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Research Article

PROTECTIVE EFFECT OF ETHANOLIC EXTRACT AND ITS ETHYLACETATE AND n-BUTANOL FRACTIONS OF *SECHIUM EDULE* FRUITS AGAINST PARACETAMOL INDUCED HEPATIC INJURY IN MICE

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ABSTRACT

Ethanolic extract of fruits of *Sechium edule* and its different fractions (200 mg/kg, p.o) showed showed a remarkable hepatoprotective activity against paracetamol induced hepatotoxicity in mice by significantly (P<0.01) reducing the levels of AST, ALT, ALP, total billirubin and significantly (P<0.01) increasing the serum total protein. The ethanolic extract of fruits of *Sechium edule* and its different fractions (200 mg/kg, p.o) also improve the histoarchitectural of the liver which was confirmed by histopathological examination. Thus, the ethanolic extract of fruits of *Sechium edule* could protect the liver cells from paracetamol induced liver damages.

Keywords: *Sechium edule*, paracetamol, hepatoprotective activity, hepatotoxicity, histoarchitectural

INTRODUCTION

Sechium edule is an edible plant that belongs to the family Cucurbitaceae also known as sayote, choko, chocho, chow-chow, and vegetable pear. The chayote is a herbaceous, perennial, monoecious, vigorous creeper or climbing plant. The fruits grow either individually or in pairs on a shared peduncle. They are fleshy or fleshy-fibrous, may have longitudinal ridges or furrows, and come in many different shapes (globose, ovoid, subovoid, pyriform) and colours (dark or light green)[1]. The fruits and the seed especially, are rich in several important amino acids[2]. A lectin from the exudate of *Sechium edule* was purified [3]. Eight flavonoids, including three *C*-glycosyl and five *O*-glycosyl flavones, were detected [4]. Twenty known Gibberellins' have been identified in extracts of the seeds of *Sechium edule* [5]. The leaves and fruits have diuretic, cardiovascular and anti-inflammatory properties, the leaves has been used in the treatment of arteriosclerosis and hypertension, and to dissolve kidney stones [6, 7]. It has been reported that the ethanolic extracts of dried leaves and water extracts of seeds were found to possess higher radical-scavenging, reducing power and antioxidant activities by the mechanism of inhibition of lipid peroxidation, free radical scavenging activity[8]. Literature reviews indicated hepatoprotective activity of this plant has not been evaluated so far. In view of this, the present study was aimed to evaluating the hepatoprotective activity of ethanolic extract and its ethylacetate, n-butanol fractions of *Sechium edule* fruits.

MATERIALS AND METHODS

Plant Material

Fruits of *Sechium edule* were collected from Reliance Fresh, Secunderabad and also from Bangalore. The fruit material was taxonomically identified and authenticated by Dr. Shiddamallayya N at Regional Research Institute (Ay.), Bangalore, where the voucher specimen is conserved under the reference number (RRCBI/MCW/7/2008). The fruits of *Sechium edule* were isolated, chopped into small pieces, dried under shade at room temperature for seven days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with ethyl alcohol to get a yield of 12.1 % w/w. Dried extract dissolved in distilled water was used for the study. The ethanolic extract was dispersed in distilled water and partitioned with ethyl acetate in a separating funnel till the colourless ethyl acetate fraction is obtained. Then the aqueous part is then partitioned with n-butanol to get the butanol fraction. Ethyl acetate and butanol fraction so obtained was concentrated by keeping in boiling water bath to get the solid residue. The dried extracts were stored in airtight container and placed in refrigerator.

Phytochemical screening

Preliminary phytochemical screening of ethanolic extract of *Sechium edule* fruits and its ethyl acetate and n-butanol fractions were performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides [9].

Drugs and chemicals

Paracetamol was procured from Dr. Reddy's lab, Hyderabad, India, silymarin were obtained from Micro Labs, Bangalore, India. ALT, AST, ALP, Total bilirubin, Totlal protein and Creatinine kits were obtained from Span Diagnostics, Surath, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animals

In-bred swiss albino mice weighing 20-25g maintained under controlled conditions of temperature (23± 2°C) and humidity (50 ± 5%) and a 12-hour light–dark cycle, were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Mice were kept overnight fasting prior to drug administration. animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of *Sechium edule* fruits and its ethyl acetate and n-butanol fractions. After the administration of *Sechium edule* fruit extract and its different fractions food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks [10].

Selection of dose of the extract and its fractions

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} of *Sechium edule* fruit extract and its different fractions as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Paracetamol induced hepatotoxicity in mice[11]

Hepatotoxicity was induced in mice by administration of paracetamol at the dose of 500 mg/kg,p.o. Mice were randomly divided into nine groups of 6 mice in each.

Group I: served as normal control and received CMC suspension only.
Group II: received paracetamol (500 mg/ kg, p.o,)
All other Groups also received paracetamol once (500 mg/ kg, p.o). 48 hours after paracetamol administration, all other groups received test and silymarin once daily for 5 consecutive days.
Group III: received standard Silymarin (25 mg/kg, p.o).
Group IV: received ethanolic extract of *Sechium edule* (200mg/kg, p.o)
Group V: received ethanolic extract of *Sechium edule* (100mg/kg, p.o)
Group VI: received ethyl acetate fraction of *Sechium edule* ethanolic extract (200mg/kg,p.o)
Group VII: received ethyl acetate fraction of *Sechium edule* ethanolic extract (100mg/kg,p.o)
Group VIII: received n-butanol fraction of Sechium *edule* ethanolic extract (200mg/kg, p.o)
Group IX: received n-butanol fraction of *Sechium edule* ethanolic extract (100mg/kg, p.o)

16 hr. after administration of last dose of drugs, animals were anaesthetized with ether and blood was collected from the retro-orbital plexus puncturing and serum was separated by centrifugation. Mice were sacrificed; livers were excised, rinsed clean in saline and preserved in 10% formalin for histopathological study.

Assessment of hepatoprotective activity

Animals were sacrificed 24 h after the last treatment. Blood was collected by retero orbital sinus puncture, under mild ether anaesthesia.and serum was separated by centrifugation 2500 rpm for 15 min and biochemical investigations were carried out. Liver was dissected out rinsed clean in saline and preserved in 10% formalin for histopathological study.

I. Serum biochemical estimations

The serum was used to estimate the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and

alkaline phosphatase (ALP) were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/liter (IU/L).The levels of total protein and total billirubin were estimated in the serum using standard commercial kits from (SPAN India Ltd, Surat, India).

II. Histopathological studies[12]

Portions of the liver from all the experimental groups were fixed in 10% formalin, dehydrated in alcohol and then embedded in paraffin. Microtome sections (5µm thick) were prepatred from each liver sample and stained with haemtoxylin- eosin (H&E) dye. The sections were examined for the pathological findings of hepatotoxicity.

STATISTICAL ANALYSIS

Data for hepatoprotective activity were expressed as Mean ± SEM from six rats in each group. Hepatoprotective activity were analysed statistically using one way analysis of variance (ANOVA), followed by Dunnett's t-test with the help of graph pad prism 4.0 soft ware. P value of < 0.05 was considered as statistically significant.

RESULT

Acute toxicity studies

In LD_{50} studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Effect of extract of *Sechium edule* and its fractions on Serum biochemical parameters:

In paracetamol induced hepatotoxicity, the activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and total billirubin level showed a significant ($p<0.01$) increase in paracetamol treated group as compared to control group and the total serum protein concentration was significantly ($p<0.01$) lower in paracetamol treated group (Table 1). Administering ethanolic extract of *Sechium edule* and its fractions significantly reduced the levels of AST, ALT, ALP and total billirubin level in paracetamol treated rats as compared to the animals treated with paracetamol alone and the total serum protein concentration was significantly increased.

Table 1: Effect of extract of *Sechium edule* and its fractions on biochemical parameters Asparate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), Total protein (TP), Total bilirubin (TB), Serum Creatinine in paracetamol induced hepatotoxicity on mice

Groups	AST (IU/l)	ALT (IU/l)	ALP (KA)	TP (g/dl)	TB (mg/dl)
Normal control	78.66±4.225	38.75±0.6292	8.31±0.2275	7.833±0.3655	1.55±0.5774
Paracetamol	290±7.284$^{\#\#}$	177.2±2.449$^{\#\#}$	28.49±1.189$^{\#\#}$	3.163±0.143$^{\#\#}$	6.98±0.567$^{\#\#}$
Silymarin (25mg/Kg)	143.3±4.535**	45.5±2.021**	12.05±0.413**	6.598±0.146**	2.15±0.514**
Ethanolic extract of S.E (200mg/Kg)	203±2.646**	50.5±3.014**	16.05±0.1991**	5.703±0.112**	2.56±0.574**
Ethanolic extract of S.E (100mg/Kg)	257±2.739**	62±1.472**	19.46±0.247**	4.09±0.107*	3.88±0.547**
Ethyl acetate fraction of S.E(200mg/Kg)	227.1±3.724**	88±3.241**	14.46±0.212**	6.638±0.299**	2.95±0.574**
Ethyl acetate fraction of S.E (100mg/Kg)	264±0.573**	112.8±1.109**	24.64±0.664**	5.008±0.043**	4.92±0.577^{ns}
n- Butanol fraction of S.E (200mg/Kg)	242±1.106**	79.25±1.548**	18.65±0.606**	6.198±0.147**	3.15±0.567**
n- Butanol fraction of S.E (100mg/Kg)	273±1.932*	116.3±1.493**	23.42±1.018**	3.95±0.108*	4.03±0.576*

Values are expressed as mean ± SEM, n=6; $^{\#\#}$ P<0.01 considered statistically significant as compared to normal control group; **P< 0.01,*P< 0.05 considered statistically significant, ns-non significant as compared to paracetamol treated group.

Histopathology of liver

Histopathological study of liver from control group animals showed a normal hepatic architecture (Figure 1). In paracetamol treated group, severe hepatotoxicity was evidenced by focal necrosis, ballooning, portal inflammation and kupffer cell hyperplasia in livers(Figure 2). Treatment with silymarin (25mg/kh, p.o.) or ethanolic extract of *Sechium edule* fruits (200mg/kg, p.o.) and its different fractions (200mg/kg, p.o.) to paracetamol treated rats exhibited almost normal architecture (Figure 3, 4, 6 & 8).

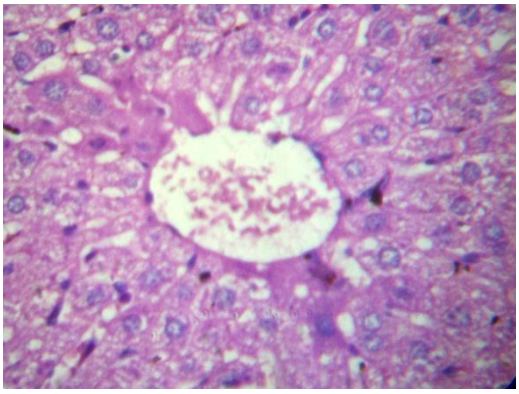

Fig 1: Group I (Normal control)

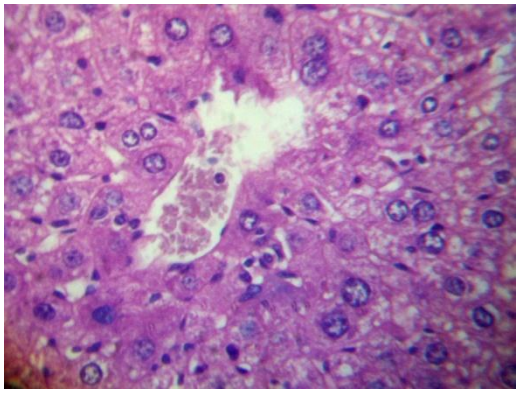

Fig 2: Group II (paracetamol)

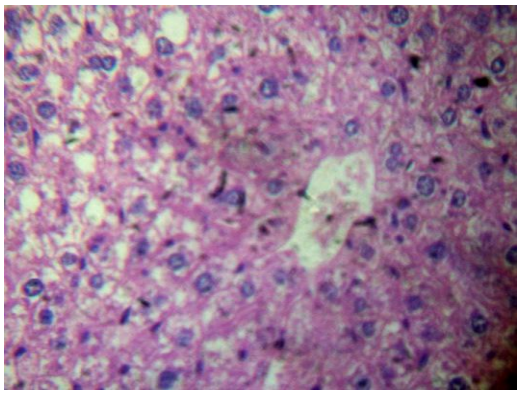

Fig 3: Group III (Silymarin, 50mg/Kg)

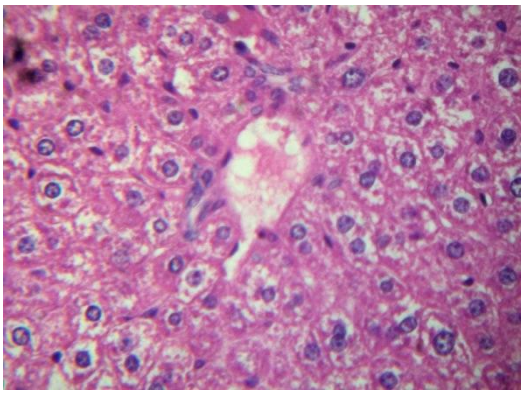

Fig 4: Group IV (Ethanolic extract of S.E, 200mg/Kg)

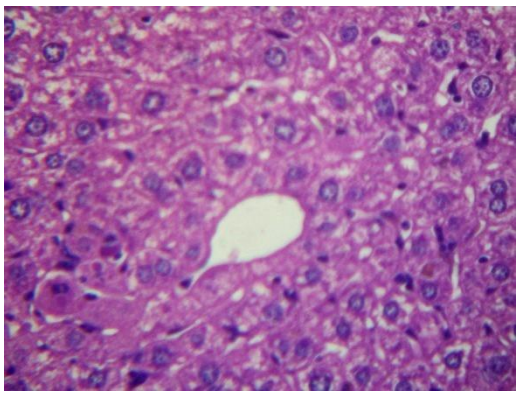

Fig 5: Group V (Ethanolic extract of S.E, 100mg/Kg)

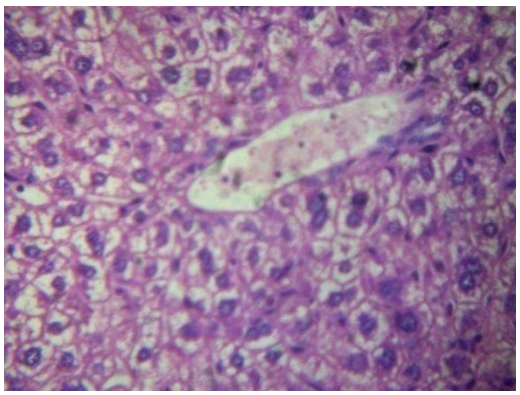

Fig 6: Group VI (Ethyl acetate fraction of S.E, 200mg/Kg)

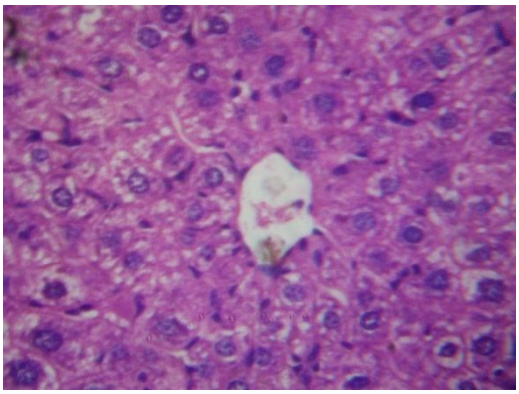

Fig 7: Group VII (Ethyl acetate fraction of S.E, 100mg/Kg)

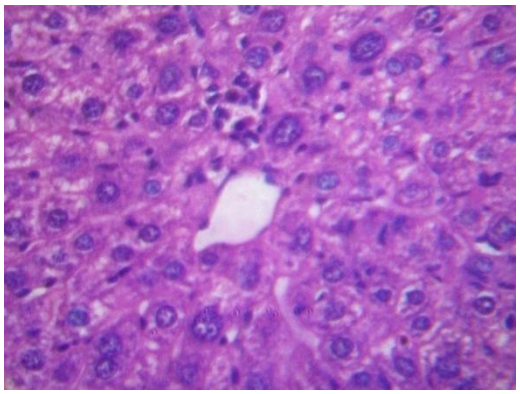

Fig 8: Group VIII (n- Butanol fraction of S.E, 200mg/Kg)

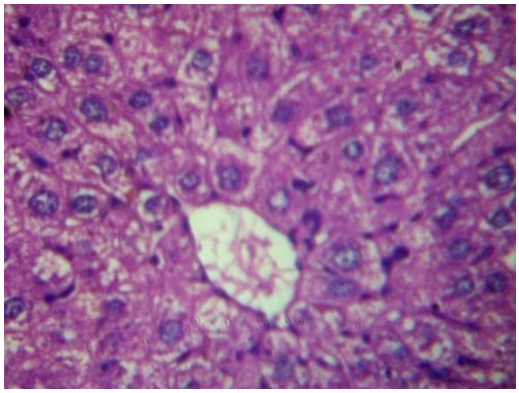

Fig 9: Group IX (n- Butanol fraction of S.E, 100mg/Kg)

DISCUSSION

Paracetamol is a known antipyretic and an analgesic which produces hepatic necrosis in high doses. Paracetamol is normally eliminated mainly as sulfate and glucuronide. Administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinemine by cytochrome-450 enzymes[13]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid[14]. Semiquinone radicals, obtained by one electron reduction of N-acetyl-p-benzoquineimine, can covalently binds to macromolecules of cellular membrane and increase the lipid peroxidation resulting in the tissue damage. Higher doses of paracetamol and N-acetyl-p-benzoquineimine can alkylate and oxidise intracellular GSH, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [15].

In paracetamol-induced hepatotoxicity in mice, our results suggest that the treatment with ethanolic extract of fruits of *Sechium edule* and its different fractions significantly reduced the enhanced level of serum ALT, AST which seem to offer the protection and maintain the functional integrity of hepatic cells. Effective control of bilirubin level and alkaline phosphatase activity by different doses of the extract and its fractions points towards an early improvement in the secretory mechanism of the hepatic cell. The significant raise in protein levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis[16]. These results indicate that the *Sechium edule* ethanolic extract and its different fractions preserved structural integrity of the hepatocellular membrane and showed dose dependant protective effect.

The preliminary phytochemical analysis of the extracts has shown the presence of flavonoids and saponins. Flavonoids and saponins are well known for its antioxidant and hepatoprotective activities which were also supported by the literature review (eg, silymarin, rutin, apigenin, catechin, quercetin, naringenin) [17-20].

There by the results reveal that the ethanolic extract and its fractions of *Sechium edule* fruits have significant hepatoprotective activities on paracetamol induced hepatic damage in rats. Histoarchitectural improvement further supported by biochemical changes in liver, and reduction in serum marker enzymes (AST, ALT, ALP, and TB) and rise in serum protein level, augmentation of endogenous antioxidants supports its hepatoprotective and antioxidant activities. This hepatoprotective activity of the extracts and its fractions of *Sechium edule* may be due to antioxidant activity which may be due to the presence of flavanoids. Further investigation is require to elucidate the hepatoprotective mechanism of *Sechium edule* fruits.

CONCLUSION

The results of our study demonstrate the hepatoprotective activity of ethanolic extract and its different fractions of *Sechium edule* fruits. The probable mechanism for its hepatoprotection may be due to its antioxidant activity.

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