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HEPATOPROTECTIVE ACTIVITY OF *PASTINACA SATIVA,* EXTRACT AGAINST ACETAMINOPHEN INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Pastinaca Sativa has been traditionally used in Indian medicine as a result of its curative results of hepatitis, gonorrhea and diabetes; it is probably not proof-founded. However, folklore has given us many powerful therapies, based on plant sources. So claims which can be made for the protective efficacy of Pastinaca Sativa (family: Apiacea) to treat hepatic diseases. The present study focused on investigating the role of alcoholic extract of Pastinaca Sativa. It appreciably prevented the increased in serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and Total serum bilirubin (SB) level in acute liver damage by Acetaminophen and elevated the activities of lipid peroxidation (LPO) and glutathione (GSH) in the liver. Histopathological observation of the liver used to be additionally performed to further support the evidence from the biochemical analysis. The observation that these significant protective effect against acute hepatotoxicity induced by Acetaminophen of Pastinaca Sativa.

KEY WORDS

Pastinaca Sativa, Hepatoprotective Activity, Acetaminophen, albino rats, histopathological studies.

1. INTRODUCTION

Pastinaca Sativa belongs to Apiacea family [1, 2]. The polyacetylene, furanocoumarin present in the plant are used as medicinal properties [3, 4]. The plant diversity is thinly distributed in India, W. Peninsula, Ceylon and China. In India it is found in tropical Himalayas, Burma and Andhra Pradesh (AP). Traditionally the plant is used as an alternative, antibacterial activity [5, 6] Though some of the plants are reputed in the indigenous systems of medicine for their activities [7], it requires scientific evaluation.

Acetaminophen (AMP) (N-acetyl-p-aminophenol, Paracetamol) is usually used as an analgesic and antipyretic drug [8, 9]. Extensive make use of AMP for therapeutic functions leads to severe hepatic damage. Toxic doses of AMP could reason changes in the morphology and function of liver mitochondria [10]. Formation of N-acetyl-p-benzoquinone imine (NAPQI) is the responsible for liver injury through depletion of glutathione (GSH) even as it binds to cellular proteins [11]. AMP induced hepatotoxicity is known to involve liver cytochrome P_{450} (CYPs) together CYP2E1, CYP3A4, and CYP1A2 and it also inhibits the mitochondrial oxidative phosphorylation, reduction of adenosine triphosphate (ATP) and produces selective mitochondrial oxidant stress [12]. Cellular necrosis of the liver cells raises the lipid peroxidation and depletion of glutathione (GSH) besides elevating the serum biochemical marker levels [8].

The present study exceptionally focused on investigating the function of alcoholic extracts from *Pastinaca Sativa* (AEPS) against Acetaminophen - induced hepatic injury of rats. To evaluate the hepatoprotective effect of AEPS in the *in vivo* study, the serum levels of different marker enzymes regarding hepatic integrity, such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Total Serum Bilirubin (SB)

were determined. And also, estimation of Glutathione (GSH) and Lipid Peroxidation (LPO) was determined in the form of Malondialdehyde (MDA) protein on the cellular degree in the liver**.** Furthermore, histological reviews had been carried out to prove the effectiveness of AEPS in a preventive and healing function against Acetaminophen (AMP)-induced toxicity of liver histopathology in rats.

2. MATERIALS AND METHODS

2.1. Chemicals:

Acetaminophen (Paracetamol) 500 mg tablets (Nirmal Prime, Mumbai, India). Silymarin was once bought as Silymarin was purchased from Micro labs, Tamilnadu, India. Moreover, saline was once bought from the nearby provider GSN pharmaceutical private limited, Hyderabad, Telangana and India. The following biochemical parameters of AST, ALT, ALP and Bilirubin were estimated through specifications kits obtained from Span Diagnostics, Surat, India. Rat's feed was once supplied from Mahaveer Endeavors, Madipally and Hyderabad, India. Other chemicals and reagents for this investigation had been of diagnostic grade.

2.2. Plant materials:

Pastinaca Sativa plant material was collected from Tirumala hills in the month of December from Chitoor district, Andhra Pradesh and identified by a Plant Taxonomist Dr.K.Madhava Chety from Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A specimen was deposited in their herbarium. Then after the plants were washed thoroughly to remove adhering soil and earthy matter, later on sliced to thin chips and dried in shade at room temperature and ground to optimal coarse powder.

2.3. Preparation of Extracts:

The powder (600 gm) used to be extracted at ambient temperature (50 - 60^{o}C) successively with alcohol (95% methanol). Throughout extraction with solvents, the solvent was changing every 24 h. The alcohol from the pooled extractions used to be removed through distillation under reduced pressure at 50-60^{o}C to withstand AEPS (62.5g). The extracts were subjected to preliminary phytochemical investigation. And subjected for hepatoprotective activity against Acetaminophen - induced liver damage.

2.4. Preliminary Phytochemical Studies:

The extract of *Pastinaca Sativa* were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, mucilage, proteins, steroids, tannins and terpenoids.

2.5. Experimental animals:

An experimental study was carried out on Wister albino rats of either sex (M/F) rat's age two months. Their body weights ranged from 150 to 200 g. Divided into 5 groups of 6 animals per cage was used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24hrs). The food in the form of dry pellets and water is provided ad libitum. All the animals were accepted by the ethics approval committee of the institute.

2.6. Acetaminophen Induced Liver Toxicity:

The Acetaminophen (AMP) was diluted with saline (vehicle) previous to oral administration (o.p). The group I: vehicle (saline) for 9 days. Group II: vehicle + AMP (1 ml/kg) on nine days [13]. Group III: AMP (1 mL/kg) + Silymarin (100 mg/kg/day, p.o) on nine days [14]. Group IV: AMP (1 mL/kg) + AEPS (250 mg/kg/day, p.o) on the ninth day. Group V: AMP (1 mL/kg) + AEPS (500 mg/kg/day, p.o) on ninth day. To enhance the acute liver damage in animals of groups V, IV, III and II, food were withdrawn 12 h before AMP administration. Animals were sacrificed 24 h after administration of Acetaminophen. Blood samples were accumulated by puncturing the retro-orbital plexus underneath mild ether anesthesia and allowed to coagulate for 30 min at 37°C. Serum was isolated by mean of centrifugation at 2500 rpm for 15 min at 35°C and analyzed for various biochemical parameters [15].

2.7. Assessment of Liver Functions:

The hepatoprotective impact of extract was assessed by the measure of liver potential, biochemical parameters, for design, Alanine Amino Transferase (ALT) [16], Aspartate Amino Transferase (AST) [17], Alkaline Phosphatase (ALP) [18], and Total Serum Bilirubin (SB) [15]' Lipid Peroxidation (LPO) as Malondialdehyde (MDA) [19] and Glutathione (GSH) [20] as per commonplace protocols.

2.8. Measurement of Antioxidant Activity:

From all the experimental groups, the element of the liver was collected and rinsed with 0.15 M Tris-HCl (pH 7.4). A 10% w/v of liver homogenate was prepared in 0.15 M Tris-HCl buffer and processed for estimation of lipid peroxidation in the form of malondialdehyde

(MDA) in the liver. And the supernatant was once used for reduced glutathione (GSH) estimation [20].

2.9. Statistical Analysis:

The Statistical analysis was performed by using One Way ANOVA test followed by Dunnet's assessment test and student t-test (unpaired). The values were expressed as mean ± SEM and the P<0.01 was considered as statistically significant.

3. RESULTS

The phytochemical constituents present in the extract of *Pastinaca Sativa* were determined as if we done. The outcome of preliminary phytochemical showing was Favonoids, phenols, Terpenoid and Steroids are presented.

3.1. Acetaminophen Induced Liver Toxicity:

Alcoholic extraction of *Pastinaca Sativa* utilized as a part of the study safeguarded the auxiliary uprightness of the hepatocellular film in a measurement subordinate way as clear from the assurance gave like that delivered by Silymarin (100 mg/kg; po) [14], an understood hepatoprotective specialist. It's obviously established that Acetaminophen affects liver harm through the activity of its dangerous metabolite, N-acetyl-P-benzoquinoneimine, delivered by the activity of cytochrome $P\text{-}_{450}$ [21]. This metabolite factors exhaustion of glutathione (GSH) prompting cell passing. It's certainly evident that the AEPS concentrate could decrease all the hoisted levels of AST, ALT, ALP and SB towards the traditional satisfactory means that adjustment of plasma layer and also repair of hepatic tissue harms brought on by hepatotoxins [22]. The similar viability of the concentrates tried for their hepatoprotective movement results outcome given a Table 1.

Table 1: Effect of AEPS on ALT, AST, ALP and SB in AMP induced liver toxicity in rats

Treatment	Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	SB (mg/dl)
Group-I: Vehicle (saline)	1ml/kg	58.33 ± 3.10	64.83 ± 2.48	81.33 ± 2.16	0.85 ± 0.03
Group-II: Control (AMP)	1 mg/kg	220.00 ± 3.58^{a}	193.00 ± 4.04^{a}	216.16 ± 3.54^{a}	2.58 ± 0.28^{a}
Group-III: AMP + Silymarin	100 mg/kg	183.5 ± 3.70***	165.5 ± 3.35**	180.00 ±3.00***	0.96 ± 0.03***
Group-IV: AMP + AEPS	250 mg/kg	172.30 ± 3.00***	157.16 ± 5.52***	173.00 ±2.48***	1.14 ± 0.04***
Group-V: AMP + AEPS	500 mg/kg	166.17 ± 3.44***	149.50 ± 5.46***	160.16 ±2.85***	1.03 ± 0.04***

Each value represents the mean ± SEM. n =6 number of animals in each group. ^{a}P<0.001 vs vehicle control, *P<0.05, **P<0.01, ***P<0.001, Compared to respective AMP treated control groups

3.2. Effect of *Pastinaca Sativa* on antioxidant activity:

There was a critical increment in MDA substance and decrease in GSH activities of AMP inebriated animals. Pre-treatment with silymarin (a hundred mg/kg) and *Pastinaca Sativa* (250 and 500 mg/kg) fundamentally P< 0.05 kept the enlargement in MDA levels and conveyed them close to typical level, while GSH levels were altogether (P< 0.01) raised, along these lines giving assurance against paracetamol toxicities. Results given Table 2.

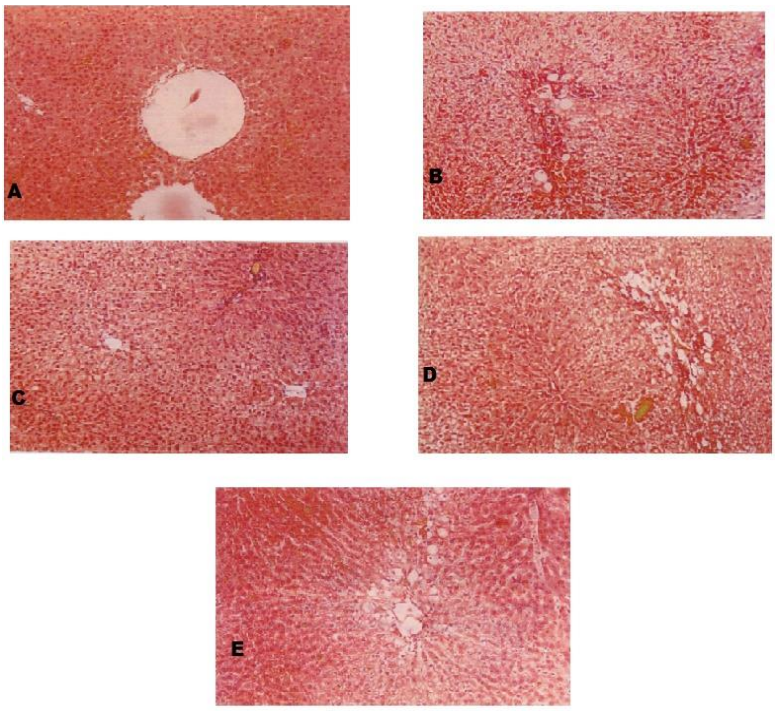
Table-2: Effect of AEPS on LPO and GSH, AMP induced hepatic damage in rats

Treatment	DOSE	LPO (nM MDA/mg protein)	GSH (µg/mg protein)
Group-I: Vehicle (saline)	1ml/kg	0.84 ± 0.06	5.37 ± 0.06
Group-II: Control (AMP)	1ml/kg	4.91 ± 0.06^{a}	2.15 ± 0.08^{a}
Group-III: AMP + Silymarin	100 mg/kg	2.29 ± 0.12***	5.10 ± 0.05***
Group-IV: AMP + AEPS	250 mg/kg	2.29 ± 0.08***	4.17 ± 0.06***
Group-V: AMP + AEPS	500 mg/kg	2.50 ± 0.04***	4.65 ± 0.04***

Each value represents the mean ± SEM. n =6 number of animals in each group. ^{a}P<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective AMP treated control groups

3.3. Histopathological examination of rat livers:
On the ninth day, blood tests were gathered from all creatures by puncturing retro-orbital plexus underneath mellow ether anesthesia, later creatures were sacrificed, and liver tissues were gathered [23]. In this study, histopathological observation of liver used to be carried out to further backing the biochemical examination proof [24]. The model gathering uncovered most extreme harm of all the groups; mainly the microscopic iewpoint of liver tissue of Silymarin and alcoholic extraction of *Pastinaca Sativa* on ALT, AST, ALP and SB in AMP affected liver lethality in rats [25]. However, histological changes in liver tissues from groups which treated at dose 250, 500 mg/kg (Fig 1 A-E).

Figure-1: Microscopic view of liver tissue of alcoholic extract of *Pastinaca Sativa* on ALT, AST, ALP and SB in AMP induced liver toxicity in rats



(A) Microscopic view of liver tissue of normal rats (Group I); (B) Microscopic view of liver tissue of AMP (Group II); (C) Microscopic view of liver tissue of AMP + Silymarin(Group III); (D) Microscopic view of liver tissue of AMP + plant extract 250 mg/kg, p.o (Group IV), (E) Microscopic view of liver tissue of AMP + plant extracts 500 mg/kg, p.o (Group V).

4. DISCUSSION

The *Pastinaca Sativa* extract has been reported to contain different types of terpenoids, the phytochemical screening. A number of compounds belonging to the class of polyphenol have been suggested to possess antioxidant activity [26]. Pretreatment of animals with alcoholic extract of *Pastinaca Sativa* and silymarin prevented the Acetaminophen induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effects of alcoholic extract of *Pastinaca Sativa* against Acetaminophen induced hepatic damage. The hepatoprotective activity of *Pastinaca Sativa* (500 mg/kg) was compared with the activity of standard silymarin (100 mg/kg). However, there was no effect on rise in serum alkaline phosphatase levels by the test extract and silymarin.

Extensive liver damage by Acetaminophen itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes [21]. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for Acetaminophen-induced toxicity [26]. The alcoholic extract of *Pastinaca Sativa* reduced the elevated stages of all the biochemical parameters through AMP. Acetaminophen induced liver necrosis was once inhibited significantly by using *Pastinaca Sativa* extract, which confirms the protective action of alcoholic extract of *Pastinaca Sativa* against

experimentally induced liver damage in rats. ALT, AST, ALP and SB are the most sensitive tests employed in the diagnosis of hepatic disease. It can be concluded from this investigation that extract of *Pastinaca Sativa* possess hepatoprotective activity. Further, detailed studies are warranted to confirm the utility profile of this drug.

CONCLUSION

The results of the present study clearly demonstrate that the various biochemical (Serum AST, ALT, ALP, and SB) histopathological transformations produced by Acetaminophen within the serum and tissue were reserved significantly by the pretreatment of extracts of *Pastinaca Sativa* and Silymarin. This study confirms its use as hepatoprotective as per the ethno pharmacological claims.

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