

Hepatoprotective Activity of Hydroalcoholic Extract of *Annona squamosa* Seeds

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Received 20th May, 17; Revised 20th June, 17, Accepted 14th July, 17; Available Online 25th July, 2017

ABSTRACT

Ethnopharmacological Relevance: Traditionally, *Annona Squamosa* Linn. has been used for treatment of cancer, diabetes, microbial infection, cardiac disease, jaundice and other liver related disorder. Hence the current work aims to explore the hepatoprotective of the *Annona Squamosa* (AS) seeds. Aim of the study: The main objective of study is to evaluate hepatoprotective activity of the hydroalcoholic extract of seeds of *Annona Squamosa* Linn. using the carbon tetrachloride induced liver toxicity in rats. Material and methods: The activity was evaluated against carbon tetrachloride induced hepatotoxicity by measuring the levels of serum enzymes such as SGOT, ALP, SGPT and total bilirubin for liver function tests. Six groups of rats were used (n=6) and administered orally once daily with normal saline (normal control), carbon tetrachloride (toxic control), or ASE (100, 200, and 400 mg/kg) for 7 days, followed by the hepatotoxicity induction. Results: The extract showed significant hepatoprotective activity at 100 mg/kg, 200 mg/kg, and 400mg /kg b.w by decreasing the levels of SGOT, SGPT, ALP and total bilirubin as compared to toxic control group. Conclusions: The hydroalcoholic ASE extract have potential of liver protection which could be attributed for further investigation.

Keywords: hepatoprotective activity, *Annona squamosa*

INTRODUCTION

The largest organ in human body, the liver play an important role in metabolism and excretion. Its function is the maintaining and regulating homeostasis of the body, fighting against disease, nutrient supply, energy production and reproduction. Due to property of active metabolite, trichloromethyl radical which covalently bind to the macromolecules and induce peroxidative degradation of endoplasmic reticulum rich in polyunsaturated fatty acids. This may cause to the formation of lipid peroxidase, which in turn gives products like malonyldialdehyde that can cause damage to the membrane. So the carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. Plants that contain phytoconstituents such as coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavanoids, organic acid, organic acid, lipids, alkaloids and xanthenes usually have been considered to have hepatoprotective activity due to which plant based liver protective drug has been in market.

In 16th century, Sitaphal (*Annona Squamosa* Linn.) belongs to the family of Annonaceae has been reported to produce cytotoxic effect³, antidiabetic activity²⁰, analgesic and anti-inflammatory activity¹⁷, hyperthyroidism¹², antimicrobial activity, antilipidemic activity, molluscicidal properties, antimicrobial activity, vasorelaxant activity, antimalarial activity, antioxidant activity, anthelmintic activity, insecticidal activity and genotoxic effect^{1,14}. The plant is traditionally used as insecticidal, dysentery,

anticancer, cardiotoxic, abortifacient, constipation, hemorrhage, and ulcers^{11,16}. It is scientifically accepted that plant *Annona Squamosa* behave towards hepatic disorders as effective remedy¹⁸. Previously, the hepatoprotective activity of leaves and bark of *Annona Squamosa* has been reported^{15,18,19}. In the ayurvedic system of medicine, herbal extract have been used from centuries, because many constituent with more than one mechanism of action are considered to be beneficial. Lots of work has been done on *Annona squamosa* leaves but the seeds have their own medicinal properties due to the presence of phytochemicals as compared to leaves. All the parts of plant possess potent bioactive principles¹³. In the tribal areas of Madhya Pradesh, the rural and traditional practitioner have been using decoction of seeds of *A. Squamosa* for the treatment of jaundice since time immemorial. There is no scientific evidence for its hepatoprotective activity which could validate its traditionally claimed healing activities.

METHODS AND MATERIALS

Collection of plant material

The seeds of *A. Squamosa* Linn. was obtained from Natural Remedies Pvt. Ltd., Bangalore (sample invoice No. DF-249) they were identified and authenticated by Dr. H S Gour University, department of botany, Sagar (M.P.). The voucher specimen No. is BOT/H/12/14/20.

Preparation of plant extract

Table 1: Effect of *A. Squamosa* extract on CCl₄ induced hepatotoxicity.

Groups	Treatment (mg/kg BW)	(U/L) SGOT	SGPT	ALP	(mg/dL) Total Bilirubin
I	Normal Saline (Control)	52.82 ± 6.40	30.24 ± 3.30	87.63±0.57	0.8±0.011
II	CCl ₄ (Toxic control)	155.22 ± 11.50	84.35 ± 5.83	194.33±0.82	3.1±0.069
III	CCl ₄ + Liv 52 (150mg/kgBW)	68.61 ± 7.55	46.24 ± 3.44	103.15±0.71	0.91±0.058
IV	CCl ₄ + AS (100mg/kgBW)	87.37±3.987***	96.22±2.940	159.25±12.894	1.47±0.145
V	CCl ₄ + AS (200mg/kgBW)	76.23±4.158	82.63±6.140	134.55±10.86**	0.98±0.059
VI	CCl ₄ + AS (400mg/kgBW)	51.22±2.56	38.21±3.46*	98.28±6.09	0.71±0.047

p value; ***p<0.001; **p<0.01; *p<0.05. ns = non significant.

AS = *Annona squamosa*

CCl₄ = Carbon tetrachloride

Liv 52 = Standard drug

SGPT = Serum glutamic pyruvic transaminase,

ALP = Alkaline phosphatase

SGOT = Serum glutamic oxaloacetic transaminase

To remove the impurities, seeds of *A. Squamosa* L. was cleaned and washed. For avoidance of microbial growth, it was dried and reduced to coarse powder and subjected to hydroalcoholic extraction using Soxhlet apparatus. The hydroalcoholic extract was filtered, concentrated under reduced pressure and lyophilized to yield 16.8%. The dried extract was stored at 4°C in air tight containers till further studies.

Phytochemical Analysis

The seeds of *A. Squamosa* L. were tested for the presence or absence of phytochemicals like alkaloids, flavonoids, tannins, terpenoids, glycosides, carbohydrates, amino acid etc by phytochemical analysis, using standard methods.

Animal Studies

Albino wister adult male and female rats, aged 2-3 months, weighing 200±25g, were housed in separate cages under controlled conditions of temperature (22 ± 2°C) and kept at 12:12 h, dark/light cycle. All animals were given standard diet (golden feed, New Delhi). All animal experiments were approved by Institutional Animal ethics Committee (IAEC certificate No. PBRI/IAEC/PN-16040) of PBRI, Bhopal.

Experiment

All animals were divided randomly into six groups with six animals in each group (n=6), where all the animal were dose with test samples p.o. for 7days. The first group was served as normal control and received normal saline solution (10ml/kg BW). Group II received CCl₄ (2ml/kg BW) diluted with olive oil (1:1v/v) which was given orally on third and sixth day, Group III received CCl₄ and standard drug Liv52 (150mg/kg BW). Similarly, Group IV, V and VI received CCl₄ and *Annona Squamosa* extract 100, 200, and 400mg/kg B.W. respectively, once daily simultaneously for 7 days. On 8th day, the rats were sacrificed and blood was collected by retro orbital puncture under mild ether anesthesia. Serum was separated from clotted blood by centrifugation at 8000 rpm for 10 min.

Biochemical estimation

The separated serum was analyzed for SGOT, SGPT, ALP and total bilirubin using commercial kit of Aspen Lab, Span diagnostic LTD, and Merck India diagnostic respectively. Analysis was done using Rapid Bioautoanalyser (Star 21). All these estimation were performed following International Federation of Clinical chemistry and Laboratory medicine (IFCC) standard procedures.

Statistical analysis

The significance of difference among the group was assed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test between the data of control and treated groups where the values was expressed in mean ±SEM, p <0.05 were considered significant.

RESULT

The result obtained by phytochemical screening showed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, carbohydrates, saponin where alkaloids, flavonoids and saponins are known to posseses hepatoprotective activity²² and hence the hydroalcoholic extract was selected in this study.

The effect of hydroalcolic seed extract of *Annona Squamosa* Linn. on CCl₄ induced liver damage in rats with reference to changes in the level of SGOT, SGPT, ALP and total bilirubin shown are in Table 1. It was observed that serum level of SGOT, SGPT, ALP and total bilirubin were increased significantly. The SGOT, SGPT, ALP and total bilirubin level was increased in toxic control group and was significantly reduced in the AS extract (100, 200 and 400mg/kg) also it was observed that the results were found to be compared with that observed for the standard drug (Liv52), indicating the recovery of hepatic cells against the damage.

DISCUSSION

The hepatoprotective efficiency of any drug depends on its efficiency to reduce the harmful reaction caused by a hepatotoxin. The phytoconstituents play important role in inducing microsomal enzymes thereby accelerating the excretion of CCl₄ or inhibiting the lipid peroxidation induced by CCl₄. The phytoconstituents such as flavanoids are known to possess hepatoprotective activity.

Serum glutamate pyruvate transaminase (SGPT) belongs to transaminases which catalyze the interconversion of amino acids and α -keto acids by the transfer of an amino group. These enzymes are released into serum during cell damage and destruction acting as hepatoprotective⁸. Alkaline phosphatase (ALP) is produced by bone, intestine, placenta and increased production in serum due to cell damage⁹. Belirubin is product of heme rises in diseases of hepatocytes⁷. SGPT, SGOT, ALP and bilirubin level increased significantly in control group while decreased significantly in all drug treated groups, so that shows efficiency of hepatoprotective of the drug.

CONCLUSION

The results obtained in the present study indicated that hydroalcoholic extract of seeds of *Annona Squamosa* shows hepatoprotective activity where the result may be due to presence of phytoconstituents flavonoids and phenols. Further studies to characterize the active principles and to elucidate the mechanism are in observed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Authors are grateful to Head, Department of Pharmacy, Banasthali Vidhyapeth University, Banasthali (Rajasthan) for providing facilities. We thank Department of Botany, Dr. H. S. Gour University, Sagar (M.P.) for identifying plant species.

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