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Arsenic Trioxide induced Hepatotoxicity in Rats - Protective role of Alcoholic Leaf Extracts of *Annona squamosa*

M. Nagalingam[1], G. Arumugam[1], N. Henita Geo[1], A. Panneerselvam[1]*
1.Department of Zoology, Thiruvalluvar University, Serkkadu, Vellore-115

ABSTRACT

Liver is the largest gland in the human body. Liver performs various vital functions in our system. The liver breaks down toxic substances and most metals products in a detoxifying manner. The present study was conducted to evaluate the hepatoprotective effects of an alcoholic leaf extract of *Annona squamosa* on Arsenic Trioxide induced liver damage in albino rats. Wistar albino rats weighing around 180-200g were used. Blood and liver tissue were collected for the assessment of serum marker enzymes such as ALT, AST and ALP.

Keywords: Annona squamosa, Arsenic, Albino rats, Biochemical tests, Histological studies

*Corresponding Author Email: sagoselvam@yahoo.co.in, bioaru.g@gmail.com
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INTRODUCTION

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics [1]. The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medical drugs, industrial chemical, natural chemicals like microcystins, herbal remedies and dietary supplements [2,3]. The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in adequate, so far not yet any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects. Therefore, that is an essential research about suitable herbal drugs that could replace the chemical ones [4]. Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure [2]. However, no effective treatment that delays disease progression and complications has yet been found. Hepatotoxicity is of Arsenic trioxide is due to the production of more free radicals. Oxidative damage plays an important role in the effects of arsenic; Arsenic disturbs natural oxidation and reduction equilibria through various mechanisms involved in complex redox reactions with endogenous oxidants and cellular antioxidant systems. Both the toxic and therapeutic effects of arsenic are mediated at least in part, by redox sensitive proteins and enzymes. The thioredoxin and glutathione systems play key regulatory roles in redox signaling, potentially cells from the damaging effects of arsenic or other related compounds [3]. Redox sensitive signaling molecules such as AP-1, NFkB, p53 and S-nitrosothiols are affected by arsenic treatment, which consequently deranges the cell signaling and alerts gene expression systems. Thus arsenic shares many property of tumor promoters.

In addition, after cells exposed to arsenic, nitric oxide production is enhanced. Arsenic also inhibits activation of extracellular signal regulated kinase by platelet derived growth factor-B in a reversible. The inhibition is attenuated by antioxidant pre treatment. Reactive oxygen species generated in response to arsenic exposure lead to accumulation of intracellular hydrogen peroxide by activation of flavoprotein dependent superoxide producing enzymes such as $NADT^{+}$ Oxidase. The critical effect of H_2O_2 in mediating apoptosis by its effects on mitochondria; arsenic trioxide acts directly on mitochondria to destroy the inner mitochondrial membrane potential to promote apoptosis.

Annona squamosa L. (Annonaceae), commonly known as the custard apple tree is a native of West Indies. In Tamil it has been called as seetapali. But the cultivation is present throughout India, because of its edible nature [4]. It is a fruit tree considered as a native of Central America also and hence has a wider cultivation throughout the regions of tropics. The taste of the pulp of the fruit is really sweet because of its higher sugar content of about 58% of dry mass, and hence it is found clear that the fruit pulp possesses a high calorie value. This plant was reputed to contain several medicinal properties.

The aqueous leaf extract of Annona *squamosa* was also reported to ameliorate hyperthyroidism, which is the major causative factor for diabetes mellitus. Though there was no such scientific evidence to prove the anti-diabetic effect of *Annona squamosa*, tribal men continued to use the plant in order to manage the diabetes [5]. Its leaves were used as the insecticidal and antispasmodic agents that were used in the treatment of rheumatism and painful spleen. The plant was reported traditionally to possess analgesic, anti-inflammatory, anti-pyretic, anti-ulcer, and antiseptic and abortifacient activities. Its utilization as an insecticidal agent was investigated by several workers and other various phytochemical, pharmacological, anti-bacterial and anti-ovulatory studies was carried out with the extracts obtained from the seeds.

In the present study was under taken to evaluate the efficacy of *Annona squamosa* on Arsenic Trioxide induced Hepatotoxicity in *albino* rats.

MATERIALS AND METHOD

Chemical Used

Arsenic Tri Oxide: Used as an inducing agent purchased from Chandhanmal & Co., Chennai.
Silymarin: Purchased from Apollo Pharmacy, Vellore.

Animals

The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by **(**CPCSEA: 1333/C/ CPCSEA**).** Rat used in this experiment were highly inbred Wister male albino rats from APCAS, Kalavai. The rats weighed between 140-180 g. The animals were housed in spacious cages under hygienic condition (12 hrs light and 12hrs dark cycle at room temperature) and maintained with commercial pellet diet purchased from Chennai as a trade name “Gold Mohr Feeds”. The rats were kept in animal house for 10 days before starting experiments. Body weights are determined at appropriate times.

Preparation of Plant Extract:

Leaves of *Annona squamosa* were collected from the Adhiparasakthi College of Agricultural and Horticulture Farm House. The plant material was air dried at room temperature and powdered coarsely. The powder obtained was macerated with 50% aqueous ethanol for a period of 24 hours and filtered. 100gm of Leaves of *Annona squamosa* was macerated with 500 ml of 50% ethanol and the yield of 22.08 mg and 21.28 mg was obtained respectively.

Experimental design

A total of 30 male Wistar albino rats were taken for this Hepatoprotective study and divided into 5 groups in each groups 6 rats were used. Group 1 - About Six Albino rats were maintained in normal condition with balanced food and water. Group 2 - The intraperitonial injection of about 40 mg Arsenic Trioxide /1kg body weight/once for 2 days is dissolved in 1 ml saline for 20 days. Group 3 - The simultaneous intraperitonial administration of Arsenic Trioxide and alcoholic extract of *Annona squamosa* (250 mg/ 1kg body weight/once for 2 days) dissolved in saline for continuous 20 days. Group 4 - The Arsenic Trioxide (40 mg Arsenic Trioxide / 1kg body weight / once for 2 days) induced rats were treated with alcoholic extract of *Annona squamosa* at a dose of (250 mg/ 1kg body weight/ once for 2 days) for 20 days intraperitonially. Group 5 - The simultaneous administration of Arsenic Trioxide and Silymarin of a dose of 250 mg / 1 kg body weight once for 2 days.

Biochemical Tests

Estimation of Serum Aspartate Transaminase (AST or SGOT) (Reitmann and Frankel`s method), Estimation of Serum Alanine Transaminase (ALT or SGPT) (Reitmann and Frankel`s method), Estimation of Serum Alkaline Phosphatse (ALP) (King and Armstrong method), Estimation of Serum Bilirubin (Malloy and Evelyn method) and Estimation of Protein (Lowry`s method).

Statistical Analysis

The difference of biochemical parameters were measured using the statistical method of analysis of variance (ANOVA). Analysis of variance refers to the examination of difference among the samples. It is a statistical technique specially designed to test whether the means of more than the quantities population are equal. All data were expressed as Mean ± SD of the experiments. The statistical significant was evaluated by one-way analysis of variance (ANOVA) using SPSS Version 16.0 obtained the individual comparisons. A value of <0.05 was considered to indicate a significant difference between groups. Values sharing a common superscript do not differ significantly with each other at $p<0.05$ and $p<0.01$.

RESULTS AND DISSCUSION

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem. Many metals are toxic to liver, in that Arsenic trioxide induces hepatotoxcity. Arsenic trioxide is a chemotherapeutic agent of idiopathic function used to treat leukemia. Arsenic trioxide is considered as toxic when it used above the therapeutic level. Sometimes it causes the centrilobular hepatic necrosis, by generating free radicals. There is no effective treatments are available that completely neutralize its toxic effects. Experimental works on several Non enzymatic anti oxidants has been carried out to evaluate the efficacy against metal induced hepatotoxcity. The present study was used to study of the efficiency of alcoholic extract of *Annona squamosa* against experimental induced hepatic injury, oxidative stress and response to the free radicals. Alcoholic extract of *Annona squamosa* acts as an antioxidant by being available for energetically favorable oxidation. Many oxidants (typically, reactive oxygen species) such as the hydroxyl radical (formed from hydrogen peroxide), contain an unpaired electron and thus are highly reactive and damaging to humans and plants at the molecular level. This is due to their interaction with nucleic acid, proteins and lipids. Reactive oxygen species oxidize (take electrons from) alcoholic extract of *Annona squamosa.* The reactive oxygen species are reduced to water, while the oxidized forms of alcoholic extract of *Annona squamosa* are relatively stable and uncreative and do not cause cellular damage. The most typical reaction leading to endogenous formation of mutagen is between nitrite and nitrosamine or amides, which can be inhibited by vitamin C. In *in-vivo* the arsenic acid acts as an antioxidant and pro oxidant mechanism, to scavenge the free radicals. Since it was water soluble accumulation was not toxic. It can easily scavenge free radicals by regeneration of vitamin E in our system.

Table -1 shows the levels of liver marker enzymes such as ALT, AST and ALP of normal and experimental groups of rats. Acute administration of arsenic trioxide produced marked elevation of the serum levels of ALT, AST and ALP in treated animals (group 2 and group 3) when compared with that of the normal group. In the assessment by arsenic trioxide the determination of enzyme into circulation; therefore, it can be increased in serum. High levels of AST indicate liver damage such as that due to viral hepatitis. ALT level also increased in similar manner. Therefore ALT is more specific to the liver, and is thus better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage of cell membrane in liver [6]. Treatment of alcoholic extract of *Annona squamosa* shows decreased levels of the

enzyme in serum and return towards the respective normal value that indicates the simultaneous treatment of ascorbic acid maintain the stability of plasma membrane, as well as prevention of liver cells from action of free radicals. The above changes can be considered as an expression of maintenance of function of hepatocytes. Liver cells participate in a variety of metabolic activities, so it contains many enzymes. Arsenic induced liver damage raises the values of ALP related to the status of the hepatic cells. Increased in serum ALP due to increased synthesis as a result of increasing bilary pressure. The simultaneous administration of alcoholic extract of *Annona squamosa* is protecting the cell from free radicals action and thereby it inhibits the leakage of marker enzyme into the circulation. Table -2 shows the level of total serum proteins in between the experimental groups decreased in the total serum proteins in arsenic treated rats, may be associated with the decrease in the number of hepatocytes, which in turn may result into the decreased hepatic capacity to synthesis protein. The administration of alcoholic extract of *Annona squamosa* simultaneously will prevent the hepatocytes from the arsenic action and this will enhance the protein synthesis. That activity was compared with the standard hepatoprotective agent, sylimarin.

Table 1: Changes in the level of ALT, AST and ALP in different experimental groups values are expressed as mean ± SD for six animals in each groups.

Parameters	Group A (Control)	Group B (AS_2O_3 Induced)	Group C(AS_2O_3 + *Annona Squamosa*) Simultaneous	Group D (AS_2O_3+ *Annona Squamosa*)	Group E (AS_2O_3+ Silymarin)	Level of Significance
SGPT (U/L)	26.26±0.824	76.26±1.065	31.65±3.53	70.16±1.72	31.09±0.78	P<0.01
SGOT(U/L)	47.19±2.61	136.72±2.533	48.98±2.34	104.5±3.27	51.11±0.92	P<0.01
ALP (U/L)	73.16±2.48	155.16±3.81	83±5.966	105.33±4.07	84.5±3.27	P<0.05

The levels of SGPT, SGOT and ALP are expressed as IU/L values are mean ± SD for six rats in each group (n=6) SGPT, SGOT and ALP (Group A & C) = $P \leq 0.01$ and $P \leq 0.05$

A – Control Group, B – AS_2O_3 induced Group, C – AS_2O_3 + *Annona Squamosa* Simultaneous, D – AS_2O_3 + Treated and E – AS_2O_3 + Silymarin

Table 2: Changes in the level of Total Protein in different experimental groups values are expressed as mean ± SD for six animals in each groups

Parameters	Group A (Control)	Group B (AS_2O_3 Induced)	Group C (AS_2O_3 + *Annona Squamosa*) Simultaneous	Group D (AS_2O_3+ *Annona Squamosa*)	Group E (AS_2O_3+ Silymarin)	Level of Significance
Total Protein (g/dl)	47.19±0.198	3.761±0.229	4.8833±0.1602	7.253±0.047	7.253±0.047	P<0.01

The levels of Total Protein are expressed as IU/L values are mean ± SD for six rats in each group (n=6) Total Protein (Group A & C) = $P \leq 0.01$

Table 3: Changes in the level of Bilirubin in different experimental groups values are expressed as mean ± SD for six animals in each groups

Parameters	Group A (Control)	Group B (AS_2O_3 Induced)	Group C (AS_2O_3 + *Annona Squamosa*) Simultaneous	Group D (AS_2O_3+ *Annona Squamosa*)	Group E (AS_2O_3+ Silymarin)	Level of Significance
Bilirubin (mg/dl)	0.555±0.047	1.608±0.0719	0.661±0.0445	1.386±0.1211	0.6617±0.0331	P<0.01

The levels of Bilirubin are expressed as IU/L values are mean ± SD for six rats in each group (n=6) Total Protein (Group A & C) = $P \leq 0.01$

Table -3 shows the level of Bilirubin of normal and experimental rats. The arsenic induced group has high Bilirubin values. Bilirubin is also one of the most useful clinical markers to the severity of necrosis and then unconjugated bilirubin is increase [7]. Simultaneous treatment of alcoholic extract of *Annona squamosa* shows decreased level of bilirubin. The treatment value of ascorbic acid was not much effective. An alcoholic extract of *Annona squamosa* treated is maintained the hepatic morphology against the arsenic action. Liver is an important organ actively involved in many metabolic functions is the target by number of toxicants. Metal induced hepatic injury accounts for approximately 2 to 5% of hospitalizations for jaundice, 10% of cases of hepatitis in all adults and more than 40% of cases in adults older than 50. Metal induced hepatotoxicity is most common cause of liver failure in the untied states. A variety of clinical presentations may be seen in patients who develop metal hepatotoxicity, ranging from asymptomatic mild biochemical abnormalities to an acute illness.

CONCLUSION

In conclusion the present study demonstrates that the simultaneous alcoholic extract of *Annona squamosa* posses hepatoprotective activity. Changes in the level of SGPT, SGOT and ALP in different experimental groups all over the world different types of hepatoprotective agents are available for hepatoprotective by the action of free radicals scavenging mechanism. Alcoholic extract of *Annona squamosa* having a more potential free radical scavenging properly by this possessions it exhibit a significant hepatoprotective activity in normal and arsenic induced groups. By analyzing the bio chemical parameters the significant reducing of the elevated levels and brought back to near to normal. After induction of hepatotoxicity by arsenic trioxide, the administered alcoholic extract of *Annona squamosa* not have a significant role in the treatment purpose. The activity of AST and ALT in serum level is increased in arsenic induced group where restored in the simultaneous administration of alcoholic extract of *Annona squamosa* and that significantly compared with Silymarin.

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