Antioxidant activities and Hepatoprotective potential of Ethanol leaf extract of *Justicia quinqueangularis* against selected Hepatotoxins induced Hepatotoxicity in Albino Wistar Rats

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 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.

***Key words*:** Hepatoprotective, *Justicia quinqueangularis*, paracetamol, ethanol, Isoniazid and Rifampicin, hepatotoxins, histopathological.

1.0 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.

2.0 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. The Research proposal was approved by the Institute's Animal Ethics Committee (224/a/18/CPCSEA). 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., 2007 [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

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.

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.

3.0 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.

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

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.

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.*

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 (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. 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 (Figures 1 (d) -1 (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).

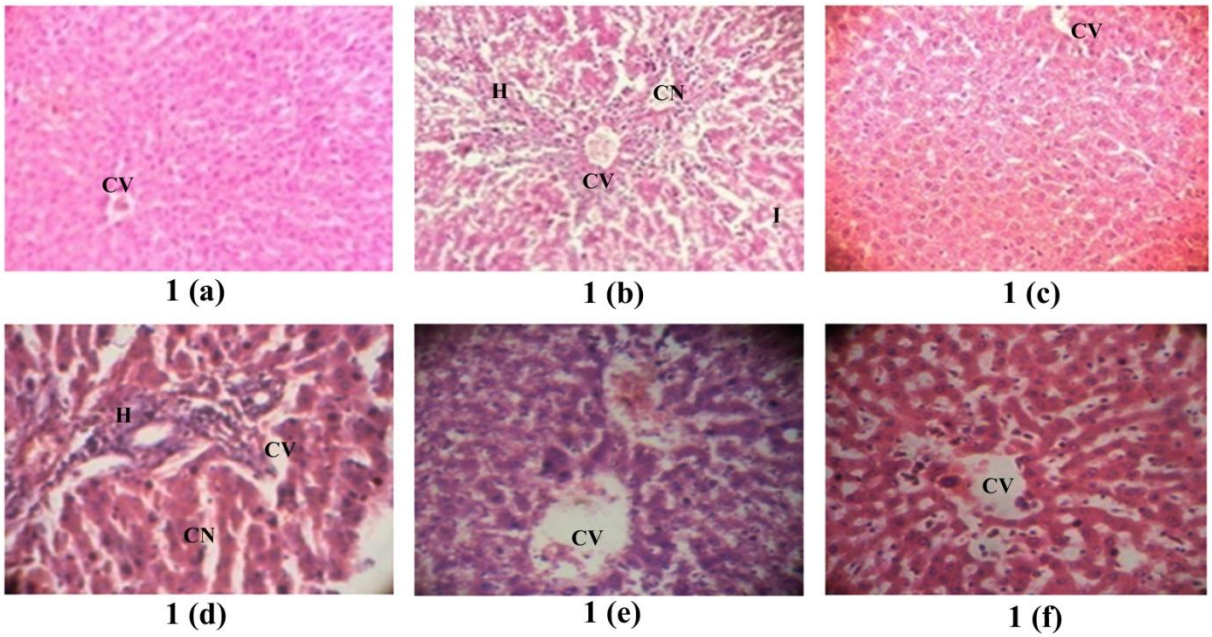

Figure 1: (a) Normal; (b) section of liver tissue of 3g/kg PCT treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ 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

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.

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±	65.83 ±	71.50 ±	0.245 ±	95.35 ±	132.8 ±	29.9 ±	43.9 ±

	1.411	0.693	0.638	0.751	1.562	0.472	0.471	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.*

Histopathological examinations confirmed the hepatoprotective effect of the ethanolic extract of JQ 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 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 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). Figure 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.

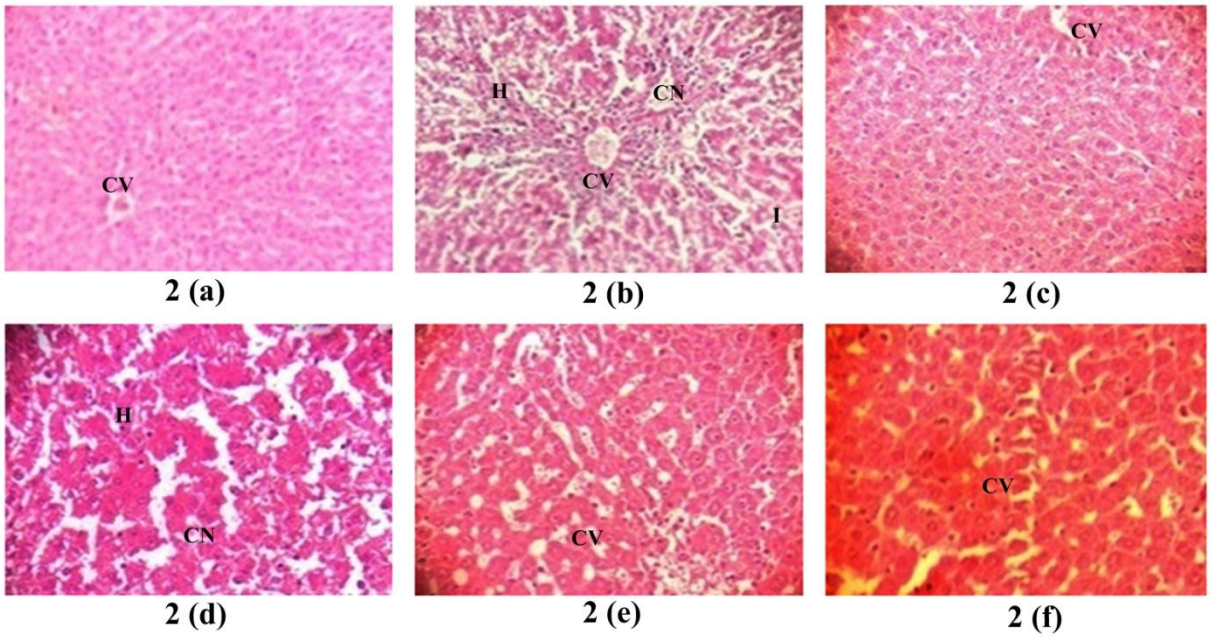

Figure 2: (a) Normal; (b) section of liver tissue of 5g/kg ETN treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by PCT showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ 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

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). 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 JQ extract (100, 200, and 400 mg/kg). (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 JQ extract (100, 200, and 400 mg/kg). (Figure 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.

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.*

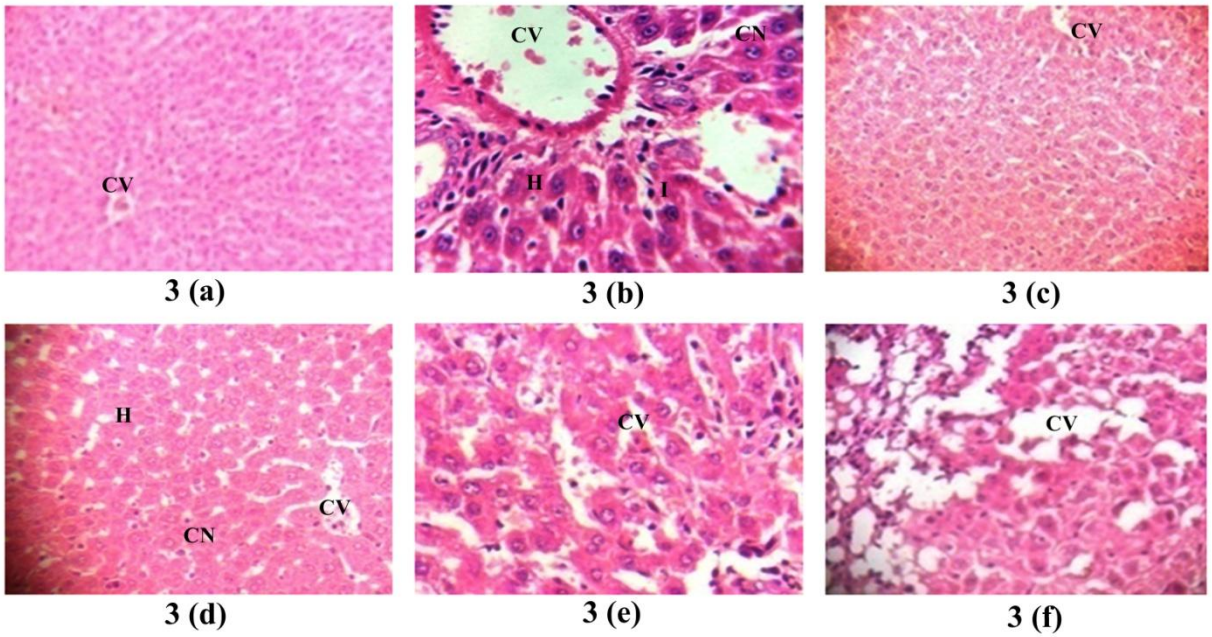

Figure 3: (a) Normal; (b) section of liver tissue of 100g/kg IR treated group showing massive coagulative necrosis, hemorrhage and inflammation; (c) Section of 25gm/kg of silymarin liver tissue pretreated on the liver followed by IR showing preservation of normal hepatocytes; (d) section 100 mg/kg JQ 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

4.0 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].

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 (Figure 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.

5.0 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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