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Hepatoprotective activity of supercritical fluid extract of *Ailanthus excelsa* leaves on paracetamol induced *Albino* rats

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Abstract
The objective of the present investigation was to study the hepatoprotective activity of supercritical fluid extract of *Ailanthus excelsa* leaf powder. The *Ailanthus excelsa* leaves were dried using dehumidified air dryer and milling was carried out using liquid nitrogen cooled pulverizer in order to retain the more bioactive compounds. The *Ailanthus excelsa* leaf extract was carried out using supercritical fluid extraction method at a pressure of 225 bar and temperature of 60 °C for extraction time of 120 min. The hepatoprotective activity of *Ailanthus excelsa* leaf extract was carried out for paracetamol (PCT) induced *Albino* rats. The effect of *Ailanthus excelsa* leaf extract on change in body weight, liver weight and biochemical parameters such as total bilirubin, SGOT, SGPT and ALP was studied. The change in body weight and liver weight of rats was found to be 4.50±0.63% and 4.21±0.05 g.100 g^{-1}, respectively for a dose of 400 $mg.kg^{-1}$ of body weight. The total bilirubin, SGOT, SGPT and ALP of rats were found to be 2.35±0.59, 52.97±0.43, 48.84±1.65 and 57.08±1.78 for a dose of 400 $mg.kg^{-1}$ of body weight. This was almost comparable to the groups treated with Silymarin a potent hepatoprotective drug used as reference standard and to the control group. Therefore, the study scientifically supports the traditional use of this drug for the treatment of liver disorders.

Keywords: *Ailanthus excelsa,* hepatoprotective, liver, SFE extract

Introduction
In India, the World Health Organization (WHO) has exhibited 2500 species with medicinal purpose and from that 150 species were used on larger scale (Umashankar and Shruti, 2011) [9]. Hence, India a "Botanical garden of the World" has traditionally well practiced knowledge on herbal medicine. Among the different species, *Ailanthus excelsa* is one of the species that has a major source of natural secondary metabolites and acts as an active component by providing a range of potential health beneficiary activities (Kalaskar *et al.,* 2019) [5]. *Ailanthus excelsa* Roxb is commonly known as Ardusa and used as a folk medicine remedy for inflammation and rheumatoid antipyretic, antifertility, antifungal, antimalarial and antibacterial, diabetes, antioxidant activity, anti-cancer activity since it has many phytoconstituents and various pharmacological activity (Verma 2016) [10].
There are different extraction methods for obtaining extract from the plants. The amount of functional compounds recovered from raw plant materials depends on the method for extraction (Garmus *et al*., 2015) [3]. Conventional extraction methods (Soxhlet and maceration) are time consuming and usually require several hours or even days to achieve a complete extraction of bioactive compounds from the plants (Azmir *et al.,* 2013) [1]. Thus, Supercritical Fluid Extraction (SFE) by using CO_2 can be employed as an alternative to conventional methods for extracting and/or fractionating functional compounds (Solona *et al*., 2014 and Ghasemi *et al*., 2011) [8, 4].
Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and wellbeing. But it is continuously and variedly exposed to environmental toxins, abused by poor drug habits and alcohol which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease (Kamlesh *et al*., 2012;

Kiran *et al.*, 2012; Deepika *et al*., 2017) [6, 7, 2]. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed (Kiran *et al*., 2012) [7]. Hence, the present study was carried out to investigate the hepatoprotective effect of supercritical fluid extract of *Ailanthus excelsa* extract against paracetamol induced liver damage in experimental rats.

Materials and Methods

Plant Materials

The fresh *Ailanthus excelsa* stems of uniform colour and freshness were harvested and procured from Yeragera, Raichur (Dist.), Karnataka, India. The fresh leaves of *Ailanthus excelsa* were separated from the stems and used for the drying and milling experiments.

Preparation of extract

The fresh *Ailanthus excelsa* leaves were dried using dehumidified air dryer (Bry air, FSD-1800, Gurgaon, Haryana, India) at 40±1 °C and 15±1% RH. The dried leaves were milled to a mesh size of 250 µm (BSS No. 60) using liquid nitrogen cooled pulverizer (M/s. Spectra Cryogenic Systems, Kota, Rajasthan) in order to retain more bioactive compounds. The extraction of bioactive compounds of *Ailanthus excelsa* leaf powder was carried out using supercritical fluid extraction system (Waters Thar; SFE 500 system, Pittsburgh, USA) at a pressure of 225 bar and temperature of 60 °C for extraction time of 120 min. The extract containing solvent was further evaporated using rotary vacuum flash evaporator (Reutech Laboratories Pvt. Ltd., New-Delhi, India) at 45 °C to completely eliminate the solvent. The concentrated extracts were refrigerated at 4±2 °C for further studies.

Acute toxicity study

The rodents used in the present study were *Albino* rats of both sex (250-300 g) for the acute toxicity studies of *Ailanthus excelsa* supercritical fluid extract. The doses were fixed as per Organization for Economic Co-operation and Development (OECD) guideline No. 423 and adopted CPCSEA protocol. The toxicological effects were assessed on the basis of mortality and behavioral changes.

Study group

The rats (3 months old) weighing between 250 to 300 g of either sex and fasted for 24 h with *ad. blitum* were taken for the experiment. Animals were divided into six groups (No. of rats in each group = 5) for paracetamol (PCT) induced hepatotoxicity studies and administered with test solutions as follows.

1. **Group I:** Served as vehicle control
2. **Group II:** Paracetamol (2 g.kg^{-1}, p. o)
3. **Group III:** Silymarin (200 mg.kg^{-1}, i. p)
4. **Group IV:** SFE extract of AE (100 mg.kg^{-1}, p. o)
5. **Group V:** SFE extract of AE (200 mg.kg^{-1}, p. o)
6. **Group VI:** SFE extract of AE (400 mg.kg^{-1}, p. o)

Group II served as PCT treated control. After 24 h of PCT administration, groups IV, V and VI received 100, 200 and 400 mg.kg^{-1} of SFE extract of *Ailanthus excelsa*, respectively, once daily for consecutive days.

Assessment of hepatoprotective activity

In the assessment of liver damage by paracetamol, the determination of enzyme levels was used. Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Alkaline phosphatase (ALP) and bilirubin are the most sensitive markers used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage (Kiran *et al.*, 2012) [7]. The treated groups of all animals were subjected to biochemical estimations. Duration of the study was 10 days. All the animals were sacrificed on the 11th day. Blood was withdrawn from retro-orbital vein, 16 h after administration of last dose of test samples. Collected blood samples were centrifuged for 10 min. at 3000 rpm and separated the plasma for biochemical (Total bilirubin, SGOT, SGPT and ALP) evaluation using assay kits.

Statistical Analysis

Statistical analysis of data results were expressed as mean ± SE (m). And the data obtained was analyzed by 'One-way ANOVA' followed by 'Dunnett's multiple comparison test'.

Results and Discussion

Acute toxicity studies

The behaviour of the treated rats was found to be normal. During this study, there were no abnormalities of any organs and no mortality of the animals was found. Hence, the *Ailanthus excelsa* leaf extract was found to be safe.

Effect of supercritical fluid extract of *Ailanthus excelsa* on hepatoprotective activity: The effect of SFE extracts of *Ailanthus excelsa* on percentage change in body weight and liver weight in *Albino* rats paracetamol (PCT) induced hepatotoxicity is shown in Table 1. The change in body weight of rats was found to be 6.25±0.41% (Control), 18.33±0.51% (PCT), 4.08±0.58% (Silymarin), whereas in case of SFE extracts of *Ailanthus excelsa*, it was found to be 11.50±1.04% (100 mg.kg^{-1}), 9.65±1.64% (200 mg.kg^{-1}) and 4.50±0.63% (400 mg.kg^{-1}). The liver weight of rats was found to be 3.58±0.08 g.100 g^{-1} (Control), 5.00±0.08 g.100 g^{-1} (PCT), 3.83±0.07 g.100 g^{-1} (Silymarin) whereas in case of SFE extracts of *Ailanthus excelsa* was found to be 5.66±0.51 g.100 g^{-1} (100 mg. kg^{-1}), 4.84±0.16 g.100 g^{-1} (200 mg.kg^{-1}) and 4.21±0.05 g.100 g^{-1} (400 mg.kg^{-1}). The rats treated with paracetamol exhibited a significant rise in body weight and liver weight when compared to the control group. This was significantly reduced after treatment with SFE extract of *Ailanthus excelsa* from a dose of 100 to 400 mg.kg^{-1}. This was almost compared to the groups treated with Silymarin.

Table 1: Effect of *Ailanthus excelsa* leaf extract on percentage change of body and liver weights in *Albino* rats PCT induced hepatotoxicity

Group	Treatment	Change in body weight (%)	Change in liver weight (g.100 g^{-1})
I	Vehicle control	6.25±0.41	3.58±0.08
II	Paracetamol (2 g.kg^{-1}, p. o)	18.33±0.51	5.00±0.08
III	Silymarin (200 mg.kg^{-1}, i. p)	4.08±0.58	3.83±0.07
IV	SFE extract of AE (100 mg.kg^{-1}, p. o)	11.50±1.04	5.66±0.51
V	SFE extract of AE (200 mg.kg^{-1}, p. o)	9.65±1.64	4.84±0.16
VI	SFE extract of AE (400 mg.kg^{-1}, p. o)	4.50±0.63	4.21±0.05

One-way ANOVA n = 6, p< 0.001, F = 325.9

Effect of supercritical fluid extract of *Ailanthus excelsa* biochemical parameters in *Albino* rats PCT induced hepatotoxicity

The Table 2 shows the effect of *Ailanthus excelsa* extract on biochemical parameters in *Albino* rats PCT induced hepatotoxicity. From the table it is observed that, the total bilirubin in rats was found to be 0.80±0.06 (control), 5.00±0.37 (Paracetamol), 2.00±0.37 (Silymarin), whereas in case of SFE extract of *Ailanthus excelsa* the bilirubin was found to be 3.56±0.40 (100 mg.kg^{-1}), 2.78±0.12 (200 mg.kg^{-1}) and 2.35±0.59 (400 mg.kg^{-1}). The SGOT was found to be 42.00±1.58 (control), 105.00±3.44 (Paracetamol), 48.00±0.67 (Silymarin), whereas in case of SFE extract of *Ailanthus excelsa* the SGOT was found to be 63.74±0.50 (100 mg.kg^{-1}), 59.01±1.26 (200 mg.kg^{-1}) and 52.97±0.43 (400 mg.kg^{-1}). The SGPT was found to be 30.00±1.34 (control), 96.00±3.50 (Paracetamol), 44.00±0.24 (Silymarin), whereas in case of SFE extract of *Ailanthus excelsa* the SGPT was found to be 55.39±0.44 (100 mg.kg^{-1}), 52.16±0.43 (200 mg.kg^{-1}) and 48.84±1.65 (400 mg.kg^{-1}). The ALP was found to be 40.00±2.37 (control), 110.00±3.67 (Paracetamol), 54.00±0.31 (Silymarin) whereas in case of SFE extract of *Ailanthus excelsa* the ALP was found to be 73.08±0.54 (100 mg.kg^{-1}), 65.07±2.17 (200 mg.kg^{-1}) and 57.08±1.78 (400 mg.kg^{-1}).

Table 2: Effect of *Ailanthus excelsa* leaf extract on biochemical parameters in *Albino* rats PCT induced hepatotoxicity

Groups	Treatment	Total bilirubin	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	Vehicle control	0.80±0.06	42.00±1.58	30.00±1.34	40.00±2.37
II	Paracetamol (2 g.kg^{-1}, p.o)	5.00±0.37	105.00±3.44	96.00±3.50	110.00±3.67
III	Silymarin (200 mg.kg^{-1}, i.p)	2.00±0.37	48.00±0.67	44.00±0.24	54.00±0.31
IV	SFE extract of AE (100 mg.kg^{-1}, p.o)	3.56±0.40	63.74±0.50	55.39±0.44	73.08±0.54
V	SFE extract of AE (200 mg.kg^{-1}, p.o)	2.78±0.12	59.01±1.26	52.16±0.43	65.07±2.17
VI	SFE extract of AE (400 mg.kg^{-1}, p.o)	2.35±0.59	52.97±0.43	48.84±1.65	57.08±1.78

One-way ANOVA n = 6, p<0.001, P-value: 0.42

The rats treated with paracetamol exhibited a significant rise in total bilirubin, SGOT, SGPT and ALP levels when compared to the control group. This was significantly reduced after treatment with SFE extract of *Ailanthus excelsa* from a dose of 100 to 400 mg.kg^{-1}. The higher dose of PCT causes oxidation and depletion of liver GSH and deviate it to augmented lipid peroxidation and liver damages (Yahya *et al.*, 2013) [11]. As a result of alteration in the level of oxidative enzymes leads to hepatocytes. The *Ailanthus excelsa* extract have preserved the structural integrity of hepatocytes membrane. The enzymes were restored as normal after the treatment with extracts of *Ailanthus excelsa*. The elevated level of enzymes by paracetamol, however prevented by the secondary metabolites of the *Ailanthus excelsa* extracts may be attributed to the presence of flavonoids. The treatment with a dose of 400 mg.kg^{-1} of body weight of *Ailanthus excelsa* extract showed high significant activity. This was almost compared to the groups treated with Silymarin, a potent hepatoprotective drug used as reference standard and to the control group. Therefore, the SFE extract of *Ailanthus excelsa* can be used as a drug for the treatment of liver disorders.

Conclusion

The results of the present work clearly demonstrate the hepatoprotective activity of SFE extract of *Ailanthus excelsa*. Paracetamol causes increase in body weight and liver weight. The paracetamol induced showed significantly increase in total bilirubin, SGOT, SGPT and ALP levels. The elevated level of enzymes by paracetamol, however prevented by the secondary metabolites of the *Ailanthus excelsa* extracts may be attributed to the presence of flavonoids. Therefore, the SFE extract of *Ailanthus excelsa* can be used as a drug for the treatment of liver disorders.

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