



HEPATOPROTECTIVE ACTIVITY OF *ANNONA SQUAMOSA* & *FICUS RELIGIOSA*

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Article Received on 11/08/2024

Article Revised on 01/09/2024

Article Accepted on 22/09/2024

ABSTRACT

Liver is the key organ of the body. It is the largest gland in the body and weighing between 1 and 2.3kg. It is situated in the upper part of the abdominal cavity. Occupying the greater part of the right hypochondriac region one part of the epigastric region and extending into the left hypochondriac region. Its upper and anterior surfaces are smooth and curved to fit the under surface at the diaphragm. The liver is enclosed in a thin capsule and incompletely covered by a layer of peritoneum. Folds of peritoneum from supporting ligaments attaching the liver to the inferior surface of the diaphragm. It is held in position partly by these ligaments and partly by the pressure of the organs in the abdominal cavity. The liver is described as having four lobes. The two most apparent are the large right lobe and the smaller, wedge-shaped left lobe. The other two caudate and quadrate lobes are areas on the posterior surface. Based on this fact after literature review two plants namely *Annona Squamosa* & *Ficus Religosa* were selected for the evaluation of hepatoprotective activity.

KEYWORDS: Liver, abdominal cavity, hepatoprotective activity.

INTRODUCTION

Liver is the key organ of the body. It is the largest gland in the body and weighing between 1 and 2.3kg. It is situated in the upper part of the abdominal cavity. Occupying the greater part of the right hypochondriac region one part of the epigastric region and extending into the left hypochondriac region. The lobes of the liver are made up of tiny lobules just visible to naked eye these lobules are hexagonal and formed by cubical shaped cells, the hepatocytes, arranged in pair of column's radiating form a central vein. Between two pairs of column's of cells these are sinusoids (blood vessel with incomplete walls) containing a mixture of blood from the tiny branches of the portal vein and hepatic artery. This arrangement allows the arterial and venous blood with a high concentration of nutritional materials to mix and come into close contact with the liver cells. Some cells, lining the sinusoids, are hepatic macrophages Kupffer-cell. Blood drains from the sinusoids into central or centrilobular veins.^[1,2,3]

Diseases

In the united state alone, liver disease is now the fifth most common causes of mortality and its incidence is rising^[3] New liver cells develop only when needed to replace damaged cells. Capacity for generation is considerable and damage is usually extensive before it is evident. The effects of disease or toxic agents are seen

when Regeneration of hepatocytes (liver cells) had not kept place with damage, leading to hepatocellular failure.

There is a gradual replacement of damaged cells by Fibrous tissue, leading to portal hypertension and is associated with an increased risk of liver cancer.^[4]

In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs effective against a variety of liver disorders because of their safety, efficacy and cost effectiveness.^[4,5,6]

Ayurveda, Siddha, and Unani are predominantly based on the plant materials. Some of the plants have chemical constituents like steroids and flavonoids which exhibit antioxidant activity thus giving hepatoprotection.

Based on this fact after literature review two plants namely *Annona Squamosa* & *Ficus Religosa* were selected for the evaluation of hepatoprotective activity.^[7]

MATERIAL AND METHODOLGY

Selection of plants

Both the plants were selected from its *hepatoprotective* investigation on the basis of exhaustive literature survey and traditional reporting on the plant.

Selection of animal species

The preferred rodent species is the rat, although other rodent species may be used. Normally females are used. This can be as a result of literature surveys of typical LD₅₀ tests show that, though there is very little distinction in sensitivity between the sexes, in those cases where variations are ascertained females are generally slightly additional sensitive. But if data of the toxicological or toxic kinetic properties of structurally connected chemicals indicates that males are possible to be additional sensitive, then this sex ought to be used. When the test is organised in males' satisfactory justification ought to be provided.

Healthy young adult animals of normally used laboratory strains ought to be used. Females ought to be nulliparous and not should be pregnant. Every animal, at the initiation of its dosing, ought to be between eight to twelve weeks recent and its weight should be between in an interval within $\pm 20\%$ of the average weight of any previously dosed animals.

Preparation of animals

The animals are randomly selected, marked to allow individual identification, and kept in their cages for a minimum of five days before dosing to permit for acclimatization to the laboratory conditions.

6.3.4 Preparation of doses

In general test substances administered in an exceedingly constant volume over the vary doses to be tested by variable the concentration of the dosing preparation. however a liquid outcome or mixture is to be tested but, the employment of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and may be a demand of of some restrictive authorities. In either case, the utmost dose volume for administration should not be exceeded. The utmost volume of liquid that may be administered at just the once depends on the scale of the test animal. In rodents, the quantity must not sometimes exceed 1ml/100g of body weight however in the case of aqueous solutions 2 ml/100g body weight can be considered. With relevance to the formulation of the dosing preparation, the employment of an aqueous solution/suspension/emulsion is recommended wherever attainable, followed thus as of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then probably solution in alternative vehicles. For vehicles other than water the toxicological characteristics of the vehicle ought to be better known. doses should be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is understood and shown to be acceptable.

Table No 03: Collection of Plant material.

S.NO	Name of Plant	Part of plant	Source
1.	<i>Annona squamosa</i>	Leaf	Local Area of Bhopal
2	<i>Ficus Religosa</i>	Leaf	Local Area of Bhopal

PROCEDURE

Administration of doses^[7]

- I. The test material is introduced in a unit dose by gavage using a tube in the stomach or a appropriate intubation canula. In the unusual condition that a unit dose is impossible, in that case the dose ought to be given in smaller divided fractions over a period of time not more than twenty four hours.
- II. Animals was fasted before dosing (e.g. with the rat, food but not water should be withheld over-night, with the albino rats, food but not water should be withheld for 3-4 hours).

Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in rats. Where a dose is administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the period.

III. Number of animals and dose levels

- (1) Three animals are used for each step. The dose level to be employed as the initial dose is chosen from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The initial dose level ought to be be that which is most likely to produce mortality in few of the dosed animals.
- (2) When available data suggests that mortality is unlikely at the highest initial dose level (2000 mg/kg body weight), then a limit test ought to be be conducted. When there is no data on a substance to be analyzed, for animal welfare reasons it is suggested to use the initial dose of 300 mg/kg total body weight.^[7]

The time gap between treatment groups is determined by the onset, duration, and severity of toxic symptoms. Treatment of animals at the next dose, ought to be delayed until one is confident of survival of the previously dosed animals. Exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered. For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.^[8]

RESULT AND DISCUSSION

Collection of Plant Material

The leaf Leaves of *Annona squamosal* & *Ficus Religiose* were collected from the local area of Bhopal. and dry under shade.

7.3 Authentication of Plant Material

The plant material was then authenticated by expert in the month of March and the certificate with Reference no.: NU/Nip/2023/524.

Table No 04: Authentication of Plants.

S.NO	Name of plant	Reference no.	Date
1.	<i>Annona squamosa</i>	NU/Nip/2023/524	16/03/2023
2	<i>Ficus Religosa</i>	NU/Nip/2023/524	16/03/2023

7.4 Extraction of Plant Material

The selection of solvent depends on the fact that how much it dissolved the required Phyto-constituents. Plant

materials were extracted by continuous hot extraction with suitable solvent as shown in table 05.

Table No 05: Extraction of o Plant materials.

S.NO	Plant Name	Part used	Solvent used	Procedure
1.	<i>Annona squamosa</i>	Leaf	Ethanol	Soxhlet extraction
2	<i>Ficus Religosa</i>	Leaf	Ethanol	Soxhlet extraction

Toxicological studies of Plant Materials

Toxicological studies revealed that the extract was not toxic up to the dose of 2000 mg/kg body weight, which is the maximum limit for investigation in general acute oral toxicity (as per OECD guidelines). Thus here 2000 mg/kg dose can be considered as NOAEL for the ASE used in present work. As per the toxicity studies 1/10th and 1/20th of NOAEL was selected as the dose for further pharmacological studies. Thus, the doses selected for investigation of hepatoprotective potential of *Annona squamosa* 200mg/kg and 300 mg/kg body weight.

Rats when treated with 250 mg/kg of the *Annona squamosa leaf* extract with silymarin (100mg/kg b.wt.), showed a prominent restoration of all the parameters when compared to the hepatotoxicant induced group, and was equal to that of control group.

Rats when treated with *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (250mg/kg b.wt.) and *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.), showed a significant restore of all the parameters, when compared to the hepatotoxicant induced group.

Table 8: Survival of test animals at different dose (acute toxicity).

S. No.	Dose	% Survival
01	05	100 %
02	50	100 %
03	300	100 %
04	2000	100 %

Rats treated with 25 mg/kg b.wt. of silymarin showed a significant restoration of all parameters were observed when compared to the hepatotoxicant induced group. Thus, the plant extract showed a dose dependent significant restoration of all altered parameters.

Annona squamosa leaf extract. with silymarin (100mg/kg b.wt.) showed a better restoration when compared to silymarin and *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.) was found to be comparatively equivalent to that of silymarin at 25mg/kg body weight.

In Vivo Hepatoprotective studies of *Annona squamosa*

The rats challenged with hepatotoxicant (Carbon tetra chloride suspension 1.0 ml/kg b.wt. i.p) were treated with

- ✓ *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (100mg/kg b.wt.)
- ✓ *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.),
- ✓ *Annona squamosa leaf* extract (250mg/kg b.wt.) with silymarin (250mg/kg b.wt.)

The hepatotoxicant group rats, on comparison with the control showed an elevation in the levels of AST, ALT, LDH, total bilirubin, direct bilirubin and there was decrease in TGL and total protein.

The effects of treatment with plant extracts and Silymarin on various biochemical parameters was recorded and are shown in table-1 .All the treated groups showed restoration of biochemical parameters, and the levels of restoration were different.

In vivo Hepatoprotective studies of *Ficus Religosa*

The rats challenged with hepatotoxicant (Carbon tetra chloride suspension 1.0 ml/kg b.wt. i.p) were treated with

- ✓ *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (100mg/kg b.wt.)
- ✓ *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.),
- ✓ *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (250mg/kg b.wt.)

The hepatotoxicant group rats, on comparison with the control showed an elevation in the levels of AST, ALT, LDH, total bilirubin, direct bilirubin and there was decrease in TGL and total protein.

The effects of treatment with plant extracts and Silymarin on various biochemical parameters was recorded and are shown in table-2. All the treated groups showed restoration of biochemical parameters, and the levels of restoration were different.

Rats when treated with 250 mg/kg of the *Ficus Religosa leaf* extract with silymarin (100mg/kg b.wt.), showed a prominent restoration of all the parameters when compared to the hepatotoxicant induced group, and was equal to that of control group.

Rats when treated with *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (250mg/kg b.wt.) and *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.), showed a significant restore all the parameters, when compared to the hepatotoxicant induced group.

Rats treated with 25 mg/kg b.wt. of silymarin showed a significant restoration of all parameters were observed when compared to the hepatotoxicant induced group. Thus, the plant extract showed a dose dependent significant restoration of all altered parameters.

Ficus Religosa leaf extract. with silymarin (100mg/kg b.wt.) showed a better restoration when compared to silymarin and *Ficus Religosa leaf* extract (250mg/kg b.wt.) with silymarin (50mg/kg b.wt.) was found to be comparatively equivalent to that of silymarin at 25mg/kg body weight.

7.9 Histopathological studies of *Annona squamos*

Histopathology of liver was studied and recorded in fig 1-6.

Group I: Rats Treated with Carboxy methyl cellulose solvent

The hepatocytes, central veins, portal triads and sinusoids appear normal – Normal histology (Figure 1)

Group II: Rats Treated with Carbon tetra chloride suspension

The hepatocytes showed larger areas id steatosis, balloon degeneration, periportal inflammation by lymphocytes and piecemeal necrosis. The central veins and sinusoids are dilated and congested. The portal triads appear normal – Acute hepatitis with piecemeal necrosis (Figure 2)

Group III: Rats Treated with Carbon tetra chloride suspension and *Annona squamosa leaf* (250mg/kg) with silymarin (250mg/kg)

While most of the hepatocytes appear normal, there is definite evidence of residual disease in the form of scattered hepatocytes with steatosis and balloon degeneration. Focal mild inflammation is also noted although no necrosis is observed. The central veins. Sinusoids and portal triads appear normal – Mild residual hepatitis (Figure 3.)

Group IV: Rats Treated with Carbon tetra chloride suspension and *Annona squamosa leaf* extract (250mg/kg) with silymarin (50mg/kg)

The hepatocytes, central veins and portal triads appear normal. No inflammation, steatosis, balloon degeneration of hepatocytes and congestion of sinusoids is observed – No pathological changes (Figure 4)

Group V: Rats Treated with Carbon tetra chloride suspension and *Annona squamosa leaf* extract (250mg/kg) with silymarin (100mg/kg)

The hepatocytes, central veins and portal triads appear normal. No inflammation, steatosis, balloon degeneration of hepatocytes and congestion of sinusoids is observed – No pathological change (Figure 5.)

Group VI: Rats Treated with Carbon tetra chloride suspension and Silymarin (25mg/kg)

The hepatocytes show focal balloon degeneration. However, no steatos necrosis is noted. The sinusoids appear congested. Central veins and portal triads appear normal. No inflammation is observed – Focal balloon degeneration of hepatocytes (Figure 6).

- ✓ Thus the vehicle CMC was safe and did not produce any pathological changes in the liver.
- ✓ Carbon tetra chloride produced adverse effects such as acute hepatitis and piecemeal necrosis in the liver.
- ✓ *Annona squamosa leaf* extract (250mg/kg) with silymarin (100mg/kg) and *Annona squamosa leaf* extract (250mg/kg) with silymarin (50mg/kg) were beneficial in ameliorating the hepatotoxic effect of Carbon tetra chloride.
- ✓ While there was some residual disease in the group treated with *Annona squamosa leaf* extract (250mg/kg) with silymarin (250mg/kg), showed less activity.
- ✓ The effect of *Annona squamosa leaf* extract (250mg/kg) with silymarin (100mg/kg) body weight was almost equal to that of silymarin, whereas the rats treated with extracts showed very mild residual disease.
- ✓ Combination of *Annona squamosa* extract with silymarin along with the CCl₄ significantly prevented all the biochemical histological alterations caused by the CCl₄. The CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases.

The lipid per oxidative degradation of bio membranes is one of the principal causes of hepatotoxicity of CCl₄. This is evidenced by an elevation in the serum maker enzymes, namely AST and ALT. *Annona squamosa* and silymarin significantly reduced these liver enzyme levels.

Histopathological studies showed that CCl₄ caused fatty degeneration and necrosis of the liver tissue. Pretreatment with *Annona squamosa* and silymarin exhibited protection, which confirmed the results of biochemical studies. These Results of our study indicate that simultaneous treatment with *Annona squamosa* and

silymarin protects the liver against CCl₄-induced hepatotoxicity.

The *Annona squamosa* and silymarin offers vast possibilities in the treatment of various liver disorders.

This may be due to the high level of antioxidant, which was claimed to be the mechanism of hepatoprotection. Further studies on any other models and extensive clinical trials are needed to confirm these results.

	ALT (U/L)	AST (U/L)	LDH (U/L)	TGL (Mg/dl)	Total Protein (g/dl)	Total Bilirubin (Mg/dl)		Direct Bilirubin (Mg/dl)	
Group I	42.30 ± 0.66	78.33 ± 1.28	239.25 ± 3.025	283.93 ± 3.23	6.98 ± 0.24	0.48	± 0.06	0.14	± 2.93
Group II	89.46 ± 1.76 ^a	248.21 ± 8.19 ^a	348.75 ± 5.035 ^a	133.43 ± 4.07 ^a	3.46 ± 0.31 ^a	1.69	± 2.41 ^a	0.52	± 5.64 ^a
Group III	50.99 ± 5.11 ^b	102.5 ± 3.8 ^b	273.59 ± 4.47 ^b	253.09 ± 8.86 ^b	6.11 ± 0.38 ^b	0.44	± 0.50 ^b	0.156 ± 2.8 ^b	
Group IV	41.83 ± 1.64 ^b	81.21 ± 1.89 ^b	245.42 ± 7.01 ^b	281.93 ± 3.76 ^b	6.69 ± 0.33 ^b	0.39	± 1.64 ^b	0.12	± 2.86 ^b
Group V	54.99 ± 3.72 ^b	116.83 ± 3.4 ^b	283.75 ± 3.77 ^b	212.26 ± 6.58 ^b	4.89 ± 0.33 ^b	0.43	± 1.90 ^b	0.176 ± 3.8 ^b	
Group VI	43.33 ± 0.69 ^b	104.33 ± 2.00 ^b	268.92 ± 4.87 ^b	211.93 ± 5.04 ^b	5.84 ± 0.36 ^b	0.43	± 0.01 ^b	0.156 ± 2.8 ^b	

A) denotes statistical significance in comparison to hepato toxicant group with normal group at

B) denotes statistical significance in comparison to hepato toxicant group with drug treated group at p < 0.0001

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The hepatocytes, central veins and portal triads appear normal. No inflammation, steatosis, balloon degeneration of hepatocytes and congestion of sinusoids is observed – No pathological changes (Figure 4)

Group V: Rats Treated with Carbon tetra chloride suspension and *Ficus Relogosa* leaf extract (250mg/kg) with silymarin (100mg/kg)

The hepatocytes, central veins and portal triads appear normal. No inflammation, steatosis, balloon degeneration

of hepatocytes and congestion of sinusoids is observed – No pathological change (Figure 5.)

Group VI: Rats Treated with Carbon tetra chloride suspension and Silymarin (25mg/kg)

The hepatocytes show focal balloon degeneration. However, no steatosis necrosis is noted. The sinusoids appear congested. Central veins and portal triads appear normal. No inflammation is observed – Focal balloon degeneration of hepatocytes (Figure 6).

- ✓ Thus the vehicle CMC was safe and did not produce any pathological changes in the liver.
- ✓ Carbon tetra chloride produced adverse effects such as acute hepatitis and piecemeal necrosis in the liver.
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- ✓ The effect of *Ficus Relogosa* leaf extract (250mg/kg) with silymarin (100mg/kg) body weight was almost equal to that of silymarin, whereas the rats treated with extracts showed very mild residual disease.
- ✓ Combination of *Ficus Relogosa* extract with silymarin along with the CCl₄ significantly prevented all the biochemical histological alterations caused by the CCl₄. The CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases.

The lipid per oxidative degradation of bio membranes is one of the principal causes of hepatotoxicity of CCl₄. This is evidenced by an elevation in the serum marker enzymes, namely AST and ALT. *Ficus Relogosa* and silymarin significantly reduced these liver enzyme levels.

Histopathological studies showed that CCl₄ caused fatty degeneration and necrosis of the liver tissue. Pretreatment with *Ficus Relogosa* and silymarin

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These Results of our study indicate that simultaneous treatment with *Ficus Relogosa* and silymarin protects the liver against CCl₄-induced hepatotoxicity.

The *Ficus Relogosa* and silymarin offers vast possibilities in the treatment of various liver disorders. This may be due to the high level of antioxidant, which was claimed to be the mechanism of hepatoprotection. Further studies on any other models and extensive clinical trials are needed to confirm these results.

	ALT (U/L)	AST (U/L)	LDH (U/L)	TGL (Mg/dl)	Total Protein (g/dl)	Total Bilirubin (Mg/dl)	Direct Bilirubin (Mg/dl)
Group I	41.30 ± 0.66	77.33± 1.28	238.25± 3.025	282.93± 3.23	5.98± 0.24	0.28 ± 0.06	0.12 ± 2.93
Group II	88.46± 1.76 ^a	247.21±8.19 ^a	347.75±5.035 ^a	132.43± 4.07 ^a	2.46± 0.31 ^a	1.29 ± 2.41 ^a	0.32 ± 5.64 ^a
Group III	49.99±5.11 ^b	101.5±3.8 ^b	272.59±4.47 ^b	252.09±8.86 ^b	5.11±0.38 ^b	0.44 ± 0.50 ^b	0.123 ± 2.8 ^b
Group IV	40.83±1.64 ^b	80.21±1.89 ^b	244.42±7.01 ^b	280.93±3.76 ^b	5.69±0.33 ^b	0.39 ± 1.64 ^b	0.10 ± 2.86 ^b
Group V	53.99±3.72 ^b	115.83±3.4 ^b	282.75±3.77 ^b	211.26±6.58 ^b	3.89±0.33 ^b	0.43 ± 1.90 ^b	0.125 ± 3.8 ^b
Group VI	42.33±0.69 ^b	103.33±2.00 ^b	267.92±4.87 ^b	210.93±5.04 ^b	4.84±0.36 ^b	0.43 ± 0.01 ^b	0.156 ± 2.8 ^b

CONCLUSION

Plant-based medicines or plant-derived natural compounds are becoming popular now days over allopathy or synthetic medicines because of less toxic effects and low cost. Synthetic drugs have therapeutic activities against liver disease but in the meantime they also have adverse side effects. Natural antioxidants are the compounds can be used to overcome this problem as they maintain the homeostasis of cell and redox potential of ROS because of the concentration of ROS crosses the threshold it activates stellate cell by activating various cytokines and ultimately it will damage hepatocytes. Medicinal plants have natural antioxidant activity and majority of the world's population are using for the treatment of various diseases and, antioxidant property of any plant is the backbone for the treatment of various ailments.

During the induced of carbon tetra chloride. it is Discussion evident that carbon tetra chloride induced liver damage in rats. The combination of extract of *Annona squamosa* and silymarin was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid carboxy methyl cellulose was thought to be involved the synergistic effect. However, its role in carbon tetra chloride induced hepatotoxicity is unclarified. The results obtained from the analysis of biochemical parameters and histopathological studies, enabled me to conclude that the extracts of *Annona squamosa* Combined extract of *Annona squamosa* and *silymarin* & *Ficus Religiosa* with *silymarin* significantly prevents and reverses Hepatotoxic damage induced by CCl₄ suspension in rats. Carbon tetra chloride, Silymarin and *Annona Squamosa* these are play important role in hepatoprotective activity of *Annona squamosa linn* on albino rats Further work need to be done on polyherbal formulation of both drug with *silymarin* and alone also provide significant result in term of Hepatoprotective activity.

ACKNOWLEDGEMENT: The Author is Thankful to RKDF school of Pharmaceutical Science, BHABHA

University, Bhopal, for providing all kind of help to complete the project.

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