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Hepatoprotective Activity of Ethanolic Fruit Extract of *A. comosus* in Paracetamol Induced Liver Toxicity in Rats

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ABSTRACT

The present study was undertaken for investigating the hepatoprotective effect of ethanolic fruit extract of *Ananas comosus* in paracetamol induced hepatotoxicity model in albino wistar rats. Silymarin was used as a standard hepatoprotective drug (100 mg/kg p.o). Ethanolic fruit extract of *A. comosus* (200 mg/kg p.o. & 400 mg/kg p.o) was administered one hour before the administration of paracetamol, once daily for 21 days. Liver biomarkers such as SGPT, SGOT, ALP, serum total bilirubin & total protein were elevated in the paracetamol group. Treatments with ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg significantly reduced liver biomarker enzymes. Histopathological reports revealed that administration of paracetamol caused degeneration of fatty cysts, infiltration of lymphocytes, proliferation of kupffer cells and congestion of liver sinusoids. Upon treatment with ethanolic fruit extract of *A. comosus*, normal hepatic globular architecture, less lymphatic infiltration and normal kupffer cells proliferation were observed, suggesting that ethanolic fruit extract of *A. comosus* protects the liver from adverse conditions. Hence, the ethanolic fruit extract of *A. comosus* possesses hepatoprotective activity against paracetamol-induced hepatotoxicity at a dose of 200 mg/kg and 400 mg/kg.

Keywords: Hepatoprotective activity; paracetamol; *A. comosus*; liver biomarkers; SGPT; total proteins.

1. INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance and performance of the body, including growth, energy provision, and reproduction. Certain chemical, natural, and medicinal agents, when taken in overdoses and within therapeutic ranges, may injure different organs (Ashish *et al*., 2012). The liver is considered as the most important organ in drug toxicity because of toxic products generated from the metabolism and elimination of foreign substances, which render it a preferred target for drug toxicity (Thonda *et al*., 2012). Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. The oxidative product of paracetamol binds to the sulfhydryl group of protein and causes necrosis of the hepatocytes, followed by excessive hepatic lesion, thereby producing cell necrosis in the liver (Yoon *et al*., 2016).

Herbal medicine has been used throughout history and within every culture to prevent and treat diseases. The Indian Traditional Medicine, like *Ayurveda*, *Siddha* and *Unani* are predominantly based on the use of plant materials. Modern medicines have little to offer to alleviate hepatic diseases, and it is chiefly the plant-based preparations that are employed for the treatment of liver disorders. But, there are meager drugs available to treat liver disorders (Sen and Chakraborty, 2017). Hence, there is an increasing need for a safe hepatoprotective agent.

A. comosus (pineapple) is a tropical plant native to South America and is the most economically important plant in the Bromeliaceae family. Various pharmacologically active phytochemicals reported in this plant are ananasate, β-sitosterol, chlorogenic acid, rutin, naringenin, glycosides, flavonoids, and neurotransmitters, such as serotonin, dopamine, adrenaline, and non-adrenaline (Mallik *et al*., 2014). Pineapple contains

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phenolic compounds, vitamins, and several proteinases such as bromelain, comosain, and ananain, which are effective antioxidants (Mohamad *et al*., 2015). *A. comosus* was reported to possess several medicinal properties such as antidiabetic, antitumor, antioxidant, hepatoprotective, anti-helmintic, anti-rheumatic, anti-microbial, anti-inflammatory, thrombolytic, wound healing, and immunomodulatory activities. Hepatoprotective profile of *A. comosus* was reported in alcohol induced hepatotoxic animal model (Lawal, 2014).

In the absence of systematic studies in literature, the present study was aimed to screen the hepatoprotective profile of ethanolic fruit extract of *A. comosus* in paracetamol induced experimental rats.

2. MATERIALS AND METHODS

2.1. Chemicals and Drugs

Paracetamol (PACT) was obtained from Jerist Biotech, Hyderabad and other chemicals used were of analytical grade and obtained from standard sources.

2.2. Collection & Authentication of Plant Material

The ripe fruits of *A. comosus* (pineapple) were collected from the village of Osman Sagar, Hyderabad in the month of August and authenticated by Dr. L. Rasingam, Scientist In-charge, Botanical Survey of India, Hyderabad, India.

2.3. Preparation of Ethanolic Extract

The fruits were cut into small pieces and dried under the shadow for one month at room temperature. The small pieces were then granulated or powdered by using a blender and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents and stored in dry, clean airtight glass jars. The powdered fruit material was mixed with absolute ethanol (99.9%) and subjected to soxhlet extraction for 18 hours to defat and remove waxy substances. The extract was dried in Petri dishes and concentrated to brownish residue by evaporation at 50°C under reduced pressure in a drying oven. The dried alcoholic extract was stored in a refrigerator until use (Mallik *et al*., 2014).

2.4. Preliminary Phytochemical Screening

The preliminary phytochemical analysis of an ethanolic extract of fruits of *A. comosus* (EEAC) was screened for the various phytochemical principles such as alkaloids, carbohydrates, saponins, glycosides, proteins, and amino acids, flavonoids, fixed oils, tannins, phytosterols and phenols using simple established methods as per standard method (Gusthinnadura *et al*., 2017).

2.5. Experimental Animals

Thirty adult albino wistar rats of either sex (160-250gm) were used in the study. The Institutional Animal Ethics Committee approved the experimental protocol, Central Animal House CPCSEA No.: 1864/PO/Re/S/17/CPCSEA, Shadan Institute of Medical Sciences, Hyderabad, India. The animals were maintained under standard laboratory conditions, 12-hr light/ dark cycle under controlled temperature. All animals were acclimatized to laboratory environment for one week and they were given standard diet pellets and free access to water before the commencement of the experiment.

2.6. Experimental Design

The present research study was designed for 21 days (Basini *et al*., 2013). Experimental animals were (Table 1) divided into 5 groups, each group containing 6 animals.

On 22nd day, blood was collected by retro-orbital plexus under light ether anesthesia into EDTA bottles and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was collected and used for the estimation of serum biomarkers such as SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), ALP (Alkaline Phosphatase), serum total bilirubin and total protein. All animals were sacrificed by cervical decapitation. Livers were weighed, washed in normal saline, stored in 10% formalin and processed for histological study.

2.7. Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's t-test using Prism 5.0 (GraphPad Software, Inc., CA). *P* value <.05 was considered as significant.

3. RESULTS

3.1. Plant Extract

The fruit of *A. comosus* was extracted with ethanol by using soxhlet apparatus. After extraction, the % yield was 24.67% and the color was deep brownish with smooth consistency.

3.2. Preliminary Phytochemical Screening

The preliminary phytochemical analysis of ethanolic extract of fruits of *A. comosus* (EEAC) revealed the presence of various phytoconstituents such as alkaloids, carbohydrates, saponins, glycosides, amino acids, flavonoids, tannins, phytosterols, and phenols.

3.3. Effect of EEAC on Liver Weight

The liver's weight was found to be increased in paracetamol treated animals (5.01 ± 0.05 g) compared to normal

group (4.25 ± 0.13 g) due to the enlargement of the organ. Weight of liver was restored to normal in EEAC treated groups (4.85 ± 0.09 and 4.74 ± 0.02 g) at a dose of 200 mg/kg and 400 mg/kg, respectively and also in silymarin treated animals (4.57 ± 0.03 g) compared to paracetamol treated group. The increase in the liver's weight was prevented by ethanolic fruit extract of A. comosus treatment, which was enhanced at a high dose (Table 2).

3.4. Effect of EEAC on Liver Biochemical Parameters

In the paracetamol treated group, SGPT, SGOT, ALP, total serum bilirubin, and serum total protein levels were significantly increased compared to the normal control group. But in groups treated with silymarin and ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg, these parameters were significantly decreased compared to hepatotoxic group respectively. The results of ethanolic fruit extract of *A. comosus* treatments were compared with the standard drug silymarin (Table 3).

3.5. Histopathological Studies

In the case of a normal control group, central vein, hepatic globular structure, portal tract and kupffer cells were normal, whereas the animals treated with paracetamol showed congested liver sinusoids and fatty degeneration like fatty cysts and proliferation of kupffer cells in hepatic cells. Focal necrosis was seen with the infiltration of inflammatory cells. Animals of standard (silymarin treated) group showed normal hepatocytes with few areas having fatty vacuoles with occasional small focus of necrosis. The animals treated with ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg have shown normal lobular architecture with minimum fatty infiltration and no change in hepatocytes architecture at a dose of 400 mg/kg. The liver was considered to nearly normal in ethanolic fruit extract of *A. comosus* treated group; results are presented in Figure 1.

4. DISCUSSION

The liver is a major target organ for the toxicity of xenobiotics and drugs, because of generation of toxic intermediates in the liver during metabolism. It is established that covalent bonding of N-acetyl-P benzoquinoneimine, an oxidation product of paracetamol, with sulfhydryl groups of protein result in cell necrosis and lipid peroxidation in the liver. Excess levels of these species can attack biological molecules such as DNA, protein and phospholipids which leads to lipid peroxidation, nitration of tyrosine

Table 1: Animal grouping

Group	*Treatment*	*Dose*
I	Normal	1 ml/kg b.w. normal saline p. o.
II	Disease	Paracetamol (2g/kg b.w.) p. o., for 21 days
III	Standard	Silymarin; 100 mg/kg b.w. in normal saline p. o. + Paracetamol for 21 days
IV	Treatment I	EEAC; 200 mg/kg b.w. in normal saline p. o. + Paracetamol for 21 days
V	Treatment II	EEAC; 400 mg/kg b.w. in normal saline p. o. + Paracetamol for 21 days

Table 2: Effect of EEAC on liver weight

S. No.	*Treatment*	*Liver Weight (g)*
1	Normal Control	4.25 ± 0.13
2	Disease Control	5.01 ± 0.05$^{\alpha}$
3	Standard (Silymarin 100 mg/kg)	4.57 ± 0.03***
4	Treatment I (EEAC 200 mg/kg)	4.85 ± 0.09***
5	Treatment II (EEAC 400 mg/kg)	4.74 ± 0.02***

Values were expressed as Mean ± SEM of six rats/treatment. Followed by ANOVA, Dunnett Multiple Comparisons Test. $^{\alpha}P$ <0.0001, Disease vs. Normal Group and $^{***}P$<.0001, Treatment vs. Disease group.

Table 3: Effect of EEAC on liver biochemical parameters

Treatment	*SGPT (IU/L)*	*SGOT (IU/L)*	*ALP (IU/L)*	*Total Bilirubin (mg/dL)*	*Total Proteins (g/dL)*
Normal control	34.33 ± 1.17	26.5 ± 3.28	83 ± 7.58	0.23 ± 0.04	6.88 ± 0.28
Disease control	82.83 ± 2.36$^{\alpha}$	65 ± 3.55$^{\alpha}$	150.33 ± 5.83$^{\alpha}$	1.65 ± 0.27$^{\alpha}$	4.83 ± 0.32$^{\alpha}$
Standard (Silymarin 100 mg/kg)	40 ± 1.82***	34.8 ± 1.97***	86.83 ± 6.51***	0.35 ± 0.04***	6.95 ± 0.32***
Treatment I (EEAC 200 mg/kg)	52.33 ± 1.08***	39 ± 2.62***	97 ± 5.75***	0.53 ± 0.07**	7.52 ± 0.25***
Treatment II (EEAC 400 mg/kg)	45.66 ± 1.49***	34.5 ± 2.11	98.16 ± 3.59**	0.43 ± 0.07***	7.07 ± 0.22***

Vales expressed as Mean ± SEM of six rats/treatment. Followed by ANOVA, Dunnett Multiple Comparisons Test. $^{\alpha}P$<.0001, Disease vs. Normal Group, $^{***}P$<.0001 & $^{**}P$<.001, Treatment vs. Disease Group.

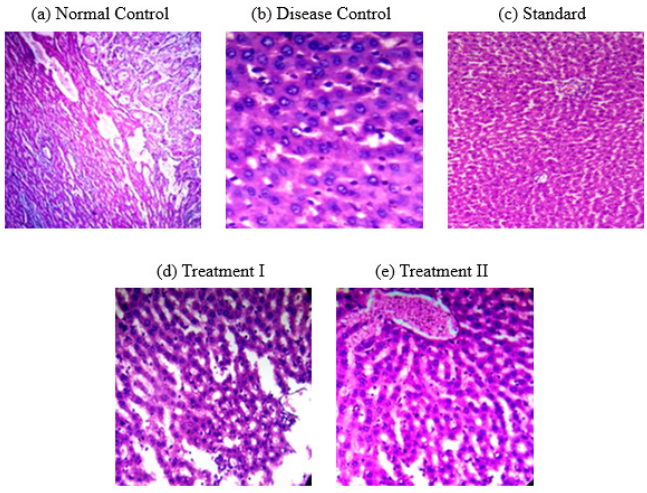

Fig. 1: Histopathological Conditions of Liver. (a) Normal liver showing the normal appearance of hepatocytes, (b) Paracetamol treated animal showing congestion, hepatocytes disorganization and hemorrhages in blood vessels, (c) Standard group animal showing normal architecture of hepatocytes and kupffer cell, (d) EEAC (200 mg/kg) treated animal showing normal hepatocyte with some leucocyte infiltration, (e) EEAC (400 mg/kg) treated animal showing normal appearance of hepatocyte.

and depletion of antioxidant enzymes that further results in oxidative stress (Yoon *et al*., 2016).

A. comosus is a tropical plant in the family Bromeliaceae. Pineapple fruits contain diverse phytochemicals such as polyphenols, including gallic acid, syringic acid, vanillin, ferulic acid, sinapic acid, coumaric acid, chlorogenic acid, epicatechin, and arbutin (Li *et al*., 2014). The fruit was reported to inhibit the activity of the cytochrome 2E1 enzyme in liver (Yantih *et al*., 2017).

The animals treated with paracetamol induce hepatocellular injury as indicated by an increase in serum biomarkers (SGPT, SGOT, ALP, and Total Bilirubin) and a decrease in total proteins in serum as compared to normal control group are shown in Table 2. However, pretreatment with silymarin and ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg dose produced a significant decrease in liver enzyme levels as compared to paracetamol treated group (Table 3). As per the obtained results, the ethanolic fruit extract of *A. comosus* showed a significant reduction in the activities of SGPT and SGOT in addition to ALP and total bilirubin concentration and increased the total proteins when compared to the paracetamol intoxicated animals. This effect was equivalent to that of silymarin at a dose of 100 mg/kg.

Histopathological reports reveal that paracetamol administration caused degeneration of fatty cysts, infiltration of lymphocytes, and proliferation of kupffer cells and congestion of liver sinusoids. This further confirms that paracetamol administration causes hepatotoxicity which is in confirmation with the earlier reports (Yoon *et al*., 2016). Upon pretreatment with ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg dose, the histopathological observations showed normal hepatic globular architecture, less lymphatic infiltration and normal kupffer cells proliferation. This observation suggests that the ethanolic fruit extract of *A. comosus* at 200 mg/kg and 400 mg/kg dose possesses hepatoprotective activity against paracetamol induced hepatotoxicity.

5. CONCLUSION

The outcomes of the current research study indicate that *A. comosus* fruit extract possess significant hepatoprotective as well as antioxidant properties. Phytochemical screening analysis indicated the presence of many biologically active ingredients in the fruits. The detailed observation of the biochemical analysis has revealed that the fruit extract significantly alters the biochemical parameters in serum. In conclusion, the secondary metabolites present in the extract may account for the hepatoprotective property of the fruit extract. Hence, further research is required to isolate and identify the phytochemicals responsible for the observed beneficial as well as pharmacological activities of *A. comosus* fruit.

6. ACKNOWLEDGEMENT

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