Pharmacological intervention of the fruit of plant *Ananas comosus* acting as hepatoprotective activity in animal models

Debashis Mallik*, Chiranjib Bhattacharjee, T. Shivraj Gouda
Sri Krupa Institute of Pharmaceutical Sciences,Velkatta, Kondapak, Medak Dist, Andhra Pradesh
***Corresponding author: E.mail:mallikcology1986@gmail.com**

ABSTRACT

In the initial step of the project work various extracts of ripe fruits have been prepared by using successive extraction procedure and thus obtained extracts were subjected to preliminary phytochemical screening. Preliminary phytochemical tests have indicated the presence of tannins, triterpene and flavonoids in aqueous and ethanolic extracts. Similarly steroids are also present in petroleum ether and chloroform extracts of ripe fruits of *Ananas comosus*. Rats treated with alcohol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against alcohol induced toxicity to liver. Groups treated with ethanolic and aqueous extracts of fruits of *Ananas comosus* showed significant effects.

Key words: Traditional Medicines, Hepatoprotective, *Ananas comosus*,

INTRODUCTION

Traditional Medicines derived from medicinal plants and 60% of the world's population rely on this traditional medicine. This review focuses on Indian Herbal drugs and plants used in the treatment of various diseases, especially in India. Ulcer, hepatic disorder, nephro disorder these are important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of organ disorder and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. In developing world there are so many causes that lead to organ disease like alcohol consumption, pollution; drug toxicity, life style etc are the most common. Therefore information on effects of these medicinal plants is also included. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named 'rasayana' are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. India is the largest producer of medicinal herbs and is called as botanical garden of the world. The current review focuses on herbal drug preparations and plants used in the treatment of organ damage. Since the plant *Ananas comosus* has not explored to significant extent and on the background of available information of the plant, the present work was planned with the following objectives.

- Collection and authentification of plant materials
- Preparation of plant extracts by using various solvents.
- To investigate preliminary Phytochemical constituents.
- Acute toxicity studies of the extracts according to the OECD guideline 420.
- To evaluate the various pharmacological activities on experimental animals.

MATERIALS AND METHODS

Collection & authentification of plant material: The ripe fruits of *Ananas comosus* (pineapple) were collected from the villages of Tripura in the month of august and after collection, the fruits were cut into small pieces and dried under the shadow around 1 month at room temperature then subjected to size reduction to a coarse powder with the help of mixer grinder. The plant is authenticated by Dr. B. K. Datta, Professor of Botany, Plant Taxonomy & Biodiversity Laboratory, Department of Botany, Tripura University (A Central University), Suryamaninagar -799022. Tripura, India.

Preparation of different extracts:

Preparation of different extracts (petroleum ether, chloroform, ethanolic and aqueous extracts): The fruits of *Ananas comosus* were extracted first with petroleum ether for 18 hours by soxhlet extraction for *defatting* and removing waxy substances. The extract was transferred into the previously weighed empty china dish and evaporated to a thick paste on the water bath, maintained at 50^{o}C to get petroleum ether extract. The extract was finally air dried thoroughly to

remove all traces of the solvent and the percentage yield was calculated each time before extracting with next solvent, marc will be dried in hot air oven below 50^oC and each extract will be concentrated by distilling off the solvent and then evaporated to dryness on rotator flash evaporator.

The aqueous extract was prepared by taking the powdered material of fruits of *Ananas comosus* in a round bottom flask (2000 ml) and macerated with distilled water with 10 ml of chloroform (preservative) for 24 h with shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50^oC. These extracts were stored in airtight containers in a refrigerator below 10^oC. The extracts were examined for their color and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Determination of acute toxicity:

Experimental animals: Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 18-25 g were procured from Sri. Venkateswara Enterprises, Bengaluru for experimental purpose and the animals were acclimatized for 7 days under standard husbandry condition as:

Room temperature: $26 \pm 2^0 C$
Relative humidity: 45-55%
Light/ dark cycle: 12:12

Method of determination of acute toxicity: The acute toxicity of extracts of Fruits of *Ananas comosus* was determined in albino mice of either sex weighing between 18-22 g those maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment and "up and down" (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extracts and observed for its mortality during 48 h study period (short term) toxicity. Based on the short-term toxicity profile of the extracts the doses of the next animals were determined as per as OECD Guidelines No: 420. All the animals were observed 14 days with special reference.

Hepatoprotective activity

Experimental design:

Evaluation of hepatoprotective activity in alcohol-induced hepatotoxicity: Albino rats (Wistar Strain) of either sex weighing 150-200g were selected and divided into 7 groups of 6 animals in each.

Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days.
Group II: 30% alcohol (1.5 ml/rat / twice a day) for 15 days.
Group III: Silymarin (25 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group IV: EEAC (200 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group V: EEAC (400 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group VI: AEAC (200 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group VII: AEAC (400 mg/kg b.w/day) and alcohol as group II, for 15 days.

This period of treatment, the rats were maintained under normal diet and water. The blood was collected from the retro orbital plexus of the rats of all groups 24 h after the last dose administration, under light anesthetic ether. The blood samples are centrifuged at 3000 rpm for 30 min to separate the serum. The serum was analyzed for various biochemical parameters such as SGPT, SGOT, ALP, ALB, BIT and BID. Their presence were determined by using Autoanalyser. Liver was dissected out and subjected for morphological study such as liver weight and liver volume of each animal. Further the liver was placed in 10% formalin solution for histopathological study.

RESULTS AND DISCUSSION

The plant material of *Ananas comosus* was extracted by using various solvent like pet.ether, chloroform, ethanol and aqueous by using soxhlet apparatus. After extraction the % yield, colour of the extracts and consistency were shown in the following table.

Table.1. Percentage yield of different extracts

Solvent	Colour of the extract	% Yield
Petroleum ether	Deep green	1.2%
Chloroform	Black	1.7%
Ethanol	Dark brown	20%
Aqueous	Dark brown	27%

Preliminary Phytochemical screening: The preliminary Phytochemical analysis of petroleum ether, chloroform, ethanolic and aqueous extracts of fruits of *Ananas comosus* revealed the presence of various phytoconstituents.

Acute toxicity study:

- Acute toxicity study was conducted for

Aqueous and Ethanolic extracts of fruits of *Ananas comosus* as per OECD guidelines 420 by using albino mice. Each animal was administered aqueous and ethanolic extracts by oral route.

- 5 mice were orally administered with 2000mg/kg of extract and observed for 14 days with special references to first 48 hours.
- They were observed for signs of toxicity and mortality for 48 hrs, with special attention given during first 4 hr. and daily thereafter for a total of 14 days.
- No mortality was observed with 2000mg/kg.

Hepatoprotective activity:
Alcohol induced hepatotoxicity:

Physical parameters: Liver weight and liver volume: Alcohol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with EEAC and AEAC showed significant effect on liver weight and liver volume compared to toxic control group. The liver weight & liver volume of ethanolic extracts of *Ananas comosus* at a dose of 200 mg/kg & 400 mg/kg.were found to be 4.06 ± 0.760, 4.33 ± 0.227 and 7.96 ± 0.08 ,7.69 ± 0.118 respectively. The liver weight & liver volume of aqueous extracts of the *Ananas comosus* at dose of 100 mg/kg & 400mg/kg were found to be 4.29 ± 0.237, 4.05 ± 0.05 & 7.67 ± 0.14, 7.37 ± 0.05 respectively. Whereas standard (silymarin) shows 3.54 ± 0.315 & 7.23 ± 0.101 respectively. The aqueous extracts were found to be most potent.

Evaluation of Bio chemical parameters:

Effect of AEAC and EEAC on SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats: Rats treated with alcohol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with silymarin had showed good protection against alcohol induced toxicity to liver. Groups treated with ethanolic and aqueous extracts of fruits of *Ananas comosus* showed significant effect which can be comparable with toxic control.

Table.2. Phytochemical study report

Tests	Petroleum ether	Chloroform	Ethanol	Water
Alkaloids	-	-	+	+
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	+
Fixed oils	+	+	-	-
Saponins	-	-	+	+
Sterols	+	+	-	-
Tannins	-	-	+	+
Glycosides	-	-	+	+

(+) Present, (-) Absent

Table.3. Liver weight and liver volume in alcohol induced hepatotoxic rats

Group	Liver weight gm/100gm	Liver volume ml/100gm
Normal Control	3.26 ± 0.322	6.81 ± 0.119
Toxic control	4.88 ± 0.315***	8.83 ± 0.162***
Silymarin (mg/kg)	3.54 ± 0.315	7.23 ± 0.101
EEAC(200gm/kg)	4.06 ± 0.760*	7.96 ± 0.089***
EEAC(400mg/kg)	4.33 ± 0.227***	7.69 ± 0.118***
AEAC(200mg/kg)	4.29 ± 0.237**	7.67 ± 0.14***
AEAC(400mg/kg)	4.05 ± 0.05*	7.37 ± 0.05

Values are expressed as mean±SEM; n=6, mean ± S.E.M, n=6, significant at*P<0.001, **P < 0.01 and *P <0.05 when compared with toxic control group, standard drug: Silymarin (25mg/kg)**

Evaluation of Bio chemical parameters:

Effect of AEAC and EEAC on SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats: Rats treated with alcohol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with silymarin had showed good protection against alcohol induced toxicity to liver. Groups treated with ethanolic and aqueous extracts of fruits of *Ananas comosus* showed significant effect which can be comparable with toxic control.

Effect on total bilirubin: The total bilirubin concentration was found to increase in animals with liver damage by alcohol. In standard group, silymarin administration reduced total bilirubin and animals

treated with EEAC and AEAC have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.

Effect on direct bilirubin: Alcohol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. Treatment with standard drug silymarin showed good reduction in direct bilirubin concentration. Groups treated with EEAC and AEAC significantly reduced direct bilirubin level in respective groups.

Effect on albumin: Induction of liver damage by administration of alcohol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with silymarin has shown significant increase while EEAC and AEAC have shown dose dependent increase in serum albumin level compared to toxic control group.

Histopathological studies of the liver in alcohol induced hepatotoxic rats:

Normal control group: Biopsy no- 6884-A/2013

Microscopic Examination: Section shows liver tissue with normal architecture. Portal area shows mild inflammatory collection. Lobules are normal.

Impression: Liver within normal limits.

Alcohol treated group: Biopsy no- 6884-B/2013

Microscopic Examination: Section shows normal architecture of liver with mild centrizonal hepatocyte necrosis and a few lymphocyte collections. Portal areas show a few plasma cells. No fibrosis.

Impression: chronic active hepatitis- moderate.

Silymarin + Alcohol treated group: Biopsy no- 6884-C/2013

Microscopic examination: Section from liver shows normal architecture tere is sinusoidal dilation and focal hepatocyte necrosis with lymphocytic collection. There is mild peripheral lymphoplasmacytic collection.

Impression: chronic active hepatitis- mild.

EEAC fruit (400mg) + Alcohol treated group: Biopsy no- 6884-D/2013

Microscopic examination: section from liver shows normal architecture. There is periportal chronic inflammatory cell collection mostly plasma cells and a few eosinophils. Lobules show occasional degenerating hepatocytes and a few lymphocytes.

Impression: chronic active hepatitis – mild.

AEAC fruit (400mg) + Alcohol treated group: Biopsy no- 6884-E/2013

Microscopic examination: Section shows normal liver architecture with mildmicrovesicular fatty change. There are few nacrotic hepatocytes and periportal chronic inflammatory cells. No fibrosis.

Impression: chronic active hepatitis- mild.

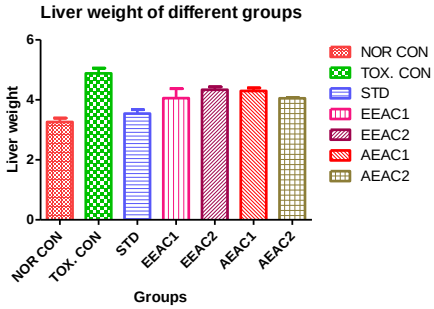


Figure.1. Liver weight in alcohol induced hepatotoxic rats

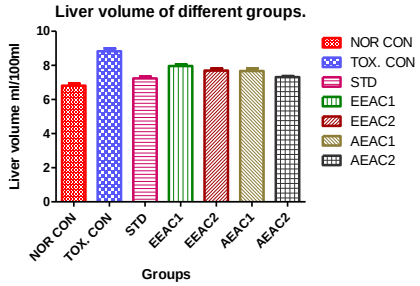


Figure.2. Liver volume in alcohol induced hepatotoxic rats

Table.4.SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	124.5±5.70	116.3±4.1	127.14±1.44
Toxic control	397.3±7.2***	341.9±27.2***	372.32±1.26
Silymarin (25mg/kg)	156.5±12.7	134.2±3.9	182.27±1,52
EEAC(200mg/kg)	257.8±16.7***	204.9±1.9***	239.92±1.32
EEAC(400mg/kg)	202.5±36.7**	184.16±1.1**	223.47±2.04
AEAC(200mg/kg)	222.14±3.7***	194.53±0.62***	225.43±2.48
AEAC(400mg/kg)	189.1±15.8**	161.17±1,68	200.51±2.29

Values are expressed as mean±SEM; n=6; * P<0.05, **p<0.01 and ***P<0.001, Comparison with toxic control, Standard drug: Silymarin (25mg/kg)

Table.5.BIT, BID & ALB levels in alcohol induced hepatotoxic rats

Group	BIT (mg/dl)	BID (mg/dl)	ALB(g/dl)
Control	0.625±0.047	0.41±0.069	4.75±0.113
Toxic control	2.92±0.057***	1.85±0.07***	1.98±0.09***
Silymarin (25mg/kg)	0.7±0.0405	0.78±0.11**	4.12±0.21*
EEAC(200gm)	1.33±0.049***	1.45±0.14***	2.33±0.32***
EEAC(400gm)	1.11±0.029**	1.22±0.106**	3.14±0.314**
AEAC(200gm)	1.26±0.038***	1.3±0.198**	2.77±0.26***
AEAC(400gm)	0.861±0.043*	1.11±0.194*	3.73±0.065*

Values are expressed as mean±SEM; n=6; * p≤0.05, **p≤0.01 and ***P<0.001, Comparison with toxic control group, Standard drug Silymarin (25mg/kg).

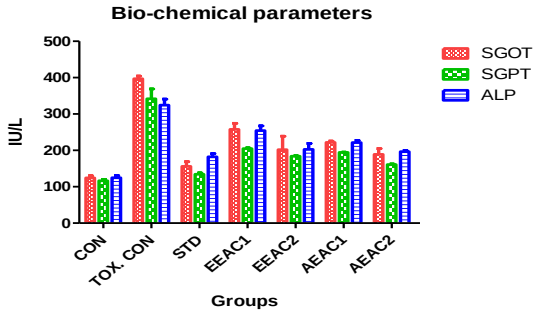


Figure.3. SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

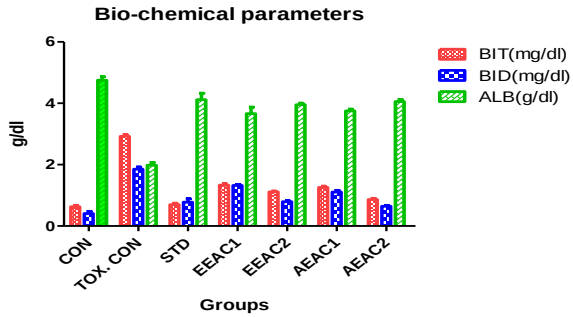
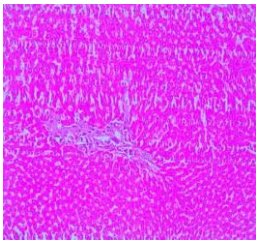
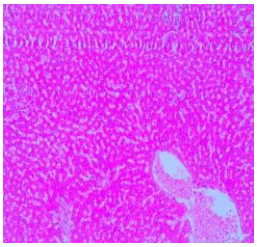


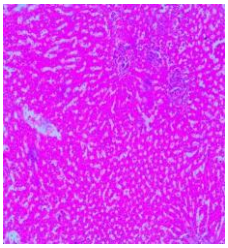
Figure.4. BIT , BID& ALB levels in alcohol induced hepatotoxic rats



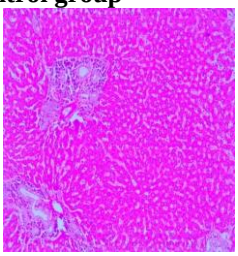
Normal control group



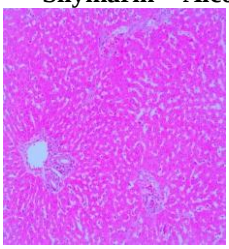
Alcohol treated group



Silymarin + Alcohol treated group



EEAC (200mg) + Alcohol



AEAC (200mg) + Alcohol

Figure.5. Histopathological studies of the liver in alcohol induced hepatotoxic rats

CONCLUSION

In the biological system oxidation plays a major role in the energy generation (that's via Crebs cycle and other system). Oxidation plays a major role in generation of energy by oxidation is the major causes of damage of the organs like heart, kidney, liver, stomach and neurons. In the present study the fruits of *Ananas comosus* possess anti-oxidant property. This anti-oxidant property of ethanolic and aqueous extracts may be due to presence of terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, polyphenolic compounds.The mechanism of organoprotective activity (hepatoprotective) may be attributed its anti-oxidant property. Further studies are required to establish the phytoconstituents responsible for organoprotective activity.

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