect was evaluated by analyzing biochemical parameters involved in liver damage. **Result:** The extract was effective in protecting the liver against the injury induced by CCl_4 in rats. This was evident from significant reduction in serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and total bilirubin content. The extract revealed antioxidant and antihyperlipidemic activity too. **Conclusion:** It was concluded from the result that the aqueous extract of asafoetida possesses hepatoprotective activity against CCl_4 induced hepatotoxicity in rats and also play a beneficial role in CCl_4associated dyslipidemia and status of oxidative stress.

Keywords: Asafoetida, hepatoprotective, liver enzymes, lipid profile, oxidative stress

Introduction

The lver is the heaviest gland, weighing about 1.4kg in human body. It plays a vital role in the biotransformation of food, drugs, endogenous and exogenous substances. It has great capacity to detoxify toxic substances and synthesis useful principal (Devraj et al, 2011). Hepatotoxicity may affect all these vital functions of the body. Chemical –induced hepatic injury depends on oxidative stress. Carbon tetra chloride is a widely used as hepatotoxic solvent and is reported to cause free radical induced damage to vital body tissues such as Kidney, lungs, brain, blood and testis (Brahma et al., 2014). Carbon tetrachloride has studied for hepatotoxic properties and it is play an important role in enhancing the accumulation of fat in liver that leads to hyperlipidemia. Cytochrome P450 converts CCl_4 to trichloromethyl ($CCl_3^{\cdot}$) which, in the presence of oxygen is further converted to a peroxy radical. Peroxy radicals initiate lipid peroxidation by abstracting hydrogen atom from polyunsaturated fatty acid of phospholipids. These free radical activate inflammatory and profibrogenic mediators which are responsible for lipid peroxidation and fibrosis which lead to liver injury (Poli et al., 1987). CCl_4 also activates tissue inhibitor of metalloproteinase 1-2(MP1-2), matrix metalloproteinase2 (MMP-2) and matrix metalloproteinase-9, these also activates profibrogenic mediators which lead to liver fibrosis (Sergio, 2015). *Ferula asafoetida* is a monoecious, herbaceous, perennial plant belongs to the Umbelliferae family. Oleo gum resin of *F. asafoetida* obtained from the exudates of roots and rhizomes of the plants. *Asafoetida* consist resin (40to 65%), volatile oil (5t0 20%), gum (20to25%). The resin of *asafoetida* comprises assaresinotannol, It acts as key constituents in the free and combined form as esters of ferulic acid. Many sesquiterpenes are present in *asafoetida* such as assafoetidnol A, assafoetidnol B. Ferulic acid and Galbanic acid are present in oleo gum resin (Kokate , 2010). Asafoetida is in use from ancient times in Indian medicine and cookery as a spice. It is also used in folk phytomedicine since antiquity in traditional medicine for the treatment of

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several neurological (epilepsy, paralysis, hysterias and depression), gastrointestinal (intestinal parasites, flatulence, weak digestion, stomach ache), respiratory (influenza, asthma), and reproductive disorders (premature labour, unusually painful, difficult and excessive menstruation, leucorrhoea, and infertility) (Mahendra and Bisht, 2012, Baitar, 2000, Kabeeruddin, 2007; Said 1997; Khare, 2007; Alqasoumi, 2012). The present study was undertaken to explore the hepatoprotective activity of *F. asafoetida* and associated dyslipidemia *in* CCl_4 induced liver damage in rats.

Material and methods

Preparation of the Extract

F. asafoetida was purchased from local market, Dehradun (UK.), India. Hundred grams of asafoetida was dissolved in 500ml distilled water for whole one day and then boil. After boiling, it was removed from the heat and allowed to stand for 15 min. Preparation was filtered and then concentrated over the water bath. It was dried under vacuum. This dry extract is referred as aqueous extract of *asafoetida* and used as test drug in present study to evaluate the hepatoprotective and hypolipidimic activity.

Experimental animals

Albino rats weighing between 150-220 g were procured from the Animal House, Department of Pharmacology, SBS PG Institute of Biomedical Sciences and Research, Balawala, Dehradun for the present study. The animals were placed at random and allocated to treatment groups in polypropylene cages with husk as bedding. Animals were housed at a temperature of 24±20 ^{0}C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pellet rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC. Animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals (CPCSEA/IAEC/ SBS/ 2016/01).

Experimental Protocol

Hepatotoxicity was induced in rats (250g -280g) by administration of carbon tetrachloride (50% CCl_4 in olive oil) 1ml/kg in alternate days for a period of 14 days. The animals were divided into 6 groups (n=6) and received the following treatment for 21 days:

Treatment protocol

Group 1. Normal control received vehicle (1ml/kg)

Group 2. Positive control received 50% CCl_4 with olive oil (1ml/kg).

Group 3. CCl_4 + *F. asafoetida* (50mg/kg/day)

Group 4. CCl_4 + *F. asafoetida* (100mg/kg/day)

Group 5. CCl_4 + *F. asafoetida* (200mg/kg/day)

Group 6. CCl_4 + Liv-52 (1ml/kg/day)

At the end of the experiment blood was collected by Retro-orbital sinus. Blood samples were allowed to clot and centrifuged at 1000 rpm for 5 min to obtain serum. The serum was used to evaluate different biochemical parameters.

The aqueous extract of *F. asafoetida* was subjected to quantitative chemical test for the identification of plant constituents such as carbohydrate, alkaloid, flavonoids, protein, tannins, sterols, tannins amino acids etc.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows statistical package version 10.0. All the data were expressed as mean ± SEM. The effect of drug treatments were evaluated statistically using one way ANOVA followed by Borforonie test for comparison of results. Statistical significance was set at the $p < 0.05$ level.

Results

Phytochemical study

The phytochemical study indicates the presence of carbohydrate, alkaloids, flavanoids, glycosides and resins in the aqueous extract of Oleogum resin of *F. asafetida*.

Liver function test

The results are summarized in Table no.1. Result has revealed that CCl_4 induces significant elevation in the level of SGOT, SGPT and total bilirubin content as compared to the normal control group. However, pretreatment with extracts (50 mg, 100 mg and 200 mg) produces dose dependent significant decrease in the serum SGOT, SGPT and total bilirubin content level i.e. biochemical indices of liver damage as compared to the CCl_4 treatment group. Standard drug LIV-52 also causes significant reduction in CCl_4induced hepatotoxicity. The results of present study are supported by some other research also where they have been shown the significant decrease in the level of SGOT, SGPT and total bilirubin content in drug treated rats in chemical induced liver damage (Bhupendra and Ashish, 2017).

Lipid profile

CCl_4 treatment induces a significant increase in serum Cholesterol and Triglyceride level summarized in Table

Table 1. Effect of *F. asafoetida* on liver function marker enzymes of rats against CCl_4 induced toxicity

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	T B Mg/dl
I.	Control	38.92±1.587	49.622±2.270	0.34±0.031
II.	CCl_4 (1ml/kg)	166.8 ±1.730	148.8±0.233	2.5±0.2046
III.	STD (1ml/kg)	35.96±0.5134**	37.98±0.2344**	0.36±0.05**
IV.	CCl_4+ *F. asafoetida* (50mg/kg)	74.1±1.069*	77.17±0.4468*	0.62±0.0182*
V.	CCl_4+ *F. asafoetida* (100mg/kg)	42.28±0.776**	43.84±0.2147**	0.40±0.040**
VI.	CCl_4 + *F. asafoetida* (200mg/kg)	40.90±0.5246**	41.32±0.7324**	0.31±0.06**

Values show the effect of treatment with different dose of *F. asafoetida* on serum SGOT level and values are given as Mean ± S.E.M. (n=5), **P<0.001, *P<0.05 when compared with positive controls groups by using ANOVA followed by Dunnet's test.

Table 2. Effect of Aqueous extract of *F. asafoetida* on Cholesterol and triglyceride level in CCl_4 intoxicated rats

Groups	Treatment	Cholesterol (Mg/dl)	Triglyceride (Mg/dl)
I.	Control	90.73±5.543	94.78±10.11
II.	CCl_4 (1ml/kg)	129±0.1503	175.7±0.2381
III.	STD (1ml/kg)	81.30±0.590**	172.9±0.5847
IV.	CCl_4+ *F. asafoetida* (50mg/kg)	77.65±0.1716**	150.5±0.2048**
V.	CCl_4+ *F. asafoetida* (100mg/kg)	73.08±0.190**	145.7 ± 0.1927**
VI.	CCl_4+*F. asafoetida* (200mg/kg)	70.15±0.0910**	144.8 ± 0.4354**

Values show the effect of treatment with different dose of *F.asafoetida* on serum Cholesterol level and values are given as Mean ± S.E.M. (n=5) , ***P<0.001, **P<0.01, *P<0.05 when compared with positive controls groups by using ANOVA followed by Dunnet's test.

No.2. It was observed that treatment with *F. asafetida* at dose 50mg/kg, significantly reduces CCl_4 augmented serum Cholesterol and triglyceride level. Further increase in the dose i.e. 100mg/kg significantly reduces the cholesterol and triglyceride level. However, further increase in dose level of aqueous extract of *F. asafetida* (200mg/kg) causes insignificant reduction in level when it is compared with positive control group. Standard drug Liv-52 reduces cholesterol and triglyceride level in liver damage significantly as compare to CCl_4 alone treated group. The result of present study is supported by some other studies also. Other studies have been also concluded increased level of lipids in CCl_4 induced hepatotoxicity and dyslipidemia. Hepatoprotective and hypolipidemic plants like *Nigella sativa causes* significant decrease in cholestrole and triglyceride level in liver damage (Essaway, 2012).

Oxidative stress

In the present study the level of LPO and SOD were evaluated for assessing the effect of treatment in the status of oxidative stress. Results proved that in groups treated with CCl_4alone, CCl_4causes significant decrease in SOD level and increase in lipid peroxidation as compares to the control group (Table 3). Animals treated with *F. asafoetida* at different dose like 50mg/kg, 100mg/kg, 200mg/kg causes significant decrease in SOD level as well as significant increase in LPO level. LIV-52 also causes significant increase in antioxidant enzyme level and decrease in lipid peroxidation. Antioxidant activity has also shown by some other hepatoprotective plants like *Pisonia aculeata* L. in hepatotoxicity (Palanivel, 2008).

Table 3. Effect of Aqueous extract of *F. asafoetida on the status of oxidative stress in* CCl_4 induced hepatotoxicity

Groups	Treatment	LPO Mol/L	SOD U/min/mg protein)
I.	Control	32.88±0.54	55.72±0.18
II.	CCl_4 (1ml/kg)	60.99±0.26	32.01±0.36
III.	STD (1ml/kg)	40.30±0.29**	53.75±0.12**
IV.	CCl_4+ *F. asafoetida* (50mg/kg)	53.29±0.60*	41.43±0.48*
V.	CCl_4+ *F. asafoetida* (100mg/kg)	48.29±0.57**	48.03±0.07**
VI.	CCl_4+*F. asafoetida* (200mg/kg)	42.83±0.41**	50.40±0.47**

Values show the effect of treatment with different dose of *F. asafoetida* on LPO and SOD level and values are given as Mean ± S.E.M. (n=5) , ***P<0.001, **P<0.01, * P<0.05 when compared with positive controls groups by using ANOVA followed by Dunnet's test.

Discussion

The present study exhibits the hepatoprotective,

antihyperlipidaemic and antioxidant capabilities of aqueous extract of the *F. Asafoetida* oleogum resin against CCl_4 induced liver damage in rats. As the liver is the organ responsible for detoxification of drugs and chemicals, it is the first target for all toxic chemicals. A number of studies have revealed the role of CCl_4 in inducing damage to the liver tissue leading to lipid peroxidation and thereby liver fibrosis. The histological features of severe CCl_4 induced liver damage include hepatocellular injury along with multilobular necrosis and a mononuclear cell infiltrates (Meddrey, 1973). The results obtained from the study reflected that the extract of oleogum resin were effectively able to protect the loss of antioxidants caused due to CCl_4 administration and preventive the formation of metabolites responsible for liver damage (Stepan, 2011). Carbon tetrachloride has studied for hepatotoxic properties and it is play an important role in enhancing the accumulation of fat in liver that leads to hyperlipidemia (Rees and Spector, 1961). Increase in levels of SGOT and SGPT in serum of the CCl_4-treated animals indicate liver damage as these enzymes leak out from liver into the blood due to tissue damage (Rees and Spector, 1961: Naik and Pand, 2008). Following the treatment of the extract, the levels of these marker enzymes were near normal or only slightly elevated, indicating protection against liver damage. The phytochemical analysis of extract has revealed the presence of flavonoids in the extract. A number of studies have been reported which suggest that flavnoids function as antioxidants and may protect against oxidative stress caused due to environmental conditions (Tattini et al., 2004; Gould and Lister, 2006). The antioxidant potential of flavones has been attributed to the higher reactivity of the hydroxyl substituents leading to its radical scavenging capacity (Heim et al., 2002).

Conclusion

The aqueous extract has shown the ability to maintain the normal functional status of the liver. From the above preliminary study, we conclude that the aqueous oleogum resin extract of F. asafetida is proved to be one of the herbal remedies for liver ailment.

Future scope

In the present study only one extract was taken at three dose level. On future the compound can be isolated from the active extract. Further detail toxicity studies can be carried out in future to establish the LD_{50} values of compound to be isolated. The compound can be evaluated to find out exact mechanism of action. Preformulation studies can be designed and carried out in different model of animals and if it shows positive responses, preclinical and clinical studies in details are required to completely evaluate the protective effect and safety profile.

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Conflict of Interests: Nil

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