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Antioxidant activity and Hepatoprotective potential of Ethanolic leaf extract of *Artabotrys hexapetalus* against various Hepatotoxins induced Hepatotoxicity in Albino wister Rats

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ABSTRACT

The objective of this research was to see whether the ethanolic extract of Artabotrys hexapetalus leaves had antioxidant and hepatoprotective properties against paracetamol (PCT), ethanol (ETN), and isoniazid and rifampicin (IR)-induced hepatotoxicity in Albino Wister rats. The materials were dried in the shade, pulverised, and extracted using ethanol. Phytochemical experiments were carried out as a first step. The ethanol extract's hepatoprotective activity was evaluated in Albino Wister rats. PCT (3 g/kg), ETN (5 g/kg), and IR (100 mg/kg) reduced the levels of SGOT, SGPT, ALP, and bilirubin, which are all biochemical indicators of liver injury. Both hepatotoxin-treated and untreated group of animals determined for their antioxidant levels. Aspartate aminotransferase (SGOT), alanine transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, an antioxidant function of DPPH (1,1-diphenyl 2-picryl hydroxyl), hydrogen peroxide (H2O2), lipid peroxidation methods, hydroxyl radicals, and nitric oxide were among the biochemical and histopathological tests performed. The altered levels of biochemical markers were restored to near-normal levels in a dose-dependent fashion after treatment with A. hexapetalus ethanolic leaf extract (100 mg/kg, 200 mg/kg, and 400 mg/kg body weight). The findings of the current research indicated that the ethanol leaf extract of A. hexapetalus had potent antioxidant and hepatoprotective properties.

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INTRODUCTION

The liver disease continues to be a public health problem. Unfortunately, drugs used to cure liver disease, whether conventional or synthetic, are ineffective and may have dangerous side effects. (Larbie *et al.*, 2012) In the absence of a reliable liver protection medication in western medicine, Ayurveda recommends a variety of herbal formulations for the treatment of liver disorders. (Shahjahan *et al.*, 2004) Because of the serious negative side effects of synthetic drugs, there is an increasing interest in using a rigorous testing approach to evaluate the scientific

basis of conventional herbal medicines that appear to have hepatoprotective properties (Dhiman, 2012; Manokaran *et al.*, 2008). Some herbal extracts and their chemical components have been shown in studies to greatly inhibit these pathological processes and protect hepatocytes from the aetiology of chronic liver damage. Due to the lack of effective liver safety medications in western medicine, a wide range of herbal preparations are prescribed for the treatment of liver diseases, with many claiming to provide substantial relief. (Zhang *et al.*, 2018) Attempts are being made around the world to gain clinical evidence for these herbal medicines that have long been published.

The plant kingdom has given a diversified range of bioactive molecules, which makes them medicinally a precious source. Due to enormous limitations in synthetic pharmaceutical products, very less or no harmful effects and increased awareness on natural products, there is a need of the hour to isolate the lead compounds from them. (David *et al.*, 2015) One such plant that is currently under investigation for its potential hepatoprotective and antioxidant activity in our laboratory is *Artabotrys hexapetalus* (family: Annonaceae). (Mathew *et al.*, 2013) Commonly known as Hari Champa and South Climbing lang-lang in English. (and, 2017; Prabhukumar *et al.*, 2017)

However, many medicinal plants used in remote villages and tribal villages of southern districts of Andhra Pradesh remain to be studied. *A. hexapetalus* is one such plant. This plant leaf is used in folklore medicine to treat liver diseases in the Kurnool and Ananthapuramu districts of Andhra Pradesh. In traditional medicine, its roots are used for treating Jaundice. Few studies have shown that the plant possesses anti-cancer and anti-microbial, anti oxidant, anti-diabetic activities. Furthermore, we also reported on the Phytochemical constituents of *A. hexapetalus*, which indicate the presence of flavonoids, tannins and triterpenes. The polyphenolic flavonoids, in particular, have proved to exhibit various pharmacological activities, including Hepatoprotective activity.

Our through literature survey shows that the hepatoprotective potential of *A. hexapetalus* ethanolic leaf extract has been proved by (Annapurna and Ganapathi, 2016) against paracetamol-induced hepatotoxicity only. No other eveidances were found to prove the hepatoprotective activity of AH against other hepatotoxicins like ethanol, Isoniazid and Rifampicin. Thus, this study was carried out to get insights into the utility of ethanolic extract of *A. hexapetalus* leaf against various hepatotoxic modles viz., paracetamol (PCT), ethanol (ETN) and Isoniazid and Rifampicin (IR) induced liver damage in rats as the animal model to develop a satisfactory hepatoprotective medicine.

MATERIALS AND METHODS

Animals

The crude extracts were tested on Albino Wister rats of both sexes. The research proposal was approved by the Institute's Animal Ethics Committee (232 / a / 19 / CPCSEA). For one week before and after the trials, the animals were held at 27±2 °C, relative humidity 44-56 %, at a light and dark periods of 10 to 14 hours, respectively. The animals were fed a normal diet (Lipton, India) and were given water ad libitum 18 hours before the experiment. All the experiments were carried out in the morning, in accordance with existing laboratory animal treatment and ethical recommendations for the study of experimental pain in conscious animals (National Research Council, 2010)

Source of plants and Preparation of crude drug extract

A. Hexapetalus leaves were collected in Tirupati, Andhra Pradesh, India. Dr K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, conducted the authentication. A specimen sample was preserved in the College's Pharmacognosy Department with the herbarium sample (voucher sample no-017 / C112 / Suresh-01). The leaves were degreased with petroleum ether and dried in the shade. Using a Soxhlet apparatus, the defatted substance was extracted with 95 % ethanol and then dried under a vacuum.

Phytochemical studies

All the extracts were subjected for Phytochemical study. (Yadav and Agarwala, 2011)

Acute toxicity studies

Albino rats were used in an acute toxicity study for the ethanolic leaf extract of *A. hexapetalus* leaves. Before the trial, the animals were fasted overnight and held in normal conditions. The extract was given orally in increasing doses and were found to be healthy up to a dosage of 2000 mg/kg. (Akhila *et al.*, 2007)

Experimental animal and design

The experiment was conducted according to the modified procedures described previously (Dash *et al.*, 2007). PCT (3g/kg), Ethanol 5mg/kg and IR (50+50 mg/kg) was dissolved in 0.5 % CMC for oral

Table 1: Animal groupings for various hepatotoxicity models

PCT Group*	ETN Group*	IR Group*	Treatment
P1	E1	IR1	Normal control and was orally given pure water for seven days, and then intra peritoneally injected with 10 ml/kg body weight isotonic 0.9% NaCl.
P2	E2	IR2	Hepatotoxicity control and was orally given pure water for seven days and then orally intoxicated.
P3	E3	IR3	Standard, and received Standard drug Silymarin 25gm/kg orally.
P4	E4	IR4	Ethanol extract of *A. Hexapetalus* leaf at 100 mg/kg, orally, for seven days.
P5	E5	IR5	Ethanol extract of *A. Hexapetalus* leaf at 200 mg/kg, orally, for seven days.
P6	E6	IR6	Ethanol extract of *A. Hexapetalus* leaf at 400 mg/kg, orally, for seven days.

*Each group contains 6 animals.

Table 2: Acute oral toxicity study of AH

Treatment	Body weight (gm)	Mortality (Animals dead)			Toxicity profile
	Rat (n=6)	After 24 hrs	After 7 days	After 14 days	
Ethanolic extract of AH leaf	160 ± 4.82	0	0	0	Safe

Table 3: *In vitro* antioxidant activity of AH

Conc.(μg/ml)	% Inhibition		Ascorbic acid
	DPPH free radical	Hydrogen Peroxide scavenging	
20	31.4 ± 0.62	44.25 ± 0.47	31.5 ± 0.15
40	42.8 ± 0.37	52.30 ± 0.27	52.9 ± 0.92
60	60.7 ± 0.45	58.35 ± 1.09	61.8 ± 0.38
80	66.79 ± 0.36	64.81 ± 0.47	72.2 ± 0.72
100	72.7 ± 0.79	70.09 ± 0.97	83.3 ± 0.69
120	76.4±0.14	75.25 ± 0.80	91.9 ± 0.45
IC_{50}	48.9	44.82	29.3

administration. Rats were randomly divided into six groups for each model and consisting of six rats. PCT intoxicated animals were grouped from P1-P6. E1-E6 represents a group of animals which were intoxicated by ethanol, and Group IR1-IR6 constitute animals intoxicated by IR. Table 1 shows the details of animal groupings for various hepato toxicity models.

The rats were given ether and then sacrificed after 48 hours of intoxication. SGOT, SGPT, ALP, and Bilirubin enzyme levels were measured using standard kits after blood was extracted via cardiac puncture into heparinized tubing. The liver was immediately removed and washed in ice-cold saline before being examined histologically. The animal grouping was shown in Table 1.

Biochemical determinations

Using test kits, biochemical parameters such as aspartate aminotransferase (AST), glutamate pyruvate transaminase (ALT) (Reitman and Frankel, 1957), serum alkaline phosphatase (ALP) (King, 1965), and gross bilirubin (Malloy and Evelyn, 1937) were determined. (Surat, Span Diagnostic).

In Vitro Anti Oxidant Activity

DPPH-scavenging activity

Hydrogen donation or radical scavenging ability using the stable radical DPPH was determined for the evaluation of the free radical scavenging activity of the extract. A 0.1 mM ethanol solution was prepared, and 1.0 ml of it was applied to 3.0 ml of

Table 4: Effect of the ethanolic extract of AH leaf on biochemical parameters in PCT induced hepatotoxicity

Group	SGOT U/L	SGPT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (μmol/g tissue)
P1	91.87± 1.411	63.83 ± 0.693	71.50 ± 0.638	0.243 ± 0.751	94.35 ± 1.562	132.8 ± 0.472	29.9 ± 0.471	42.9 ± 0.592
P2	241.5 ± 2.349	291.5 ± 1.763	208.5 ± 0.458	0.959 ± 0.392	37.93 ± 1.095	66.2 ± 0.928	61.2 ± 0.091	13.2 ± 0.184
P3	106.5 ± 0.763**	86 ± 0.577**	85.50 ± 0.763**	0.294 ± 0.613**	86.79 ± 1.373	116.3 ± 1.537	32.2 ± 0.927	37.2 ± 0.316
P4	211.5 ± 0.763	243 ± 0.572	173.5 ± 0.763	0.617 ± 0.105	45.9 ± 0.872	74.2 ± 0.945	54.2 ± 0.921	19.36± 0.210
P5	176.5 ± 0.763**	196± 0.600**	139.8 ± 0.577**	0.564 ± 0.019**	61.1 ± 1.147 **	83.5 ± 1.032 **	47.3 ± 0.762 **	21.81 ± 0.462 **
P6	135.7 ± 0.462**	108.5 ±0.691**	95.50 ± 0.825**	0.398 ± 0.179**	75.81 ± 0.986 **	102.9 ± 0.712 **	37.2 ± 0.371 **	36.73 ± 0.064 **

***P < 0.001significant with respect to Control group. Values are expressed as mean ± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test.*

Table 5: Effect of the ethanolic extract of AH leaf on biochemical parameters in ETN induced hepatotoxicity

Group	SGOT U/L	SGPT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (μmol/g tissue)
E1	91.87± 1.411	63.83 ± 0.693	71.50 ± 0.638	0.247 ± 0.751	97.35 ± 1.562	132.8 ± 0.472	29.9 ± 0.471	43.9 ± 0.592
E2	241.5 ± 2.349	292.5 ± 1.763	208.5 ± 0.458	0.959 ± 0.392	39.93 ± 1.095	66.2 ± 0.928	61.2 ± 0.091	13.2 ± 0.184
E3	104.5 ± 0.763**	84± 0.577**	85.50 ± 0.763**	0.296 ± 0.613**	86.79 ± 1.373	116.3 ± 1.537	32.2 ± 0.927	38.2 ± 0.316
E4	209.2 ± 0.253	251.5 ± 0.467	187± 0.577	0.676 ± 0.092	43.7 ± 1.482	72.6 ± 0.852	54.2 ± 0.482	17.23 ± 0.502
E5	191.5 ± 0.727**	205.2 ± 0.579**	156 ± 1.145**	0.552± 0.010**	57.8 ± 0.927 **	82.9 ± 0.692 **	49.7 ± 0.379 **	23.60 ± 0.714 **
E6	148.8 ± 0.632**	126.5 ± 0.763 **	108.8 ± 0.945**	0.461 ± 0.149**	75.82 ± 0.871 **	103 ± 0.921 **	38.2 ± 0.321 **	36.17 ± 0.861 **

***P < 0.001significant with respect to Control group. Values are expressed as mean ± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test.*

the entire solution of extracts in water at various concentrations (10–100 g / ml). The absorbance was estimated after 30 mins at 517 nm. The reaction mixture's lower absorbance means a higher free radical removal activity. The standard drug was ascorbic acid (Marinova and Batchvarov, 2011).

Scavenging Of Hydrogen Peroxide (H_2O_2)

A 20 mM hydrogen peroxide solution in phosphate-buffered saline (pH 7.4) was prepared, and different amounts of extract or standard in methanol (1 ml) were added to 2 ml of peroxide solution buffer saline solution containing hydrogen. The absorbance was estimated at 230 nm after 10 minutes. (Sroka and Cisowski, 2003)

Determination of Biochemical parameters

Various biochemical serum markers such as serum oxaloacetic glutamic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline

Table 6: Effect of the ethanolic extract of AH leaf on biochemical parameters in IR induced hepatotoxicity

Group	SGOT U/L	SGPT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (μmol/g tissue)
IR1	91.87± 1.411	63.83 ± 0.693	71.50 ± 0.638	0.247 ± 0.751	97.35 ± 1.562	132.8 ± 0.472	29.9 ± 0.471	43.9 ± 0.592
IR2	241.5 ± 2.349	292.5 ± 1.763	208.5 ± 0.458	0.959 ± 0.392	39.93 ± 1.095	66.2 ± 0.928	61.2 ± 0.091	13.2 ± 0.184
IR3	104.5 ± 0.763**	84± 0.577**	85.50 ± 0.763**	0.296 ± 0.613**	86.79 ± 1.373	116.3 ± 1.537	32.2 ± 0.927	38.2 ± 0.316
IR4	209.2 ± 0.253	251.5 ± 0.467	187± 0.577	0.676 ± 0.092	43.7 ± 1.482	72.6 ± 0.852	54.2 ± 0.482	17.23 ± 0.502
IR5	191.5 ± 0.727**	205.2 ± 0.579**	156 ± 1.145**	0.552± 0.010**	57.8 ± 0.927 **	82.9 ± 0.692 **	49.7 ± 0.379 **	23.60 ± 0.714 **
IR6	148.8 ± 0.632**	126.5 ± 0.763 **	108.8 ± 0.945**	0.461 ± 0.149**	75.82 ± 0.871 **	103 ± 0.921 **	38.2 ± 0.321 **	36.17 ± 0.861 **

***P < 0.001significant with respect to Control group. Values are expressed as mean ± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test.*

phosphate (ALP), bilirubin, superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (reduced) (GSH) were assessed using commercially available kits for each sample, and all analyses were carried out in triplicate. (Haldar *et al.*, 2011; Dash *et al.*, 2007; Acharya *et al.*, 2012)

Histopathological studies

Dissected liver tissue was frozen in 10% formalin, dehydrated in 50% ethanol, eliminated in xylene, and embedded in paraffin. Photomicroscopic observations of cell necrosis, fat displacement, hyaline regeneration, and balloon degeneration were made using sections stained with hematoxylin and eosin dye (H-E).

Statistical analysis

The mean and standard deviation of the mean are used to express the data (SEM). Data were evaluated using one-way analysis of variance (ANOVA), and discrepancies between groups were calculated using Graph pad PRISM V5.02 software's Dunnett's post hoc test. The $p<0.05$ significance level was chosen.

RESULTS

Phytochemical study

All extracts subjected for the phytochemical study showed the presence of alkaloids, proteins, amino acids, phenolic compounds, glycosides and flavonoids.

Acute toxicity studies

Up to doses of 2000 mg/kg, the ethanolic and aqueous extracts displayed no signs or symptoms of toxicity or mortality. The findings of the acute toxicity trials as seen in Table 2.

In vitro antioxidant study

Before proceeding for *in vivo* activity, the efficacy of the plants were tested i*n vitro.* The *in vitro* antioxidant activity was performed by using DPPH free radical and Hydrogen Peroxide scavenging. Results were tabulated in Table 3.

Effect of the ethanolic extract of AH leaf on biochemical parameters against PCT induced hepatotoxicity

The liver markers SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH, are all very responsive, and their elevated levels indicate liver damage. The effects of the ethanolic extract of the HA leaf on different biochemical parameters are shown in Table 4. In standard control rats, there were no significant improvements in the levels of these parameters. PCT was injected into rats with mediated liver damage, resulting in significantly higher SGOT, SGPT, ALP, bilirubin, SOD, CAT, MDA, and GSH behaviours than the usual control group. However, as compared to the PCT-treated population, the AH treatment (400 mg/kg) showed a substantial reduction in the levels of elevated serum enzymes. The effect of HA on a dose-dependent basis is equal to that of silymarin therapy. These findings suggested that an ethanolic extract of HA leaves could protect rats from PCT-induced liver injury.

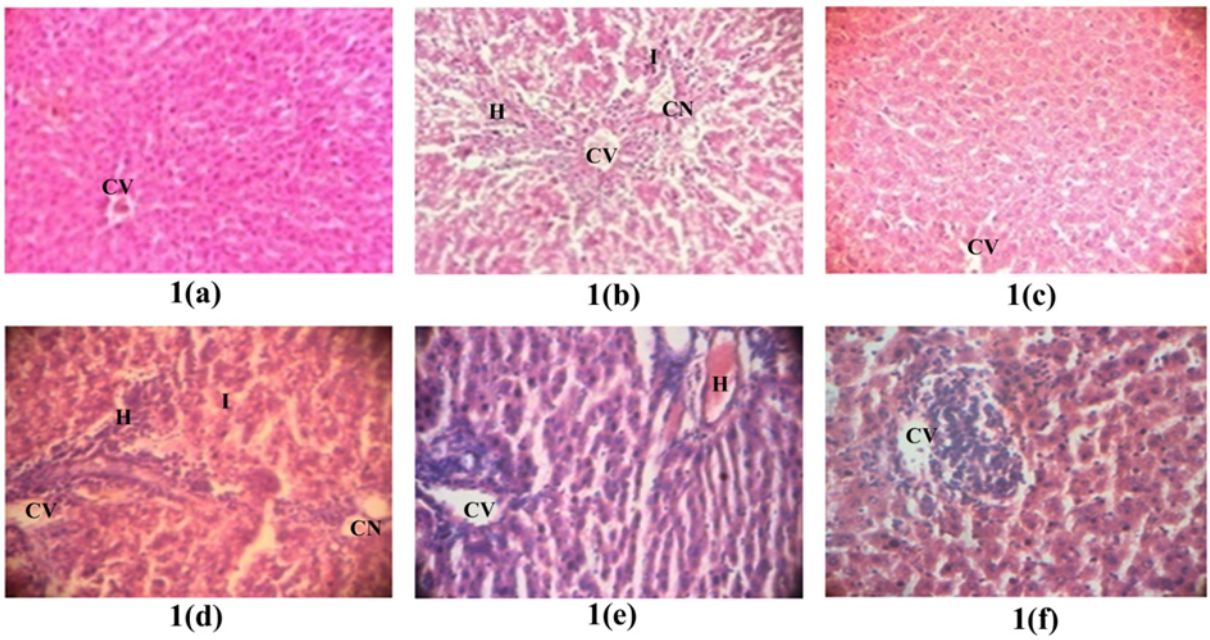

Figure 1: (a-f): Sections of liver tissue of groups P1-P6 (100x Magnification) CV: centrilobular.CN: coagulative necrosis. I: inflammation. H: haemorrhage.

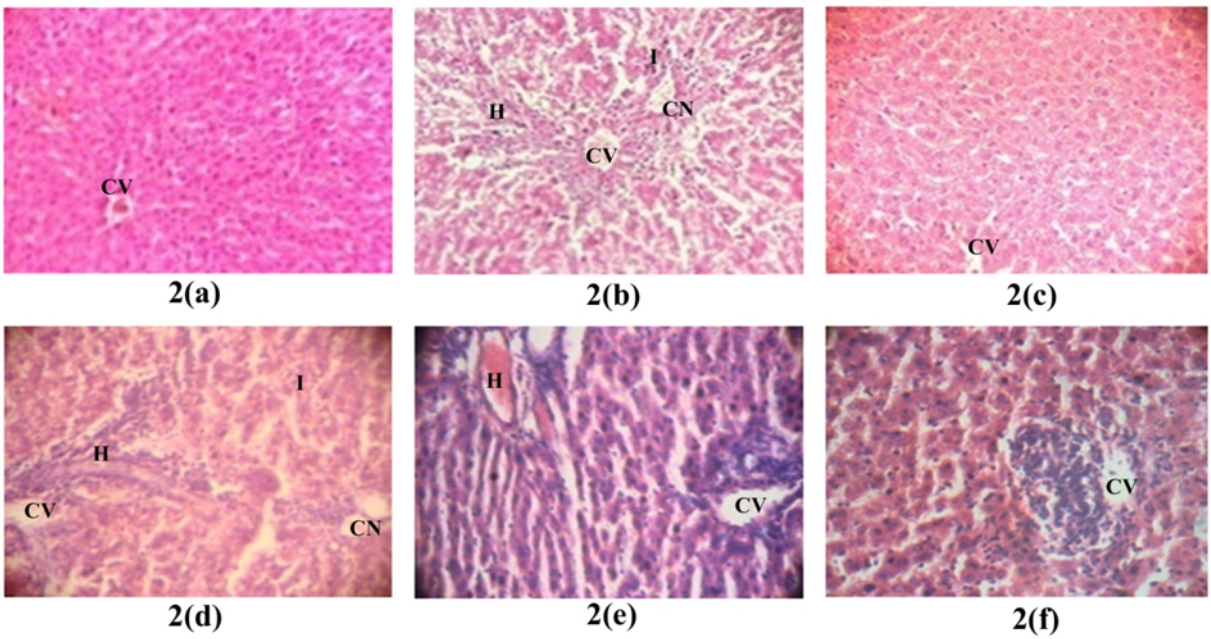

Figure 2: (a-f): Sections of liver tissue of groups E1-E6 (100x Magnification) CV: centrilobular.CN: coagulative necrosis. I: inflammation. H: haemorrhage

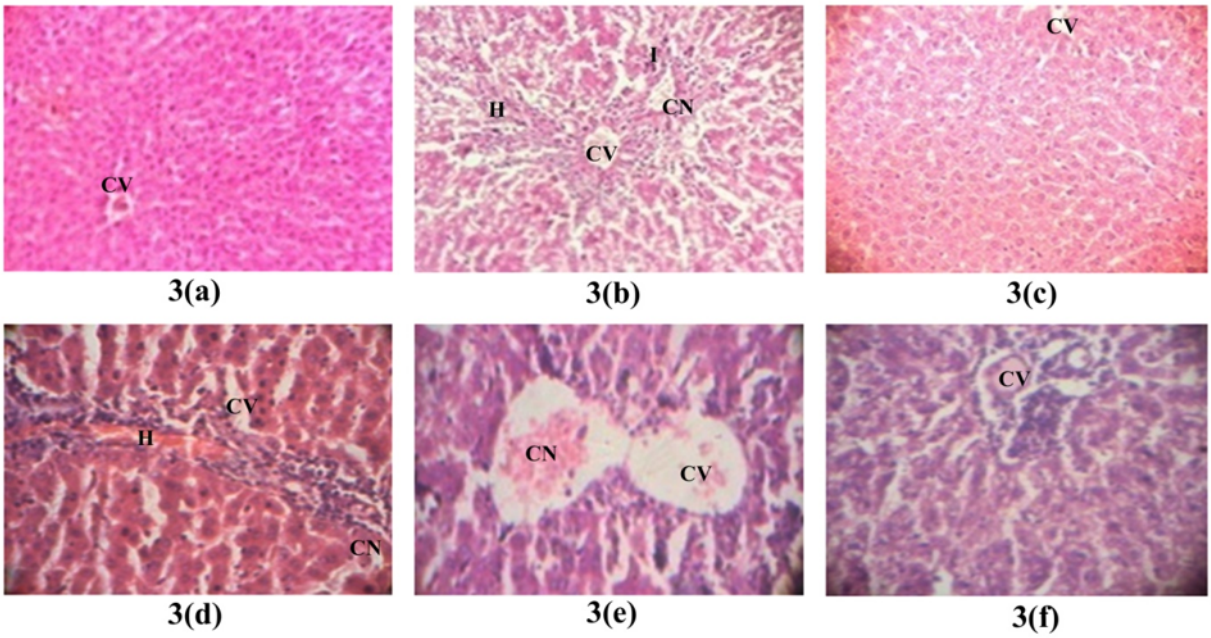

Figure 3: (a-f): Sections of liver tissue of groups IR1-IR6 (100x Magnification) CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage

The non- PCM- intoxicated liver pretreated with 10% DMSO (normal) has normal lobular morphology and normal liver cells with well-preserved cytoplasm, a well-defined sinusoidal line, and a nucleus across the perivenular region (Figure 1 (a)). Figure 1 (b) reveals lymphocyte penetration, haemorrhage, and severe coagulative necrosis of the perivenular and midline regions with periportal preservation in a PCM-poisoned liver segment pretreated with 10% DMSO. The perivenular zone is primarily affected by coagulant necrosis of hepatocytes in PCM-induced liver toxicity (zone 3). With increasing AH dosage, these pathological improvements were found to be minimal, meaning that the extract would reverse PCM-induced intoxication (Figure 1 (d) - (f)). Pretreatment with the extract or silymarin greatly decreased the presence of marked necrosis, inflammation, and bleeding during PCM treatment (as seen in the negative control group).

Effect of the ethanolic extract of AH leaf on biochemical parameters against ETN induced hepatotoxicity

Increased amounts of liver biomarkers such as SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH revealed that the hepatotoxic agent ethanol-induced substantial liver harm. When compared to ETN-treated rats, rats given doses of 100, 200, and 400 mg/kg had slightly lower levels of biochemical markers. The maximum dose (400 mg/kg) had greater hepatoprotective efficacy than the lowest doses. The effect of the ethanolic extract of the HA leaf on biochemical parameters against ETN-induced hepatotoxicity is detailed in Table 5.

Histopathological examinations confirmed the hepatoprotective effect of the ethanolic extract of HA leaves on ETN-induced liver injury. Figure 2 (a) shows natural lobular morphology and regular liver cells with well-preserved cytoplasm, a well-defined sinusoidal axis, and a nucleus around the perivenular region in the non-intoxicated liver with ETN pretreated with 10% DMSO (standard). Figure 2 (b) shows normal histological structures in the livers of rats infected with silymarin (25 g / kg). In rats given ETN, abnormal liver cells, necrosis, and inflammation were observed (Figure 2 (c)). Rats given HA extract (100, 200, and 400 mg/kg) demonstrated a reduction in body weight. Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with HA extract (100, 200, and 400 mg/kg). Figure 2 (d, e, and f) Lower doses of ethanolic extract of HA leaves (100 mg/kg) provided less safety than higher doses of 400 mg/kg.

Effect of the ethanolic extract of AH leaf on biochemical parameters against IR induced hepatotoxicity

The ethanolic extract of HA demonstrated significant hepatoprotective activity ($p<0.05$) against the toxicity caused by isoniazid and Rifampicin (IR) (50 mg/kg + 50 mg/kg) by enhancing liver function, as shown by lower liver enzyme levels relative to the control group. The full effects of hepatopro-

tective activity against the IR-induced hepatotoxicity model are seen in Table 6. The liver architecture of IR-induced rats pretreated with 10% DMSO was significantly damaged ($p<0.05$), with extreme hepatocyte necrosis, according to histopathological tests of liver removed from the rats. Regular lobular morphology and normal liver cells with non-IR intoxicated liver pretreated with 10% DMSO (normal). Figure 3 (a) shows natural lobular morphology and normal liver cells with well-preserved cytoplasm and well-defined sinusoidal line and nucleus across the perivenular region in non-IR intoxicated liver pretreated with 10% DMSO (normal). Figure 3 (b) shows normal histological structures in the livers of rats infected with silymarin (25 g / kg). In the IR-treated rats, changes in liver cells, necrosis, and inflammation were observed (Figure 3 (c)). Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with HA extract (100, 200, and 400 mg/kg). (See Figure 2 (d, e, and f)). Lower doses, on the other hand, Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with HA extract (100, 200, and 400 mg/kg). (See Figure 2 (d, e, and f)). Lower doses of ethanolic extract of HA leaves (100 mg/kg) provided less safety than higher doses of 400 mg/kg.

DISCUSSION

Because of its metabolic and detoxifying capacities, the liver is an essential part of life. When people are exposed to a variety of endogenous and xenobiotic compounds, they develop a vast amount of intermediate and final products, which can induce hepatocellular death and are the leading causes of liver disease (Meharie *et al.*, 2020; Armeni and Principato, 2020). Traditional medicine relies on symptom control and liver transplantation in acute cases of liver failure in order to sustain liver function. (Hoofnagle *et al.*, 1995) However, no medications are actually being used to improve the organ's detoxification ability. As a result, the use of botanical hepatoprotective agents is becoming increasingly common. Therefore, it would be absolutely imperative to demonstrate the efficacy of plant extracts in the presence of chemical-induced hepatotoxicity. (Meharie *et al.*, 2020)

Paracetamol (PCT) and ethanol (ETN) were generally consumed by a human for the reasons like pyrexia and those who have a habit of taking alcohol, respectively. Isoniazid and Rifampicin (IR) are the most widely used drugs to treat tuberculosis. All these agents were known to induce hepatotoxicity. So, the same hepato toxins were chosen to induce hepatotoxicity in rats and evaluate the hepatoprotective activity of *Artabotrys hexapetalus*. The rats were given an ethanolic extract of the leaves of A. hexapetalus. In humans and laboratory animals, PCT, ETN, and IR have been shown to cause hemorrhagic liver necrosis in many trials. In this study, rats treated with PCT, ETN, and IR developed infiltration, vacuolation, and inflammation in the liver, resulting in increased rat liver weight (Figure 1 b, Figure 2 b and Figure 3 b). The hepatoprotective ability of plant extracts in different animal models was evaluated using PCT, ETN, and IR mediated hepatotoxicity. Bioactivation of these hepatotoxins by cytochrome P450 results in strongly unstable reactive free radicals. These can kill cells by peroxiding membrane lipids and binding covalently with other macromolecules in hepatocytes. When the membrane is damaged, cytosolic and endoplasmic enzymes are released, indicating that the liver's structure and function have been compromised. Elevated amounts of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH are signs of this. As a result, measuring the amounts of these biomarkers of liver injury will show the plant extract's and solvent fractions' hepatoprotective function. The ethanolic extract reduced the levels of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH in a dose-dependent manner in the current sample. At the lowest dosage, 100 mg/kg ethanolic extract of HA leaves had little effect on all biomarkers of liver damage, but medium and high doses resulted in substantial reductions in AST, ALT, and ALP levels (Tables 4, 5 and 6). This may indicate that the lower dose is smaller than the minimal effective dose and cannot induce a substantial decrease in liver enzyme levels, whereas the other two doses are high enough to do so. Percent reduction in hepatic injury biomarkers revealed that 200 mg/kg and 400 mg/kg of ethanolic extract had an effect that was almost identical to the normal (Tables 4, 5 and 6). With the exception of the 100 mg/kg dosage, pre-and post-treatment with ethanolic extract in all doses (200 mg/kg and 400 mg/kg) significantly reduced the severity of the liver injury. The ethanolic extract can stabilise liver cell membranes and avoid enzyme degradation, as shown by the return of enzyme levels to near-normal levels in ethanolic rats before and after surgery.

Other possible explanations for the therapeutic activity of *A. hexapetalus* leaf extract include preventing the formation of free radicals and neutralising them, as well as the plant's ability to defend against hepatotoxins. The crude ethanolic extract was fractionated to concentrate or isolate the active

ingredients. The majority of the polar components of the plant leaf may be attributed to the available flavonoids material, according to this report. Since the active theory or ingredients responsible for the hepatoprotective behaviour of the ethanolic extract and solvent fractions of *A. hexapetalus* are unclear, it is impossible to pinpoint the compounds are responsible for the antioxidant and hepatoprotective effects. Alkaloids and flavonoids have been found to have antioxidant properties in previous research. The crude ethanolic extract and the solvent fractions were subjected to preliminary phytochemical analysis, which showed a number of secondary metabolites that seemed to be dispersed differently in the extract. It is fair to believe that the phytochemicals found in the plant work individually or in concert to create *A. hexapetalus* hepatoprotective function. It's likely that the flavonoids and alkaloids in the raw leaf extract have a hepatoprotective impact by scavenging free radicals and preventing lipid peroxidation and cell injury, as has been proposed with some other plants. Alkaloids and flavonoids are sometimes classified as natural antioxidants because of their ability to scavenge free radicals.

In conclusion, this analysis added to the growing body of evidence that the ethanolic extract has hepatoprotective properties comparable to the regular treatment. Both biomarkers of liver damage were reduced in a dose-dependent manner before and after surgery, according to the findings. As a result, these findings suggest that the plant's hepatoprotective effect is spread to the polar bioactive concepts contained in the ethanolic fraction. While the plant extract's hepatoprotective function is yet to be discovered, one of the expected mechanisms is its antioxidant activity. Overall, according to the findings of the acute oral toxicity report, the ethanolic extract of the leaf of *A. hexapetalus* is considered safe. In addition, future experiments will use HPLC / LC-MS / MS strategies to isolate and characterise new antioxidants.

CONCLUSIONS

The current study's experimental evidence showed that the leaf of *A. hexapetalus* has hepatoprotective function against PCT, ETN, and IR-induced liver toxicity. The presence of flavonoids and other components in the plant may be responsible for this behaviour. To confirm the mechanism underlying this hepatoprotective effect, additional in vitro and in vivo studies will be needed.

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ORIGINAL ARTICLE

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A Comparison of Polyherbal Tablets to Treat Type II Diabetes

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ABSTRACT

The most pervasive problems today include the burden of disease and its complexities. While diabetes is not an infectious disease, it is caused by a healthy diet and bad eating habits. The causes of diabetes are unhealthy dietary patterns, attributable to insufficient pancreas insulin secretion and insulin receptor insensitivity, caused by process wisdom. It results in improper glucose metabolism and reuptake into the muscles. Various synthetic drugs are used successfully to regulate diabetes. The drugs have those side effects, which make their use limited, because of the fear of creating such complications. Different diseases have been treated with herbs and medicinal plants in this respect, and are often considered to be effective and safer. Many herbs are now used to treat diabetes, and it has also been researched and shown the exact mode of action of all those herbs. Chemical leads have been isolated and shown to be effective against diabetes from plants. The tablet formulation can control the amount of blood sugar in the current study for diabetes caused by STZ. This was prepared using extracts from *Withania somnifera, Psidium guava, Trigonella foenumgracum,* and *Piper nigrum* and anti-diabetic property testing revealed that compared to standard and individual extracts, the tablets showed greater activity.

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INTRODUCTION

Diabetes Mellitus is a metabolic condition affecting digestion, usually caused by poor eating habits. Countless hours should be spent regarding elevated blood sugar levels. About one in every ten people worldwide is affected by this disease. DM is subdivided into D and M. Two types of diabetes first one diabetes and type two diabetes [1]. Type 1 diabetes is caused by the destruction of beta cells, and type 2 diabetes is caused by the problems in the production of sugar in the body. The mechanism of action reveals how reduced insulin production results in an inability to control blood glucose and how insensitivity to insulin results in improper processing by the muscles. DM is more common in older and obese individuals. CVD, stroke, hyperglycemia, chronic kidney disease, nerve injury, neurological dysfunction, and foot ulcers can occur as complications. The DM can impact a person's lifestyle choices [2].

Synthetic medications are currently being used to treat diabetes, but also have side effects and complications. In treating diabetes, many of the medicinal plants are found for their therapeutic effects on the body. The herbal medication contains beneficial chemicals that are effective against diabetes [3]. The experiment was carried out using various tech-

Table 1: Ingredient and composition of anti-diabetic formulations

Sl no.	Ingredients of formulation	Amount
1	*Withania* Extract	100mg
2	*Psidium* Extract	75mg
3	*Trigonella* Extract	75mg
4	*Piper nigram* powder	60mg
5	Starch	60mg
6	Talc	Qs
6	Acacia	40mg

Table 2: Effect of tablet composition on the anti-diabetic activity

Groups	Sugar level in blood(units-mg/dL)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal control	106.12±6.871	112.42±7.83	110.23±4.613	104.54±1.12	102.89±3.517
DM control	327±7.125	329.61±6.156	327.45±8.290	318.81±7.916	319.94±8.367
Withania Extract	333.01±8.326	288.41±9.459	264.1±7.691	15.12±6.7	141.4±7.980
Psidium Extract	331.2±9.427	273.16±6.72	241±7.814	179±5.871	122.73±6.254
Anti-diabetic formulation	327.45±7.172	264.92±3.10	223±2.225	165±8.278	106.12±7.018
Std. drug	324.73±6.289	291.2±7.914	276.90±6.278	204.64±6.21	137.8±7.23

niques such as the use of alloxan and streptozotocin, which are the most commonly, used DM inducement process. Withania *somnifera, Psidium guava, Trigonella foenumgracum,* and *Piper nigrum* are used to make the anti-diabetic medication. The leaves of each plant are removed to produce the medication [4].

Preparation of Tablets

The dried plant parts were powdered for use in extraction. The plant powder was treated with purification and run of alcohol at a 2:1 ratio. The plant material was immersed in the combination solution long enough to ensure proper blending of the material with the solvent. After filtering, the macerate was filtered [5]. The concentrate was dried, ground, and then used to produce a tablet of the filtrate, and the streptozotocin process was investigated [Table 1].

Lab animals

To assess the efficacy of the anti-diabetic intervention used in the trial, Albino Wistar rats were used. The rats were distributed at a scale from 180 to 190 grams, and they were housed in an air-controlled and humidified environment in a laboratory setting [6]. Rats were kept in plastic cages where they had access to water and a free diet of rodent meat.

Animal segregation

Based on body weight and sex, optimal dosages were administered. Six animals were assigned to six animal groups with four members per group and the animals were randomly paired. The two sexes were divided in the primate lineage [7, 8]. Group-I (Normal/control): 1gm in 10ml suspension of sod.CMC in double distilled water that is given to the rats given at 5ml/kg; Group-II-STZ'cin-induced diabetes in rats which received only 1gm in 10ml suspension of sod.CMC in distilled water that is given at 5ml/kg; Group-III-STZ'cin-induced diabetes in rats received Glycerrhia extract-250mg/kg/day p.o suspended in 1 percent w/v of CMC; Group-IV-STZ'cin-induced diabetes in rats received Tribulus-250mg/kg/day p.o suspended in 1 percent w/v of CMC; Group-V-STZ'cin-induced diabetes in rats received tablet powder-250mg/kg/day p.o suspended in 1 percent w/v of CMC; Group-VI-Standard-group-STZ'cin-induced diabetes in rats received rosiglitazone-2mg/kg p.o) suspended in 1 percent w/v of CMC.

Diabetes mellitus induced procedure

In a sample of rats, streptozotocin-induced diabetes when it was dosed at 45 mg per kg per rodent. The compound dissolved in citrate buffer at pH 4.5 with the use of IP to cause diabetes. The sum offered of the medication was very limited. The glucose solution was supplied to the rats in an attempt to avoid the development of hypoglycemia as an adaptive reaction. Ten rats with a blood glucose level of

245 mg/dL were selected and started with analyses. All the animal experiments were approval of the Institutional Animal Ethical Care committee (IAEC), Mother Theresa Post Graduate and Research and Research Institute of Health Sciences, Puducherry (Registration number: 1923/GO/Re/S/16/CPSEA).

The scientific testing was undertaken for 30 days and the checked formulation was given once in the morning [9, 10]. To monitor for the amount of blood glucose, the animals were tested with a glucometer, and the blood tests were performed at the beginning of each day, every 7th day, and every 14th day. It noticed the reading and registered it.

RESULTS AND DISCUSSION

There was an increase in the elevation of blood glucose levels in these rats which resulted in an improvement in blood sugar levels and diabetes was caused by the drug. Group 1 sugar levels were normal since they were not replaced with DM.

The quantities and levels of blood glucose are listed in Table 2. The extracts were tested for their anti-diabetic efficacy, and the findings were remarkable: they were able to substantially decrease blood sugar levels. Yet, there was less exhaustion with the tablets and, relative to the ordinary medication, more rest.

In that category, the daily therapy reduced fasting blood sugar by a significant amount. Blood sugar levels were considerably lowered by the tablet formulations [Figure 1].

That is, they were dramatically increased over the rug and individual extracts. The tablet formulation is reliable, and the tablet has not yet been standardized for its chemical constituents and pharmacological activity.

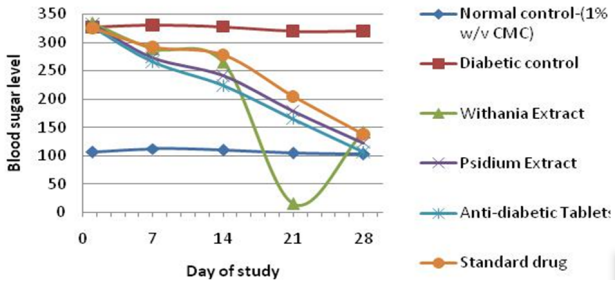

Figure 1: Effect of Formulation in the blood sugar level of rats

CONCLUSION

This research focuses on the structure of STZ tablets to assess how much they reduce blood sugar. This research studied the anti-diabetic activity of various extracts of *Withania somnifera, Psidium guava, Trigonella foenumgracum,* and *Piper nigrum*. The tablets demonstrated stronger action than the individual extracts.

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Evaluation of Hepatoprotective and Antioxidant Activity of Ethanolic Extract of *Artabotrys zeylanicus* Stem against Various Hepatotoxins Induced Hepatotoxicity in Albino Wister Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author KS has designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HAA and SVS managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of the present study was to investigate the antioxidant and hepatoprotective activity of ethanolic stem extract of *Artabotrys zeylanicus* against paracetamol (PCT), Ethanol (ETN) and Isoniazid and Rifampicin (IR) induced hepatotoxicity in Albino wister rats.
Methodology: The material was dried in shade, they were powdered and extracted with ethanol. Preliminary Phytochemical tests were done. The hepatoprotective activity of the ethanol extract was assessed in Albino wister rats. PCT (3 g/kg), ETN (5 gm/kg) and IR (100 mg/kg) has enhanced

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the levels of various biochemical markers of hepatic damage like Serum Glutamic Oxaloacetic Trasaminase (SGOT), Serum Glutamic pyruvic transaminase (SGPT), Alkaline phosphatise (ALP), bilirubin. Antioxidant levels were tested in all the Hepatotoxins treated and untreated groups.

Results: The various biochemical and Histopathological investigations done were Serum Glutamic Oxaloacetic Trasaminase (SGOT), Serum Glutamic pyruvic transaminase (SGPT), Alkaline phosphatise (ALP), Bilirubin, antioxidant activity by 1,1-diphenyl 2-picryl hydrazyl (DPPH), Nitro Blue Tetrazolium (NBT), Hyderogen peroxide (H_2O_2), lipid perioxidation, hyderoxil radical and nitric oxide. Treatment of ethanolic extract of stem of *A. zeylanicus* (100mg/kg, 200mg/kg and 400mg/kg body weight) has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner. Ethanolic extract of *A. zeylanicus* were observed to inhibit oxidant stress with the maximum value of 71% and 62% at the concentration of 100 µg/mL. The crude ethanolic extract of *A. zeylanicus* had a calculated IC_{50} value of 62.2 and 63.25 µg/mL, which is nearly similar to the calculated IC_{50} value of the known antioxidant, ascorbic acid, ie 65.3 µg/mL. While the rats treated with AZ extract (100, 200 and 400 mg/kg) which were shown as reduction/absence of inflammatory cells, vascular congestion, cellular degeneration, necrosis and vacuoles. In contrast, the lower doses (100 mg/kg) of ethanolic extract of AZ stem shown low protection than at higher dose 400 mg/kg.

Conclusion: Our findings suggested that *A. zeylanicus* ethanol stem extract possessed a potent antioxidant and hepatoprotective activity.

Keywords: Hepatoprotective; artabotrys zeylanicus; paracetamol; ethanol; isoniazid and rifampicin; hepatotoxins; histopathological.

1. INTRODUCTION

The liver is the main homeostatic organ in the body. It maintains homeostasis by regulating various physiological functions such as metabolism, secretion and storage [1]. It serves as a first line of defense and prevents toxic effects by detoxifying toxic substances. Various toxic chemicals, alcohols, drugs, infections and autoimmune diseases, through the process of lipid peroxidation and other mechanisms damage liver cells, leading to hepatotoxicity [2].

The liver plays a vital role in the metabolism of various substances, such as carbohydrates: glycogenesis (storage of glucose as glycogen), glycogenolysis (breakdown of glycogen into glucose), gluconeogenesis (production of glucose from a non-carbohydrate source) and protein - deamination amino acids (main detoxification mechanism) [3]. Together with the spleen, it helps reuse the proteins that make up old red blood cells. Liver damage is a key disease process in most chronic liver disease, and long-term liver damage causes liver fibrosis, liver cirrhosis, and even hepatocellular carcinoma [4,5]. Research has indicated that certain herbal extracts and their chemical components can significantly inhibit these pathological processes mentioned above and protect hepatocytes from the etiology of chronic liver damage. In the absence of effective liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver ailments and are most often believed to offer significant relief [6]. Attempts are being made globally to obtain scientific evidence for these traditionally reported herbal drugs.

The plant kingdom has provided a diverse array of bioactive molecules which make them a valuable source, from a medical point of view. Due to the enormous limitations of synthetic drugs, little or no harmful effects and increased awareness of natural products, it takes an hour to isolate lead compounds from them [7]. One of those plants currently under investigation for its potential hepatoprotective and antioxidant activity in our laboratory is Artabotrys zeylanicus (family: Annonaceae) [8]. Commonly known as Ceylon Green Champa and South Indian tail grape in English [9,10].

Recent studies have shown varying levels of hepatoprotective prosperity in traditional plants found in southern India such as Phyllanthus maderaspatensis, [11] Phyllanthus rheedii [12], Thespesia populena [13], Momordica subangulata [14], Naregamia alata [15], Lygodium flexuosum[16], Cheilanthes farinose [17], Physalis peruviana [18] and Trichopus zeylanicus [19]. However, many medicinal plants used in remote villages and tribal villages in the southern districts of Andhra Pradesh remain to be studied. A. zeylanicus is one of those plants.

This stem of the plant is used in folk medicine to treat liver disease in the Kurnool and Ananthapuramu districts of Andhra Pradesh. In traditional medicine, its roots are used to treat jaundice. Few studies have shown that the plant has antitumor and antimicrobial, antioxidant and antidiabetic activities. In addition, the phytochemical components of A. zeylanicus are also reported, indicating the presence of flavonoids, tannins and triterpenes. Polyphenolic flavonoids, in particular, have been shown to exhibit various pharmacological activities, including hepatoprotective activity.

Our study through the literature revealed that to date no attempt has been made to study the hepatoprotective activity of A. zeylanicus roots. Therefore, this study was conducted to obtain information on the utility of the ethanolic extract of the A. zeylanicus stem against various hepatotoxic models, namely, paracetamol (PCT), ethanol (ETN) and isoniazid and rifampicin-induced liver injury (IR) in rats. as an animal model for developing a successful hepatoprotective drug.

2. MATERIALS AND METHODS

2.1 Animals

Albino Wister rats of both sexes were used to study the crude extracts. The Institute's Animal Ethics Committee approved the project (831/a/19/CPCSEA). Animals were kept at 27 ± 2°C, relative humidity 44-56%, and light and dark cycles of 10 and 14 hours, respectively, for 1 week before and during the experiments. The animals were given a standard diet (Lipton, India) and food was removed 18 hours before the start of the experiment and water ad libitum. All experiments were performed in the morning according to current laboratory animal care guidelines and ethical guidelines [20] for the study of experimental pain in conscious animals.

2.2 Plant Resources and Preparation of Crude Drug Extract

The stems of A. Zeylanicus was collected from Tirupati, Andhra Pradesh, India. And the authentication was performed by Dr. K. Madhava Chetty, assistant professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. The herbarium sample was submitted to the College's Department of Pharmacognosy (Voucher specimen no-017 / C112 / suresh-01). The stems were dried in the shade and degreased with petroleum ether. The defatted material was extracted with 95% ethanol using a soxhlet apparatus and then dried under vacuum.

2.3 Phytochemical Studies

All the extracts were subjected for Phytochemical study [21].

2.4 Acute Toxicity Studies

The acute toxicity study for ethanolic extract of stem of *A. Zeylanicus* stems were performed using albino rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administrated orally in increasing dose and found safe up to dose of 2000 mg/kg for all extracts [22]

2.5 Experimental Animal and Design

The experiment was conducted according to the modified procedures described above [5]. PCT (3 g / kg), ethanol 5 mg / kg and IR (50 + 50 mg / kg) were dissolved in 0.5% CMC for oral administration. The rats were randomly divided into six groups for each model and consisted of six rats. PCT poisoned animals were pooled from P1-P6. E1-E6 represents a group of animals intoxicated by ethanol and the group IR1-IR6 constitutes animals intoxicated by IR. Table 1 shows details of animal groupings for various hepatotoxicity models.

After 48 hours of intoxication, the rats were sacrificed with ether and then sacrificed. Blood was dissected by cardiac puncture into heparinized tubes for evaluation of different levels of AST, ALP and bilirubin enzymes using standard kits available. The liver was immediately removed and washed with ice-cold saline for histological observation. Table 1 shows the groups of animals.

2.6 Biochemical Determinations

The biochemical parameters like serum enzymes: aspartate aminotransferase (AST), Alanine aminotransferase (ALT) [23] serum alkaline phosphatase (ALP) [24] and total bilirubin [25] were assayed using assay kits (Span Diagnostic, Surat).

Table 1. Animal groupings for various hepato toxicity models

PCT Group*	ETN Group*	IR Group*	Served with
P1	E1	IR1	Normal control and was orally given pure water for seven days, and then intraperitoneally injected with 10 ml/kg body weight isotonic 0.9% NaCl
P2	E2	IR2	Hepatotoxicity control and was orally given pure water for seven days and then orally intoxicated.
P3	E3	IR3	Standard, and received Standard drug Silymarin 25gm/kg, orally.
P4	E4	IR4	Ethanol extract of *A. Zeylanicus* stem at 100 mg/kg, orally, for seven days.
P5	E5	IR5	Ethanol extract of *A. Zeylanicus* stem at 200 mg/kg, orally, for seven days.
P6	E6	IR6	Ethanol extract of *A. Zeylanicus* stem at 300 mg/kg, orally, for seven days.

**Each group contains 6 animals*

2.7 In Vitro Anti-Oxidant Activity

2.7.1 DPPH-scavenging activity

The free radical scavenging activity of the extract was measured in terms of radical scavenging or hydrogen donation capacity using the stable radical DPPH [26]. A 0.1 mM solution in ethanol and 1.0 ml of this solution was prepared to 3.0 ml of all the extract solution in water at different concentrations (10–100 µg / mL). Thirty minutes later, the absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates a greater elimination activity of free radicals. Ascorbic acid was used as a standard drug [26].

2.7.2 Scavenging of hydrogen peroxide (H_2O_2)

A solution of hydrogen peroxide (20 mm) in phosphate buffered saline (pH-7.4) was prepared, various concentrations of extract or standard in methanol (1 ml) were added to 2 ml of peroxide solution. hydrogen in PBS. After 10 min the absorbance was measured at 230 nm [27-30].

2.8 Histopathological Studies

Liver tissue was dissected and fixed in 10% formalin, dehydrated in stepwise ethanol (50-100%), eliminated in xylene and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation, including cell necrosis, fat displacement, hyaline regeneration, balloon degeneration [31].

2.9 Statistical Analysis

The data are expressed as mean ±standard error of mean (SEM). The data were analysed using the one – way analysis of variance (ANOVA), and the differences between the groups were determined using the Dunnett post hoc test as provided by the graph pad PRISM V5.02 software. The limit of significance was set at $p<0.05$ [32].

3. RESULTS

3.1 Phytochemical Study

All extracts subjected for phytochemical study showed the presence of alkaloids, proteins, amino acids, phenolic compounds, glycosides and flavonoids.

3.2 Acute Toxicity Studies

Ethanolic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose. Table 2 shows the results of acute toxicity studies.

3.3 *In vitro* Antioxidant Study

Before proceeding for in vivo activity the efficacy of the plants was tested in vitro. The *in vitro* antioxidant activity was performed by using DPPH free radical and Hydrogen Peroxide scavenging. Results were tabulated in Table 3.

Table 2. Acute oral toxicity study of AZ

Treatment	Body weight (gm)	Mortality (Animals dead)			Toxicity profile
	Rat (n=5)	After 24 hrs	After 7 days	After 14 days	
Ethanolic extract of AZ stem	150 ±10.50	0	0	0	Safe

Table 3. *In vitro* antioxidant activity of AZ

Conc. (µg/ml)	% Inhibition		Ascorbic acid
	DPPH free radical	Hydrogen peroxide scavenging	
20	21.8 ± 0.47	25.15± 0.92	31.5 ± 0.15
40	39.6 ± 0.72	28.68± 1.57	54.9 ± 0.92
60	48.3 ± 0.57	32.94± 0.58	61.8 ± 0.38
80	58.1 ± 0.41	48.23± 0.39	70.2 ± 0.72
100	67.35 ±0.29	57.29± 0.91	81.3 ± 0.69
120	71.5 ± 0.47	62.23± 1.26	91.9 ± 0.45
Half maximum inhibitory concentration (IC_{50})	62.2	63.25	65.3

3.4 Effect of the Ethanolic Extract of AZ Stem on Biochemical Parameters against PCT Induced Hepatotoxicity

AST, ALT, ALP, Bilurubin, SOD, CAT, MDA, and GSH are highly sensitive liver markers and their elevated levels are indicative of liver damage. Table 4 shows the results of the AZ stem cell ethanol extract on various biochemical parameters. There were no marked changes in the levels of these parameters detected in normal control rats. Rats with induced liver injury were injected with IP PCT, representing significantly elevated SGOT, SGPT, ALP, bilurubin, SOD, CAT, MDA, and GSH activities compared to the normal control group. However, a significant decrease in elevated serum enzyme levels can be observed in the AZ treatment (400 mg / kg) compared to the PCT treated group. The dose-dependent effect of AZ is comparable to that of silymarin treatment. These results indicated a protective effect of the AZ stem ethanolic extract on PCT-induced liver injury in rats.

Histopathologically, non-PCM-intoxicated liver pretreated with 10% DMSO (normal) shows normal lobular architecture and normal liver cells with well-preserved cytoplasm and well-defined sinusoidal line and nucleus around the perivenular area (Fig. 1 (a)). The PCM-intoxicated section of the liver, pretreated with 10% DMSO, shows infiltration of lymphocytes, presence of hemorrhage and extensive coagulative necrosis of the perivenular and mid-zonal region with periportal sparing (Fig. 1 (b)). Coagulative necrosis of hepatocytes in PCM-induced liver toxicity is predominantly present in the perivenular zone (zone 3). These pathological changes were found to be less with increasing MEBP dose, indicating the extract's ability to reverse PCM-induced intoxication (Figs. 1(d) - 1(f))). Interestingly, the presence of marked necrosis, inflammation and hemorrhage following PCM treatment (shown by the negative control group) was significantly reduced when pretreated with the extract or silymarin.

3.5 Effect of the Ethanolic Extract of AZ Stem on Biochemical Parameters against ETN Induced Hepatotoxicity

The hepatotoxic agent ethanol caused significant liver damage as indicated by the increased levels of liver chemistry biomarkers such as AST, ALT, ALP, Bilirubin, SOD, CAT, MDA and GSH. Rats treated with 100, 200 and 400 mg/kg doses significantly reduced levels of biochemical markers when compared with ETN administered controls. As compared to the lower doses, the higher one (400 mg/kg) demonstrated an improved hepatoprotective activity. Table 5 shows the detailed results of effect of ethanolic extract of AZ stem on biochemical parameters against ETN induced hepatotoxicity.

The hepatoprotective effect of the ethanolic extract of the AZ stem on ETN-induced liver injury was further confirmed by histopathological

examinations. Non-intoxicated liver with ETN pretreated with 10% DMSO (normal) shows normal lobular architecture and normal liver cells with well preserved cytoplasm and well-defined sinusoidal line and a nucleus around the perivenular area (Fig. 2(a)). Normal histological structures were observed in the liver of rats treated with silymarin (25 g / kg) as shown in Fig. 2(b). Liver cell abnormalities, necrosis and inflammation were observed in ETN-treated rats (Fig. 2(c)). While rats treated with AZ extract (100, 200 and 400 mg/kg) showed reduction/absence of inflammatory cells, vascular congestion, cell degeneration, necrosis and vacuoles. (Fig. 2(d,e,f)). In contrast, lower doses (100 mg/kg) of ethanolic extract of AZ stems showed lower protection than a higher dose of 400 mg/kg.

3.6 Effect of the Ethanolic Extract of AZ Stem on Biochemical Parameters against IR Induced Hepatotoxicity

The AZ ethanolic extract showed significant hepatoprotective activity ($p < 0.05$) against the toxicity patterns induced by isoniazid and rifampicin (50 mg / kg + 50 mg / kg) by improving liver function, as indicated by the reduction of liver enzyme levels compared to the control group. Table 6 shows the detailed results of hepatoprotective activity against the IR-induced hepatotoxicity model. Histopathological studies in liver extracted from IR-induced rats pretreated with 10% DMSO revealed significant damage ($p < 0.05$) in liver architecture, with severe hepatocyte necrosis. Non-IR intoxicated liver pretreated with 10% DMSO (normal) shows normal lobular architecture and normal liver cells with well-preserved cytoplasm and well-defined sinusoidal line and nucleus around the perivenular area (Fig. 3 (a)). Normal histological structures were observed in the liver of rats treated with silymarin (25 g / kg) as shown in Fig. 3 (b). Alterations of liver cells, necrosis, and inflammation were observed in the rats given IR (Fig. 3 (c)). While rats treated with AZ extract (100, 200 and 400 mg / kg) showed reduction / absence of inflammatory cells, vascular congestion, cell degeneration, necrosis and vacuoles. (Fig. 2 (d,e,f) respectively). In contrast, lower doses (100 mg / kg) of ethanolic extract of AZ stems showed lower protection than a higher dose of 400 mg / kg.

4. DISCUSSION

The liver plays an indispensable role in life thanks to its metabolic and detoxifying capabilities. As it is exposed to various endogenous and xenobiotic agents, a large number of intermediate and final products are produced which can cause hepatocellular death and are the main causes of liver disease.[33,34] To ensure an individual's survival and maintain liver function, conventional treatment focuses on symptom management and liver transplantation in severe cases of liver disease.[35] However, no drugs are currently used to increase the detoxifying power of the organ. Therefore, the tests and use of herbal hepatoprotective agents are increasing dramatically. Therefore, it would be absolutely imperative to demonstrate the efficacy of plant extracts in the presence of chemical induced hepatotoxicity [33].

Paracetamol (PCT) and ethanol (ETN) have generally been consumed by humans for reasons such as pyrexia and those who have a habit of drinking alcohol, respectively. Isoniazid and rifampicin (IR) are the most commonly used drugs to treat tuberculosis. All of these agents were known to induce hepatotoxicity.[33] Therefore, the same hepatotoxins were chosen to induce hepatotoxicity in rats and to evaluate the hepatoprotective activity of Artabotrys zeylanicus. The rats were treated with ethanolic extract of the A. zeylanicus stem. Several studies indicate that PCT, ETN and IR can cause hemorrhagic liver necrosis in humans and experimental animals.[33] Thus, in this study, rats treated with PCT, ETN, and IR resulted in increased rat liver weight through the development of infiltration, vacuolation and inflammation in the liver (Fig. 1b 2b and 3b). PCT, ETN and IR induced hepatotoxicity was used to evaluate the hepatoprotective potential of plant extracts in various animal models. These hepatotoxins are bioactivated by cytochrome P450 into highly unstable reactive free radicals. These can cause cell damage through peroxidation of membrane lipids and covalently bind with other macromolecules within hepatocytes.[34] Damage to the membrane causes the release of cytosolic and endoplasmic enzymes, which show damage to the structure and function of the liver. These manifest as elevations of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA and GSH levels.

Table 4. Effect of the ethanolic extract of AZ stem on biochemical parameters in PCT induced hepatotoxicity

Group	AST U/L	ALT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)
P1	91.87± 1.411	63.83± 0.693	71.50± 0.638	0.245± 0.151	95.35± 1.562	132.8± 0.472	29.9 ± 0.471	42.9 ± 0.592
P2	243.5± 2.349	291.5± 1.763	208.5± 0.458	0.957± 0.392	37.93± 1.095	66.2 ± 0.928	61.2 ± 0.091	13.2 ± 0.184
P3	104.5± 0.763**	87 ± 0.577**	85.50± 0.763**	0.296 ± 0.113**	86.79± 1.373	116.3± 1.537	32.2 ± 0.927	38.2 ± 0.316
P4	203.8± 0.534	261.8± 0.609	181.5± 0.428	0.677 ± 0.071	45.3 ± 0.947	68.2 ± 0.638	56.6 ± 0.972	17.83± 0.853
P5	198.3± 0.494**	211.3± 0.482**	161.3± 0.631**	0.574 ± 0.048**	58.6 ± 0.381**	82.3 ± 0.753**	48.2 ± 0.642 **	21.53± 0.851**
P6	171.2± 0.600**	141.2± 0.081**	116.3± 0.286**	0.476 ± 0.514**	74.86 ± 0.719 **	98.4 ± 1.084 **	37.6 ± 0.371 **	32.91± 0.640 **

***P < 0.001 significant with respect to Control group. Values are expressed as mean± S.E; n=6 in each group. statistical analysis one-way ANOVA followed by t-test*

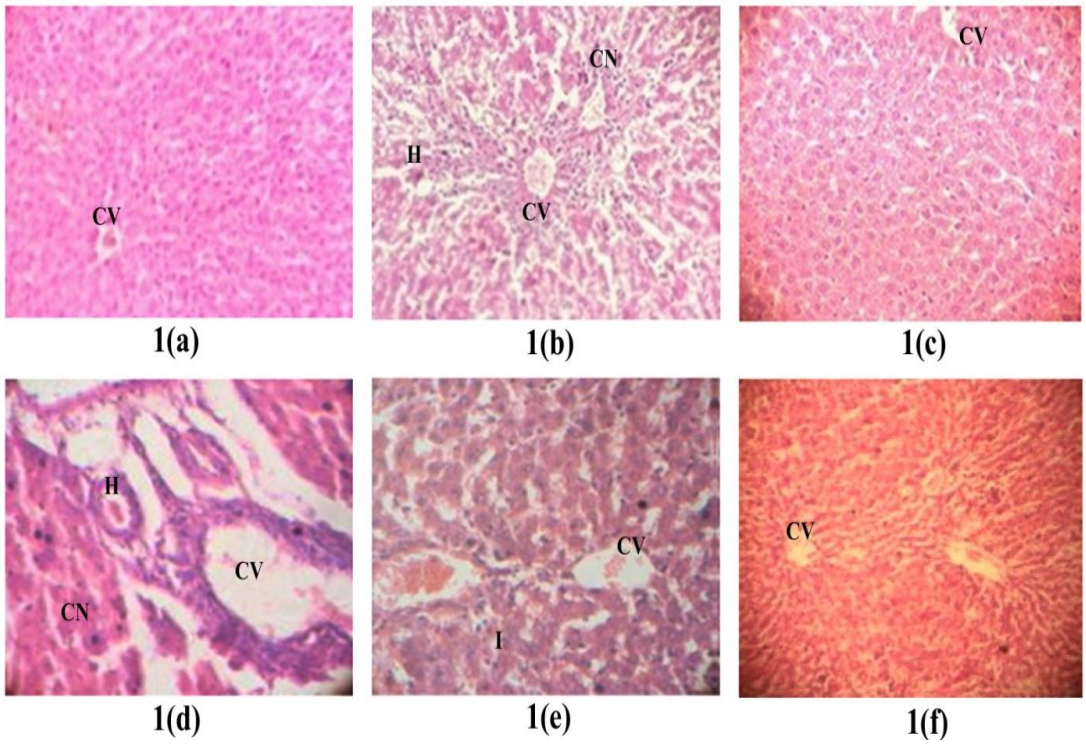

Fig. 1. (a) Normal; (b) Section of liver tissue of 3g/kg PCT treated group showing massive coagulative necrosis, hemorrhag e and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) Section 100 mg/kg AZ liver tissue intoxicated with PCT showing tissue necrosis and inflammation; (e) Section 200 mg/kg AZ liver tissue intoxicated with PCT showing tissue necrosis and inflammation; (f) Section 400 mg/kg AZ liver tissue intoxicated with PCT showing normal histology with mild inflammation. (100x Magnification) CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage

Table 5. Effect of the ethanolic extract of AZ stem on biochemical parameters in ETN induced hepatotoxicity

Group	AST U/L	ALT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)
E1	91.87± 1.411	63.83 ± 0.693	71.50 ± 0.638	0.245 ± 0.151	96.35 ± 1.562	131.8 ± 0.472	28.9 ± 0.471	42.9 ± 0.592
E2	241.5 ± 2.349	291.5 ± 1.763	208.5 ± 0.458	0.957 ± 0.392	38.93 ± 1.095	65.2 ± 0.928	60.2 ± 0.091	14.2 ± 0.184
E3	104.5 ± 0.763**	84 ± 0.577**	85.50 ± 0.763**	0.296 ± 0.113**	85.79 ± 1.373	115.3 ± 1.537	31.2 ± 0.927	39.2 ± 0.316
E4	212.2± 0.577	258.5 ± 0.763	183.8 ± 1.169	0.693 ± 0.068	43.9 ± 0.731	75.2± 0.521	54.6 ± 1.003	17.07 ± 0.635
E5	194.2 ± 0.703**	208.5 ± 0.412**	157.8 ± 0.703**	0.565 ± 0.192**	58.2 ± 1.179 **	83.1± 0.052 **	48.2 ± 0.521 **	21.94 ± 0.168 **
E6	158.5 ± 0.763**	131.3 ± 0.881**	112.8 ± 0.792**	0.461 ± 0.168**	70.12 ± 1.153 **	103.9± 0.581 **	39.2 ± 0.257 **	34.86 ± 0.921 **

*** P <0.001 significant compared to the control group. Values are expressed as mean ± S.E; n = 6 in each group. One-way ANOVA statistical analysis followed by t-test*

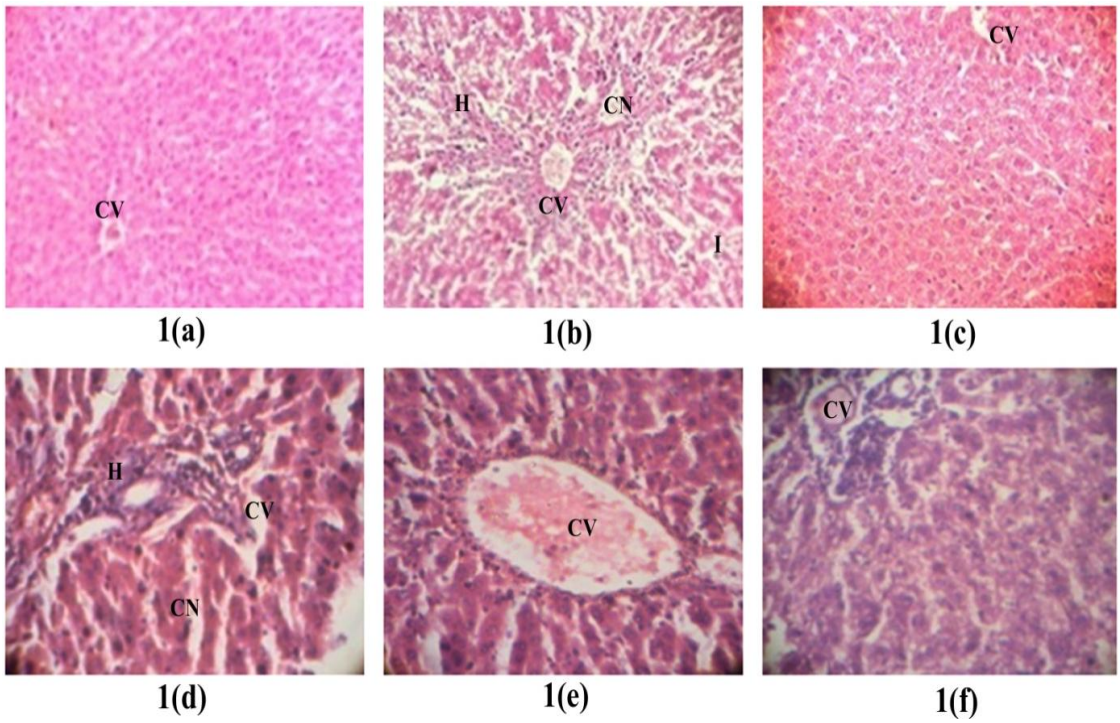

Fig. 2. (a) Normal; (b) Section of liver tissue of 5g/kg ETN treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) Section 100 mg/kg AZ liver tissue intoxicated with ETN showing tissue necrosis and inflammation; (e) Section 200 mg/kg AZ liver tissue intoxicated with ETN showing tissue necrosis and inflammation; (f) Section 400 mg/kg AZ liver tissue intoxicated with ETN showing normal histology. (100x Magnification) CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage

Table 6. Effect of the ethanolic extract of AZ stem on biochemical parameters in IR induced hepatotoxicity

Group	AST U/L	ALT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)
IR1	91.87± 1.411	63.83 ± 0.693	71.50 ± 0.638	0.244 ± 0.151	95.35 ± 1.562	132.8 ± 0.472	29.9 ± 0.471	43.9 ± 0.592
IR2	243.5 ± 2.349	291.5 ± 1.763	208.5 ± 0.458	0.954 ± 0.192	39.93 ± 1.095	64.2 ± 0.928	62.2 ± 0.091	13.2 ± 0.184
IR3	104.5 ± 0.763**	84 ± 0.577**	83.50 ± 0.763**	0.294 ± 0.113**	86.79 ± 1.373	114.3 ± 1.537	33.2 ± 0.927	38.2 ± 0.316
IR4	206.5 ± 1.176	256.5 ± 0.834	186.5 ± 0.496	0.657± 0.050	47.2 ± 1.156	71.2 ± 0.947	52.8 ± 0.731	18.90± 0.680
IR5	187 ± 0.575**	203.8± 0.369**	151.5 ± 0.436**	0.570± 0.068**	61.3 ± 0.961 **	83.71 ± 0.567 **	48.5 ± 0.570 **	22.37 ± 0.421 **
IR6	143.8 ± 0.912**	121.8± 0.945**	103.5 ± 0.672 **	0.423± 0.154**	72.80 ± 0.915 **	99.2± 0.269 **	37.7 ± 0.358 **	32.48 ± 0.631 **

***P < 0.001 significant with respect to control group. Values are expressed as mean± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test*

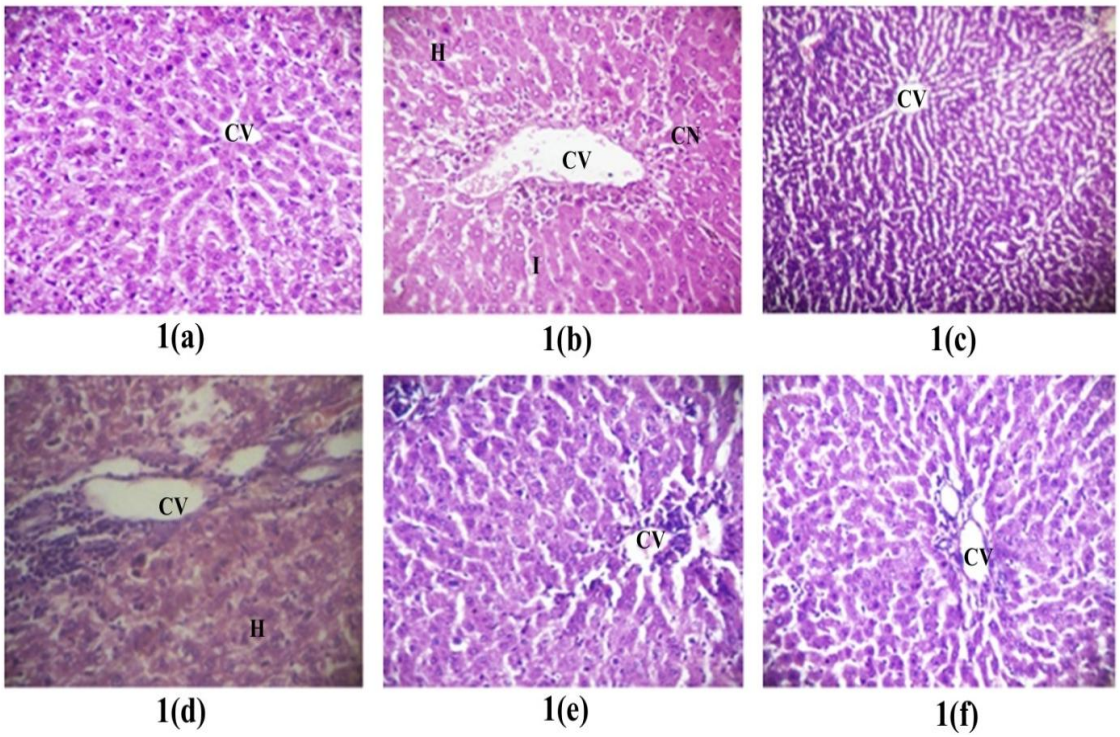

Fig. 3. (a) Normal; (b) Section of liver tissue of 100g/kg IR treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by IR showing preservation of normal hepatocytes; (d) Section 100 mg/kg AZ liver tissue intoxicated with IR showing tissue necrosis and inflammation; (e) Section 200 mg/kg AZ liver tissue intoxicated with IR showing tissue necrosis and inflammation; (f) Section 400 mg/kg AZ liver tissue intoxicated with IR showing normal histology. (100x Magnification) CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage

Therefore, measuring the levels of these biomarkers of liver damage can reveal the hepatoprotective activity of the plant extract and solvent fractions. In the present study, the ethanolic extract showed a reduction in the levels of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA and GSH in a dose-dependent manner. 100 mg / kg of AZ ethanolic stem extract did not produce a visible effect on all biomarkers of liver injury at their lowest dose, but medium and high doses managed to produce a significant reduction in AST, ALT and ALP levels (Tables 4-6). This could likely suggest that the lower dose may be less than the minimum effective dose, which cannot cause a significant reduction in liver enzyme levels, and the other two doses may be large enough to cause a significant reduction. Percent reduction of hepatic injury biomarkers showed that 200 mg / kg and 400 mg / kg of the ethanolic extract exerted an effect almost similar to that of the standard (Tables 4-6). The pre and post treatment with ethanolic extract in the two doses (200 mg / kg and 400 mg / kg) except for the 100 mg / kg dose strongly modulated the severity of the liver damage. The return of enzyme levels to near-normal levels in ethanolic rats before and after treatment shows that the ethanolic extract can stabilize liver cell membranes and prevent the escape of enzymes.

The prevention of free radical production and their neutralization, as well as the protection potential of this plant from hepatotoxins, may be other probable reasons for the healing effect of A. zeylanicus stem extract. To concentrate or separate the active ingredients, the crude ethanolic extract was fractionated. This study showed that most of the polar components of the plant stem could be due to the available flavonoidal content. The active principle or the principles responsible for the hepatoprotective activity of the ethanolic extract and the solvent fractions of A. zeylanicus are, until now, unknown, therefore it has not been identified which compounds are exactly responsible for the antioxidant and hepatoprotective activities. Previous studies have shown that alkaloids and flavonoids have antioxidant activity. Preliminary phytochemical analysis was performed on the crude ethanolic extract and the solvent fractions revealed a variety of secondary metabolites which appeared to be differentially distributed throughout the extract. It is reasonable to suggest that the phytochemicals present in the plant may act individually or synergistically to produce the observed hepatoprotective activity of A. zeylanicus. Probably, the flavonoids and alkaloids present in the raw stem extract exerted a hepatoprotective effect due to their free radical scavenging activity, prevention of lipid peroxidation and cell damage, as such action has been suggested for some other plants. Furthermore, alkaloids and flavonoids are known as natural antioxidants due to their free radical scavenging activity [34].

As the liver is continuously exposed to oxidative stress, the release of free radicals is the main hepatotoxicity mechanism of toxicants. In oxidative stress, the balance between the formation of reactive oxygen species and the amount of antioxidants is disturbed. Oxidative stress causes damage to cell components, such as proteins, lipids and nucleic acids.[33] To confirm the antioxidant activity of the plant extract, in vitro DPPH radical scavenging and hydrogen peroxide scavenging assya were carried out. In these scavenging assay, ethanolic extract of *A. zeylanicus* were observed to inhibit with the maximum value of 71% and 62% at the concentration of 100 µg/mL (Table 3). The crude ethanolic extract of *A. zeylanicus* had a calculated IC_{50} value of 62.2 and 63.25 µg/mL, which is nearly similar to the calculated IC_{50} value of the known antioxidant, ascorbic acid, ie 65.3 µg/mL. As it is explained for other plants ethanolic extract might act via their free radical scavenging, neutralization of free radicals and inhibition of necrosis via several pathways.

To sum up, this study provided further evidence that the ethanolic extract, possessed a comparable hepatoprotective activity with that of the standard drug. Results obtained revealed that there was a dose-dependent reduction in all biomarkers of liver injury in pre- and post-treatment. Therefore, this data seems to indicate that the hepatoprotective effect of the plant is distributed to polar bioactive principles contained in the ethanolic fraction. Even though the hepatoprotective mechanism of the plant extract is yet not elucidated, the observed antioxidant activity is one of the anticipated mechanisms. Above all, the ethanolic extract of the stem of *A. zeylanicus* would be rewarded as safe based on the results of acute oral toxicity study. Moreover, isolation and characterization of novel antioxidants will be done in future studies by using HPLC/LC-MS/MS techniques.

5. CONCLUSION

Experimental evidence obtained in the present study demonstrated that the *A. zeylanicus* stem

possesses hepatoprotective activity against PCT, ETN and IR induced liver toxicity. This activity may be due to the presence of flavonoids and other components present in the plant. However, complementary in vitro and in vivo studies will be necessary to confirm the mechanism responsible for this hepatoprotective effect.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors declare that the study was approved by institutional animal ethical committee with the project no. (831/a/19/CPCSEA)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Antioxidant Activities and Hepatoprotective Potential of Ethanol Leaf Extract of *Justicia quinqueangularis* against Selected Hepatotoxins Induced Hepatotoxicity in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The objective of this research was to see whether the ethanolic extract of *Justicia quinqueangularis* leaves had antioxidant and hepatoprotective properties against paracetamol (PCT), ethanol (ETN), and isoniazid and rifampicin (IR)-induced hepatotoxicity in Albino Wistar rats. **Methods:** The leaves of *Justicia quinqueangularis were* dried in the shade at room temperature, pulverised, and extracted by soxhlet using ethanol. Quantitative phytochemical experiments were carried out as a first step. The ethanol extract's hepatoprotective activity was evaluated in Albino Wistar rats. PCT (3 g/kg), ETN (5 g/kg), and IR (100 mg/kg) reduced the levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin, which are all biochemical indicators of liver injury. Both

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hepatotoxin-treated and untreated group of animals determined for their antioxidant levels. **SGOT, SGPT, ALP**, bilirubin, antioxidant function of DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide (H_2O_2), lipid peroxidation methods, hydroxyl radicals, and nitric oxide scavenging activities were among the biochemical and histopathological tests performed.

Results: The altered levels of biochemical markers were restored to near normal levels in a dose-dependent fashion after treatment with *J. quinqueangularis* ethanol leaf extract (100 mg / kg, 200 mg / kg, and 400 mg / kg body weight).

Conclusion: The findings of the current research indicated that the ethanol leaf extract of *J. quinqueangularis* had potent antioxidant and hepatoprotective properties against standard drug.

Keywords: Hepatoprotective; Justicia quinqueangularis; paracetamol; ethanol; Isoniazid and Rifampicin; hepatotoxins; histopathological.

1. INTRODUCTION

Liver disease continues to be a public health problem. Unfortunately, drugs used to cure liver disease, whether conventional or synthetic, are ineffective and may have dangerous side effects [1]. In the absence of a reliable liver protection medication, Ayurveda recommends a variety of herbal formulations for the treatment of liver disorders [2] because of the serious negative side effects of synthetic drugs, there is an increasing interest in using a rigorous testing approach to evaluate the scientific basis of conventional herbal medicines that prove to have hepatoprotective properties [3,4]. Some herbal extracts and their chemical components have been shown in studies to greatly inhibit these pathological processes and protect hepatocytes from the aetiology of chronic liver damage [5] Due to the lack of effective liver safety medications in western medicine, a wide range of herbal preparations are prescribed for the treatment of liver diseases, with many claiming to provide substantial relief [6]. Attempts are being made around the world to gain clinical evidence for these herbal medicines that have long been published.

Plant kingdom has given a diversified range of bioactive molecules which makes them medicinally, a precious source. Due to enormous limitations in synthetic pharmaceutical products, very less or no harmful effects and increased awareness on natural products, there is a need of hour to isolate the lead compounds from them [7]. One such plant that is currently under investigation for its potential hepatoprotective and antioxidant activity in our laboratory is *Justicia quinqueangularis* (family: Acanthaceae). Justicia quinqueangularis is a five-angled prostrate herb with slender, four-angled branches that root at nodes. Leaves are opposite, 1.5-3 x 0.2-0.4 cm, rough, linear-oblong or linear-lanceshaped, pointed at top, wedge-shaped at base; leaf stalks can be up to 3 mm long. Pink flowers bloom in spikes up to 7 cm long at branch ends. Bracteoles are similar to bracts but narrower, measuring 4 mm long, linear, blunt, and with scarious margins. The calyx is profoundly 4-partite, linear, blunt, and scarious at the margin. Flowers are 8 mm long and hairless, with a 2-fid upper lip and a 5-mm long and deep lower lip that is almost oval and minutely 3-lobed. Capsules are 8 mm long, oblong, and hairless with a rounded tip. Five-Angled Justicia can be seen all over India. August to December are the months when the flowers bloom [8].

However, many medicinal plants used in remote villages and tribal villages of southern districts of Andhra Pradesh remain to be studied. J. quinqueangularis is one such plant. This plant leaf is used in folklore medicine to treat liver diseases in Rayalaseema districts of Andhra Pradesh. In traditional medicine, its roots are used for treating Jaundice.

Literature survey reviles no major pharmacological activity reported. Furthermore, we also disclose on the phytochemical constituents of J. quinqueangularis, which indicate the presence of alkaloids, glycosides, flavanoids, steroids and proteins. The polyphenolic flavonoids, in particular have proved to exhibit various pharmacological activities including anti oxidant and Hepatoprotective activity.

Our through literature survey shows no evidences were found to prove the hepatoprotective activity of J. quinqueangularis against other hepatotoxicins like ethanol, Isoniazid and Rifampicin. Thus, this study was carried out to get insights into the utility of ethanolic extract of J. quinqueangularis leaf against various hepato toxic agents viz.,

paracetamol (PCT), ethanol (ETN) and Isoniazid and Rifampicin (IR) induced liver damage in rats as the animal model to develop a satisfactory hepatoprotective medicine.

2. MATERIALS AND METHODS

2.1 Source of Plants and Preparation of Crude Extract

J. quinqueangularis (JQ) leaves were collected in Tirupati, Andhra Pradesh, India. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, conducted the authentication. A specimen sample was preserved in the College's Pharmacognosy Department with the herbarium sample (voucher sample no-024/ C112 /suresh-04). The fresh leaves were degreased with petroleum ether and dried in the shade at room temperature. Using a Soxhlet apparatus, the defatted substance was extracted with 95 % ethanol and then dried under vacuum using a rotavapor.

2.2 Animals

The crude extracts were tested on Albino Wistar rats of both sexes. Animals and their feed were purchased from Sinivasa agencie, Bangalore, India. For one week before and after the trials, the animals were held at 27±2 °C, relative humidity 44-56 %, at light and dark periods of 10 to 14 hours, respectively. The animals were fed a normal diet (Lipton, India) and were given water ad libitum 18 hours before the experiment. All the experiments were carried out in the morning, in accordance with existing laboratory animal treatment and ethical recommendations for the study of experimental pain in conscious animals [9-10].

2.3 Phytochemical studies

All the extracts were subjected for Phytochemical study as per described by Akhila et al. [11].

2.4 Acute Toxicity Studies

Albino rats weighing between150-180 gm were used in an acute toxicity study for the ethanolic leaf extract of *J. quinqueangularis* leaves. Before the trial, the animals were fasted overnight and held in normal conditions. Extract was given orally in increasing doses and were found to be healthy up to a dosage of 2000 mg/kg bw [11].

2.5 Experimental Animal and Design

PCT (3g/kg), Ethanol 5mg/kg and isoniazid and rifampicin (50+50 mg/kg) was dissolved in 0.5 % CMC for oral administration. Rats were randomly divided into six groups for each model and consisting of six rats. PCT intoxicated animals were grouped from P1-P6. E1-E6 represents group of animals which were intoxicated by ethanol and Group IR1-IR6 constitute animals intoxicated by IR. Table 1 shows the details of animal groupings for various hepato toxicity models.

The rats were given ether and then sacrificed after 72 hours of intoxication. SGOT, SGPT, ALP, and Bilirubin enzyme levels were measured using standard kits after blood was extracted via cardiac puncture into heparinized tubing. The liver was immediately removed and washed in ice cold saline before being examined histologically.

2.6 *In Vitro* AntiOxidant Activity

2.6.1 DPPH-scavenging activity

Hydrogen donation or radical scavenging ability using the stable radical DPPH was determined for the evaluation of the free radical scavenging activity of the extract [12]. A 0.1 mM ethanol solution was prepared, and 1.0 ml of it was applied to 3.0 ml of the entire solution of extracts in water at various concentrations (10–100 µg/ml). The absorbance was estimated after 30 mins at 517 nm. The reaction mixture's lower absorbance means a higher free radical removal activity. The standard drug was ascorbic acid [13].

2.6.2 Scavenging of Hydrogen Peroxide (H_2O_2)

A 20 mM hydrogen peroxide solution in phosphate buffered saline (pH 7.4) was prepared, and different amounts of extract or standard in methanol (1 ml) were added to 2 ml of peroxide solution buffer saline solution containing hydrogen. The absorbance was estimated at 230 nm after 10 minutes [14].

2.7 Determination of Biochemical Parameters

Various biochemical serum markers such as serum oxaloacetic glutamic transaminase (SGOT), serum glutamic pyruvic transaminase

(SGPT), alkaline phosphate (ALP), bilirubin, superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (reduced) (GSH) were assessed using commercial available kits for each sample, all analyses were carried out in triplicate [15,16].

2.8 Histopathological Studies

Dissected liver tissue was frozen in 10% formalin, dehydrated in 50% ethanol, eliminated in xylene, and embedded in paraffin. Photomicroscopic observations of cell necrosis, fat displacement, hyaline regeneration, and balloon degeneration were made using sections stained with hematoxylin and eosin dye (H-E).

2.9 Statistical Analysis

The mean and standard deviation of the mean are used to express the data. Data were evaluated using one-way analysis of variance (ANOVA), and discrepancies between groups were calculated using Graph pad PRISM V5.02 software's Dunnett's post hoc test. The $p<0.05$ significance level was chosen.

3. RESULTS

3.1 Phytochemical study

All extracts subjected for phytochemical study showed the presence of Alkaloids, Glycosides, Flavanoids, Steroids and Proteins.

3.2 Acute toxicity studies

Up to doses of 2000 mg/kg bw, the ethanolic and aqueous extracts displayed no signs or symptoms of toxicity or mortality. This proves that, a dose of as higher as 2000 mg/Kg bw was safe to administer. The LD50 was found to be 3800 mg/kKg bw

3.3 *In vitro* antioxidant study

Before proceeding for *in vivo* activity the efficacy of the plants were tested i*n vitro.* The *in vitro* antioxidant activity was performed by using DPPH free radical and Hydrogen Peroxide scavenging. Results were tabulated in Table 2.

3.4 Effect of the Ethanolic Extract of JQ Leaf on Biochemical Parameters against PCT Induced Hepatotoxicity

The liver markers SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH are all very responsive, and their elevated levels indicate liver damage. The effects of the ethanolic extract of the JQ leaf on different biochemical parameters are shown in Table 3. In standard control rats, there were no significant improvements in the levels of these parameters. PCT was injected into rats with mediated liver damage, resulting in significantly higher SGOT, SGPT, ALP, bilirubin, SOD, CAT, MDA, and GSH behaviors than the usual control group. However, as compared to the PCT-treated population, the JQ treatment (400 mg/kg) showed a substantial reduction in the levels of elevated serum enzymes. The effect of JQ on a dose-dependent basis is equal to that of silymarin therapy.

The non- PCM- intoxicated liver pretreated with normal saline has normal lobular morphology and normal liver cells with well-preserved cytoplasm, a well-defined sinusoidal line, and a nucleus across the perivenular region (Fig. 1 (a)). Fig. 1 (b) reveals lymphocyte penetration, haemorrhage, and severe coagulative necrosis of the perivenular and midline regions with periportal preservation in a PCM-poisoned liver segment. The perivenular zone is primarily affected by coagulant necrosis of hepatocytes in PCM-induced liver toxicity (zone 3). With increasing JQ dosage, these pathological improvements were found to be minimal, meaning that the extract would reverse PCM-induced intoxication (Figs. 1 (d) -1 (f)). Pretreatment with the extract or silymarin greatly

Table 1. Animal groupings for various hepato toxicity models

PCT Group*	ETN Group*	IR Group*	Treatment
P1	E1	IR1	Normal control fed with 10 ml/kg body weight isotonic 0.9% NaCl
P2	E2	IR2	Selected Hepatotoxicity control.
P3	E3	IR3	Standard, Silymarin 25gm/kg, orally.
P4	E4	IR4	Ethanol extract of *JQ* leaf at 100 mg/kg, orally, for seven days.
P5	E5	IR5	Ethanol extract of *JQ* leaf at 200 mg/kg, orally, for seven days.
P6	E6	IR6	Ethanol extract of *JQ* leaf at 400 mg/kg, orally, for seven days.

**Each group contains 6 animals*

Table 2. *In vitro* antioxidant activity of JQ

Conc. (µg/ml)	% Inhibition		Ascorbic acid
	DPPH free radical	Hydrogen Peroxide scavenging	
20	33.56 ±0.24	25.31 ±0.57	31.5 ± 0.15
40	43.36 ±0.58	32.63 ±0.62	54.9 ± 0.92
60	51.12 ±0.28	38.26 ±0.63	61.8 ± 0.38
80	58.9 ± 0.43	55.21 ±0.58	70.2 ± 0.72
100	62.8 ± 0.27	62.83 ±0.49	81.3 ± 0.69
120	69.8 ± 0.56	74.28 ±0.42	91.9 ± 0.45
IC_{50}	59.8 mol/L	61.23 mol/L	29.3 mol/L

Table 3. Effect of the ethanolic extract of JQ leaf on biochemical parameters in PCT induced hepatotoxicity

Group/Markers	P1	P2	P3	P4	P5	P6
SGOT U/L	91.87± 1.411	243.5 ± 2.349	104.5 ± 0.763**	205.7 ± 0.663	153.7 ± 0.833**	138.8 ± 0.536**
SGPT U/L	65.83 ± 0.693	291.5 ± 1.763	86 ± 0.577**	251 ± 0.577	198.5± 0.913**	116.3± 0.881**
ALP U/L	71.50 ± 0.638	208.5 ± 0.458	85.50 ± 0.763**	178.8 ± 1.249	144 ± 1.371**	101.5 ± 1.138**
BILURUBIN mg/dL	0.245 ± 0.751	0.959 ± 0.392	0.296 ± 0.613**	0.642 ± 0.038	0.571 ± 0.075**	0.416 ± 0.639**
SOD (units/mg liver protein)	95.35 ± 1.562	37.93 ± 1.095	86.79 ± 1.373	46.8 ± 1.456	58.31 ± 0.835 **	74.29 ± 1.149 **
CAT (units/mg liver protein)	132.8 ± 0.472	66.2 ± 0.928	116.3 ± 1.537	68.2 ± 1.032	82.3 ± 0.731 **	95.4 ± 0.925 **
MDA (nmol/g tissue)	29.9 ± 0.471	61.2 ± 0.091	32.2 ± 0.927	55.9 ± 0.935	48.6 ± 0.637 **	41.6 ± 0.531 **
GSH (µmol/g tissue)	44.9 ± 0.592	13.2 ± 0.184	38.2 ± 0.316	17.62± 0.715	22.29± 0.471**	31.52 ± 0.491**

***P < 0.001 significant with respect to Control group. Values are expressed as mean± SD; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test*

Table 4. Effect of the ethanol extract of JQ leaf on biochemical parameters in ETN induced hepatotoxicity

Group	SGOT U/L	SGPT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)
E1	91.87± 1.411	65.83 ± 0.693	71.50 ± 0.638	0.245 ± 0.751	95.35 ± 1.562	132.8 ± 0.472	29.9 ± 0.471	43.9 ± 0.592
E2	243.5 ± 2.349	291.5 ± 1.763	208.5 ± 0.458	0.957 ± 0.392	37.93 ± 1.095	64.2 ± 0.928	61.2 ± 0.091	13.2 ± 0.184
E3	104.5 ± 0.763**	84 ± 0.577**	85.50 ± 0.763**	0.296 ± 0.613**	84.79 ± 1.373	116.3 ± 1.537	32.2± 0.927	38.2 ± 0.316
E4	231± 0.066	282± 0.312	203± 0.982	0.942± 0.038	42.58± 1.062	68.38± 0.726	58.17± 0.061	16.26± 0.508
E5	229± 0.521	273± 0.190	193± 0.395	0.937± 0.031	48.91± 1.086	68.21± 0.291	58.78± 0.027	18.48± 0.291
E6	224± 0.471	272± 1.121	183± 0.291	0.897± 0.098	52.28± 1.038	71.27± 0.832	59.21± 0.019	18.37± 0.751

***P < 0.001 significant with respect to Control group. Values are expressed as mean± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test*

Table 5. Effect of the ethanolic extract of JQ leaf on biochemical parameters in IR induced hepatotoxicity

Group	SGOT U/L	SGPT U/L	ALP U/L	BILURUBIN mg/dL	SOD (units/mg liver protein)	CAT (units/mg liver protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)
IR1	91.87± 1.411	65.83 ± 0.693	71.50 ± 0.638	0.245 ± 0.751	95.35± 1.562	132.8 ± 0.472	29.9 ± 0.471	43.9 ± 0.592
IR2	243.5 ± 2.349	291.5 ± 1.763	208.5 ± 0.458	0.959 ± 0.392	39.93 ± 1.095	66.2 ± 0.928	61.2 ± 0.091	15.2 ± 0.184
IR3	104.5 ± 0.763**	84 ± 0.577**	85.50 ± 0.763**	0.294 ± 0.613**	86.79 ± 1.373	116.3 ± 1.537	32.2± 0.927	38.2 ± 0.316
IR4	233± 0.231	275±1.210	201±0.398	0.913 ±0.041	44.21± 1.021	71.32± 0.931	59.29± 0.047	15.38± 0.932
IR5	223± 0.275	252±0.981	195±0.108	0.825± 0.052	49.92± 1.038	73.86± 0.431	58.91± 0.051	19.72± 0.831
IR6	222± 0.176	243±0.881	185±0.291	0.793±0.027	54.29± 1.048	75.37± 0.725	56.81± 0.052	22.63± 0.261

***P < 0.001 significant with respect to Control group. Values are expressed as mean± S.E.M; n=6 in each group. Statistical analysis one-way ANOVA followed by t-test*

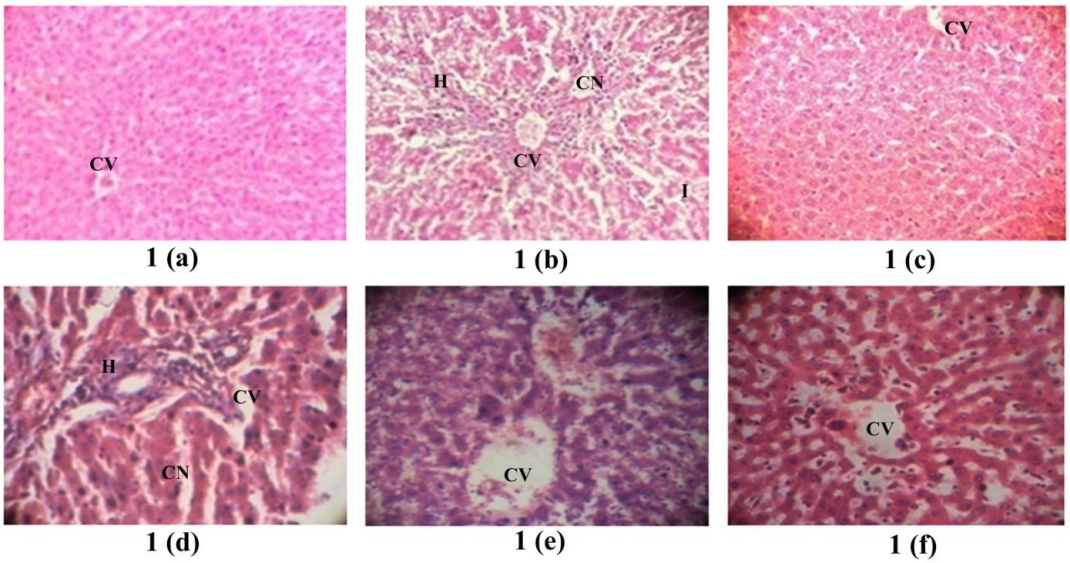

Fig. 1. (a) Normal; (b) section of liver tissue of 3g/kg PCT treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ liver tissue intoxicated with PCT showing tissue necrosis and inflammation; (e) section 200 mg/kg JQ liver tissue intoxicated with PCT showing tissue necrosis and inflammation; (f) section 400 mg/kg JQ liver tissue intoxicated with PCT showing normal histology with mild inflammation. (100x Magnification)

CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage. Stain: hematoxylin and eosin dye

decreased the presence of marked necrosis, inflammation, and bleeding during PCM treatment (as seen in the negative control group).

3.5 Effect of the Ethanol Extract of JQ Leaf on Biochemical Parameters against ETN Induced Hepatotoxicity

Increased amounts of liver biomarkers such as SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH revealed that the hepatotoxic agent ethanol induced substantial liver harm. When compared to ETN-treated rats, rats given doses of 100, 200, and 400 mg/kg had slightly lower levels of biochemical markers. The maximum dose (400 mg / kg) had greater hepatoprotective efficacy than the lowest doses. The effect of the ethanolic extract of the JQ leaf on biochemical parameters against ETN-induced hepatotoxicity is detailed in Table 4.

Histopathological examinations confirmed the hepatoprotective effect of the ethanolic extract of JQ leaves on ETN-induced liver injury. Fig. 2 (a) shows natural lobular morphology and regular liver cells with well-preserved cytoplasm, a well-defined sinusoidal axis, and a nucleus around the perivenular region in non-intoxicated liver with ETN pretreated with 10% DMSO (standard). Fig. 2(b) shows normal histological structures in the livers of rats infected with silymarin (25 g / kg). In rats given ETN, abnormal liver cells, necrosis, and inflammation were observed (Fig. 2 (c)). Rats given JQ extract (100, 200, and 400 mg/kg) demonstrated a reduction in body weight. Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with JQ extract (100, 200, and 400 mg/kg). Fig. 2 (d, e, and f) Lower doses of ethanolic extract of JQ leaves (100 mg/kg) provided less safety than higher doses of 400 mg/kg.

3.6 Effect of the Ethanol Extract of JQ Leaf on Biochemical Parameters against IR Induced Hepatotoxicity

The ethanol extract of JQ demonstrated significant hepatoprotective activity ($p<0.05$) against the toxicity caused by isoniazid and Rifampicin (IR) (50 mg/kg + 50 mg/kg) by enhancing liver function, as shown by lower liver enzyme levels relative to the control group. The

full effects of hepatoprotective activity against the IR-induced hepatotoxicity model are seen in Table 5. The liver architecture of IR-induced rats pretreated with 10% DMSO was significantly damaged ($p<0.05$), with extreme hepatocyte necrosis, according to histopathological tests of liver removed from the rats. Regular lobular morphology and normal liver cells with non-IR intoxicated liver pretreated with 10% DMSO (normal). Fig. 3 (a) shows natural lobular morphology and normal liver cells with well-preserved cytoplasm and well-defined sinusoidal line and nucleus across the perivenular region in non-IR intoxicated liver pretreated with 10% DMSO (normal). Fig. 3(b) shows normal histological structures in the livers of rats infected with silymarin (25 g / kg). In the IR-treated rats, changes in liver cells, necrosis, and inflammation were observed (Fig. 3 (c)). Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with JQ extract (100, 200, and 400 mg/kg). (Fig. 2 (d, e, and f)). Lower doses, on the other hand, Inflammatory cells, artery congestion, cell degeneration, necrosis, and vacuoles were reduced or absent in rats treated with JQ extract (100, 200, and 400 mg/kg). (Fig. 2 (d, e, and f)). Lower doses of ethanolic extract of JQ leaves (100 mg/kg) provided less safety than higher doses of 400 mg/kg.

4. DISCUSSION

Liver is an essential part of life, because of its metabolic and detoxifying capacities. When people are exposed to a variety of endogenous and xenobiotic compounds, they develop a vast amount of intermediate and final products, which can induce hepatocellular death and are the leading causes of liver disease [17,18]. Liver transplantation in acute cases of liver failure in order to sustain liver function [19]. As a result, the use of botanical hepatoprotective agents is becoming increasingly common. Therefore, it would be absolutely imperative to demonstrate the efficacy of JQ in the presence of chemical induced hepatotoxicity [17].

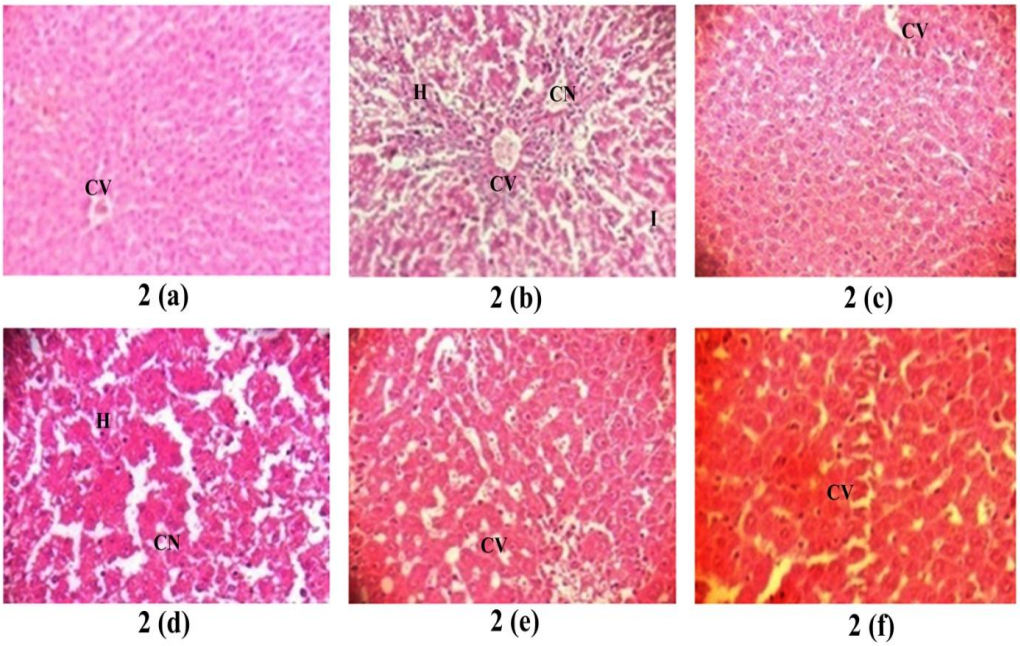

Fig. 2. (a) Normal; (b) section of liver tissue of 5g/kg ETN treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ liver tissue intoxicated with ETN showing tissue necrosis and inflammation; (e) section 200 mg/kg JQ liver tissue intoxicated with ETN showing tissue necrosis and inflammation; (f) section 400 mg/kg JQ liver tissue intoxicated with ETN showing normal histology. (100x Magnification)

CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage. Stain: hematoxylin and eosin dye

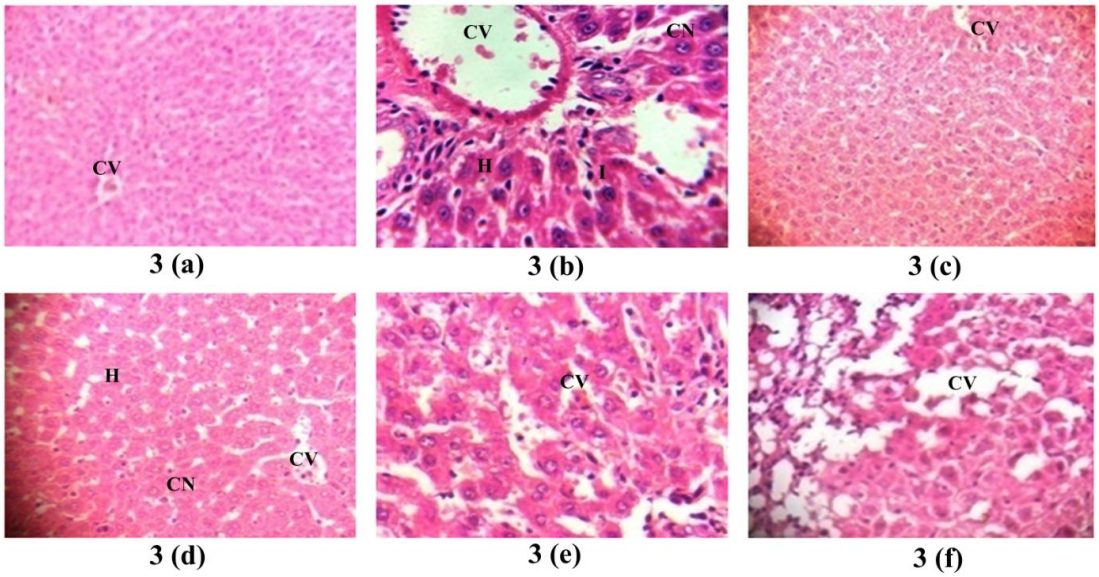

Fig. 3. (a) Normal; (b) section of liver tissue of 100g/kg IR treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by IR showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ liver tissue intoxicated with IR showing tissue necrosis and inflammation; (e) section 200 mg/kg JQ liver tissue intoxicated with IR showing tissue necrosis and inflammation; (f) section 400 mg/kg JQ liver tissue intoxicated with IR showing normal histology. (100x Magnification)

CV: centrilobular. CN: coagulative necrosis. I: inflammation. H: haemorrhage. Stain: hematoxylin and eosin dye

Humans often take paracetamol (PCT) and ethanol (ETN) for pyrexia and those who have a tendency of using alcohol, respectively. The most often used medicines to treat TB are isoniazid and rifampicin (IR). All these agents were known to induce hepatotoxicity [20]. So, the same hepato toxins were chosen to induce hepatotoxicity in rats and evaluate the hepatoprotective activity of *Justicia quinqueangularis*. The rats were given an ethanolic extract of the leaves of *J. quinqueangularis*. In humans and laboratory animals, PCT, ETN, and IR have been shown to cause hemorrhagic liver necrosis in many trials. In this study, rats treated with PCT, ETN, and IR developed infiltration, vacuolation, and inflammation in the liver, resulting in increased rat liver weight (Fig. 1b, 2b and 3b). The hepatoprotective ability of plant extracts in different animal models was evaluated using PCT, ETN, and IR mediated hepatotoxicity. Bioactivation of these hepatotoxins by cytochrome P450 results in strongly unstable reactive free radicals. These can kill cells by peroxiding membrane lipids and binding covalently with other macromolecules in hepatocytes. When the membrane is damaged, cytosolic and endoplasmic enzymes are released, indicating that the liver's structure and function have been compromised. Elevated amounts of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH are signs of this. As a result, measuring the amounts of these biomarkers of liver injury will show the plant extract's and solvent fractions' hepatoprotective function. The ethanolic extract reduced the levels of SGOT, SGPT, ALP, Bilurubin, SOD, CAT, MDA, and GSH in a dose-dependent manner in the current sample. At the lowest dosage, 100 mg/kg ethanolic extract of JQ leaves had little effect on all biomarkers of liver damage, but medium and high doses resulted in substantial reductions in AST, ALT, and ALP levels (Table 3 - 5). This may indicate that the lower dose is smaller than the minimal effective dose, and cannot induce a substantial decrease in liver enzyme levels, whereas the other two doses are high enough to do so. Percent reduction in hepatic injury biomarkers revealed that 200 mg/kg and 400 mg/kg of ethanolic extract had an effect that was almost identical to the normal (Table 4-6). With the exception of the 100 mg/kg

dosage, pre- and post-treatment with ethanolic extract in all doses (200 mg/kg and 400 mg/kg) significantly reduced the severity of liver injury. The ethanolic extract can stabilise liver cell membranes and avoid enzyme degradation, as shown by the return of enzyme levels to near-normal levels in ethanolic rats before and after the treatment.

Other possible explanations for the therapeutic activity of *J. quinqueangularis* leaf extract include preventing the formation of free radicals and neutralising them, as well as the plant's ability to defend against hepatotoxins. The crude ethanolic extract was fractionated to concentrate or isolate the active ingredients. The majority of the polar components of the plant leaf may be attributed to the available flavonoids material, according to this report. Since the active theory or ingredients responsible for the hepatoprotective behaviour of the ethanolic extract and solvent fractions of *J. quinqueangularis* are unclear, it is impossible to pinpoint the compounds are responsible for the antioxidant and hepatoprotective effects. Alkaloids and flavonoids have been found to have antioxidant properties in previous research. The crude ethanolic extract and the solvent fractions were subjected to preliminary phytochemical analysis, which showed a number of secondary metabolites that seemed to be dispersed differently in the extract. It is fair to believe that the phytochemicals found in the plant work individually or in concert to create *J. quinqueangularis* hepatoprotective function. It's likely that the flavonoids and alkaloids in the raw leaf extract have a hepatoprotective impact by scavenging free radicals and preventing lipid peroxidation and cell injury, as has been proposed with some other plants. Alkaloids and flavonoids are sometimes classified as natural antioxidants because of their ability to scavenge free radicals.

In conclusion, this analysis added to the growing body of evidence that the ethanolic extract has hepatoprotective properties comparable to the regular treatment. Both biomarkers of liver damage were reduced in a dose-dependent manner before and after the treatment, according to the findings. As a result, these findings suggest that the plant's hepatoprotective effect is spread to the polar bioactive concepts contained in the extract. While the plant extract's hepatoprotective function is yet to be discovered, one of the expected mechanisms is its antioxidant activity. Over all, according to the findings of the acute oral toxicity report, the ethanolic extract of the leaf of *J. quinqueangularis* is considered safe. In addition, future experiments will use HPLC and LC-MS/MS strategies to isolate and characterise new phytoconstituents.

5. CONCLUSION

The current study's experimental evidence showed that the leaf of *J. quinqueangularis* has hepatoprotective function against PCT, ETN, and IR-induced liver toxicity. The presence of flavonoids and other components in the plant may be responsible for this behavior. To confirm the mechanism underlying this hepatoprotective effect, additional in vitro and in vivo studies will be needed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Research proposal was approved by the Institute's Animal Ethics Committee (224/a/18/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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