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Hepatoprotective activity of 2-piperidone isolated from leaf extracts of *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf in carbon tetrachloride induced hepatotoxicity

[Actividad hepatoprotectora de 2-piperidona aislada de extractos de hojas de *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf en hepatotoxicidad inducida por tetracloruro de carbono]

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Abstract

Context: Liver disorders have become common problem worldwide. The drugs available currently for the treatment are few with serious side effects. Since phytochemicals have proven to be potential therapeutic agents, an attempt has been made to screen novel hepatoprotective agents from the leaves of the medicinally ignored plant *Talinum portulacifolium*.

Aims: To evaluate the phytoconstituents of *Talinum portulacifolium* responsible for hepatoprotective activity in carbon tetrachloride-induced hepatotoxicity models both *in vitro and in vivo*.

Methods: The hepatic damage was assessed *in vitro* by serum marker enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase followed by *in vivo* histopathological examination.

Results: The results of the study indicate that the plant hydroalcoholic and acetone extracts at 500 mg/kg and compound 2-piperidone at 0.5 mg/kg exhibited equipotent results in the reduction of biochemical marker enzymes ($p<0.01$. $p<0.05$ and $p<0.001$) significantly compared to standard drug silymarin. The histopathological studies further supported that compound 2-piperidone showed better regeneration of damaged hepatocytes compared to standard. The possible mechanism of action may be due to inhibition of cytochrome P450 2E induced endoplasmic reticulum and oxidative stress.

Conclusions: The present study reveals that the hepatoprotective activity of leaf hydroalcoholic and acetone extracts may be due to the presence of 2-piperidone. As it showed equipotent potential to standard drug silymarin, it can be further developed as a hepatoprotective drug.

Keywords: hepatoprotective; 2-piperidone; *Talinum portulacifolium*.

Resumen

Contexto: Los trastornos hepáticos se han convertido en un problema común en todo el mundo. Los medicamentos disponibles actualmente para el tratamiento son pocos con efectos secundarios graves. Dado que los fitoquímicos han demostrado ser agentes terapéuticos potenciales, se ha intentado seleccionar nuevos agentes hepatoprotectores de las hojas de *Talinum portulacifolium*.

Objetivos: Evaluar los fitoconstituyentes de *Talinum portulacifolium* responsables de la actividad hepatoprotectora en modelos de hepatotoxicidad inducida por tetracloruro de carbono tanto *in vitro* como *in vivo*.

Métodos: El daño hepático se evaluó *in vitro* mediante las enzimas marcadoras séricas alanina aminotransferasa, aspartato amino-transferasa y fosfatasa alcalina, seguido de un examen histopatológico *in vivo*.

Resultados: Los resultados del estudio indican que los extractos hidroalcohólicos y de acetona de la planta a 500 mg/kg y el compuesto 2-piperidona a 0,5 mg/kg mostraron resultados significativos ($p<0,01$. $p<0,05$ y $p<0,001$) equipotentes en la reducción de las enzimas marcadoras bioquímicas en comparación con la droga estándar de silimarina. Los estudios histopatológicos respaldaron además que el compuesto 2-piperidona mostró una mejor regeneración de hepatocitos dañados en comparación con el estándar. El posible mecanismo de acción puede deberse a la inhibición del retículo endoplásmico inducido por el citocromo P450 2E y al estrés oxidativo.

Conclusiones: El presente estudio revela que la actividad hepatoprotectora de los extractos hidroalcohólicos y de acetona de las hojas puede deberse a la presencia de 2-piperidona. Como mostró un potencial equipotente a la silimarina estándar, pudiera desarrollarse como un fármaco hepatoprotector.

Palabras Clave: hepatoprotector; 2-piperidona; *Talinum portulacifolium*.

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INTRODUCTION

Medicinal plants are valuable resources of medicinal agents since decades for various kinds of ailments (Alagesboopathi, 2011). Synthetic pharmaceutical products offer limited ability to control major diseases. Hence, there has been a revival of interest in developing novel lead compounds to discover new drugs from plant kingdom (Hussain et al., 2011). Alcohol abuse and nonalcoholic fatty liver disease (NAFLD) are the most common causes of hepatic damage. NAFLD is a metabolic syndrome linked to multiple risk factors such as obesity, insulin resistance, high blood sugar, high triglyceride in the blood particularly in the fatty abdomen, polycystic ovary syndrome, hypothyroidism, and hypopituitarism (Marchesini et al., 2001). It leads to accumulation of triglycerides in the liver (hepatic steatosis), inflammation and later fibrosis (nonalcoholic steato hepatitis, NASH) (Day and James, 1998; Tarantino et al., 2010). Chronic damage to the liver results in scarring (cirrhosis), which can lead to liver failure, a life-threatening condition (Costa et al., 2004). The current pharmaceutical market finds few drugs available for liver problems. The field of hepatology demands novel therapeutic agents to treat hepatic disorders (Guntupalli et al., 2006).

The plant commonly known as the flame flower, *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. (*Talinaceae*) is widely grown in tropical regions as a leafy vegetable. The family is cosmopolitan with 19 genera (Heywood, 1978), and 500 species across the world (Anonymous, 1974). *T. portulacifolium* grows in deciduous forests of Tirumala region and coastal regions of Andhra Pradesh, India. The tribal people of the Rayalaseema region in Andhra Pradesh, use the leaves of the plant to keep away from diabetes, inflammatory skin problems, gastrointestinal disturbance, arthritis, ulcer, and fevers (Seetharami Reddy et al., 2004). Review of literature indicates reports on antidiabetic, antioxidant (Ramesh et al., 2009; Nageswara Rao et al., 2008), antidepressant (Babu Rao et al., 2015), antibacterial, antifungal (Godwin et al., 2014), antiulcerogenic (Gundamaraju et al., 2014) and analgesic (Satyanarayana et al., 2012) properties of the plant species. The phytochemicals isolated and characterized from leaf methanolic extract were luteolin and kaempferol (Sunil et al., 2010). The leaf extracts of the plant were subjected to gas chromatogram-mass spectrum (GC-MS) analysis, followed by *in vivo* antiasthmatic study by anticholinergic and antihistaminic models (Vani et al., 2017). The leaf and stem methanolic extracts of the plant were subjected to standardization studies for the flavonoid quercetin by HPLC (Adithya et al., 2012).

The literature review reports suggest that no scientific reports were available on the hepatoprotective activity of the plant species. Therefore, the present study was aimed at exploring the phytochemicals responsible for hepatoprotective activity of the plant extracts.

MATERIAL AND METHODS

Chemicals and reagents

Carnoy, azocarmine aniline blue and silymarin were purchased from Sigma-Aldrich Chemical Co. (Bangalore, India), biochemical kits were purchased from Span diagnostic limited, Surat, India. All other chemicals used were of analytical grade and commercially available.

Collection of plant material

The plant (Fig. 1) material was collected from local grounds of Prasadampadu and Enikepadu co-ordinates 16°32′45″N 80°34′12″E of Vijayawada rural region, Krishna district, Andhra Pradesh, India. Dr. P. Satya Narayana Raju, Plant Taxonomist, Dept. of Botany & Microbiology, Acharya Nagarjuna University (ANU), Guntur (Dt.), Andhra Pradesh, India, identified and authenticated the plant specimen. A voucher specimen 004/VIPW was deposited for future reference in the Department of Pharmacognosy, Vijaya Institute of Pharmaceutical Sciences for Women, Vijayawada.

Figure 1. *Talinum portulacifolium* plant.

Preparation of extracts

The leaves of the plant were dried, powdered coarsely, to 10 kg using a mechanical grinder (Kanchan Trendy, Thane, Maharashtra, India). Then, 2 kg of powder were extracted with 50:50 methanol, water (2 L) and acetone (2 L) alone separately using Soxhlet apparatus (Jain Scientific glass works, Haryana, India) at 50 °C for 48 hrs. The extracts obtained were dried under vacuum (rotary evaporator, Buchi R-100, Harrisons Pharma, New Delhi, India), and preserved in refrigerator (Godrej Champion, Mumbai, India) for future use.

Isolation of phytoconstituents

The collected chloroform fraction of *Talinum portulacifolium* acetone extract (TPAE) on GC-MS analysis showed the presence of ten types of alkaloids depending up on basic nucleus present. i.e., piperidine, quinazoline, imine, isatin, oxazole, pyrrole, diazo, hydrazine, indole, and isoquinoline groups (Vani et al., 2017). The present study was planned to isolate the piperidine group alkaloid, i.e., 2-piperidone. The chloroform fraction (30 mL) was acidified with 2 M hydrochloric acid in methanol and subjected to column chromatography (silica gel 60 for column, 60-120 mesh, Merck, India) in chloroform. The eluate was made alkaline with 4 M sodium hydroxide at pH 13 (Manske, 1965). It was further, extracted with ether and pentane in 1:2 ratios. Brown liquid was found floating over organic phase and separated using separating funnel. The organic phase was concentrated and removed under reduced pressure. The residues were kept in the refrigerator for future study (Viro, 1984). The obtained product was determined by physicochemical studies and structural characterization by UV (UV 3200, Lab India Ltd, Hyderabad, India), IR (FT-IR, Bruker IFS-28, Ettlingen, Germany), Mass (FTMS Bruker Daltonics-apex-Qe-Qh-FTMS, Chennai, India), and NMR (Pulsar NMR spectrometer, Oxford 60 and 90 MHz, Abingdon, Oxford, UK) spectroscopy (Figs. 2-7).

Characterization of phytoconstituents

The UV absorbance of 0.01% w/v solution of the drug was determined between 200-400 nm. State: Brown colored liquid, UV absorbance 322 nm (UV-visible double beam spectrophotometer U-2900/U-2910, Lab India). IR, V_{max} cm^{-1} 3311.43 - (N-H str); 2948.65, 2872.75 - (C-H str); 1621.67 - (C=O str); 1494.38, 1409.04 (C-N str) (Fig. 2). MS APCI-MS (m/z; %): 199.1 ($2M+H^+$; 15) 100.2 ($M+H^+$; 100) (Bruker optics-IFS 66 *vs.* vacuum FT-IR) (Fig. 3). ^{1}H NMR, (in ppm); δ=4.79 (S, 1H, NH); δ=3.27 (t, J=5.7Hz, 2H, H-3); δ=2.30 (t, J=6.4 Hz, 2H, H-6); δ=1.76 (m, 4H, H-4 & *H-5)* (Fig 4).^{13}CNMR ($CDCl_3$) (in ppm); δ=173 (C=O); δ=41.61 (C-3); δ=30.72 (C-6); δ=21.76 (C-4); δ=20.40 (C-5) (Fig. 5). DEPT *^{13}CNMR* (in ppm); δ=41.61, 30.72, 21.76, 20.40 (C-3, C-6, C-4 and C-5) (Fig. 6) (Oxford 60 and 90 MH_z NMR spectrometer, deuterated chloroform ($CDCl_3$) as solvent.

Experimental animals

Wistar rats (100–150 g) of either sex were purchased from Mahaveer enterprises, Hyderabad, Telangana, India, housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (12 h light/dark cycles). They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur district, Andhra Pradesh, India, approval No. 012/IAEC/NCPA/PhD/2016-17, nominated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Department of Animal Husbandry, Ministry of Environment and Forests, Government of India.

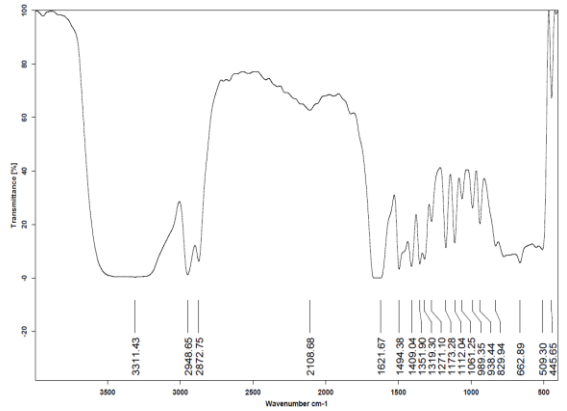

Figure 2. IR spectrum of 2-piperidone.

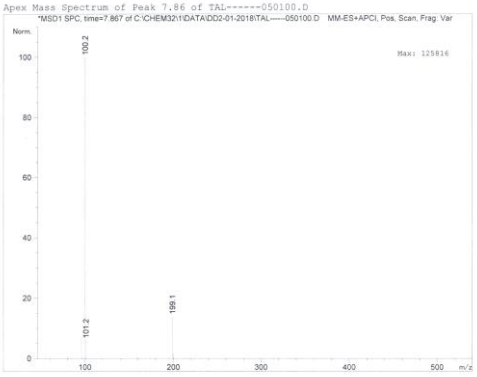

Figure 3. Mass spectrum of 2-piperidone.

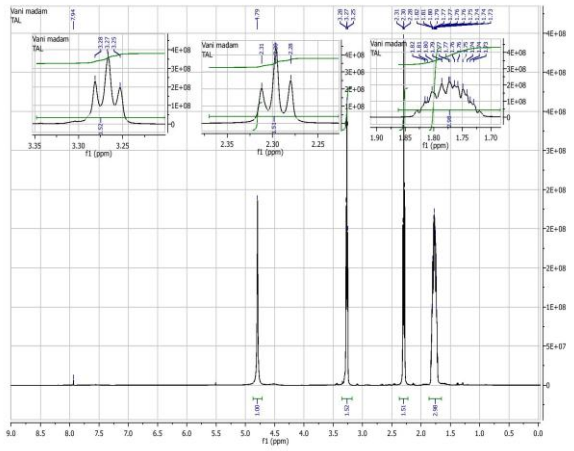

Figure 4. [1]H NMR spectrum of 2-piperidone.

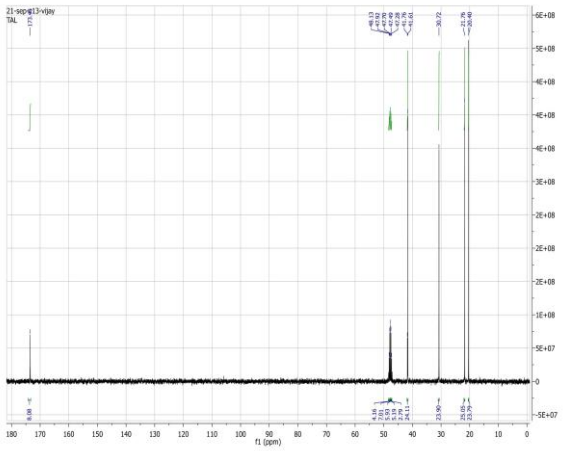

Figure 5. [13] CNMR spectrum of 2-piperidone.

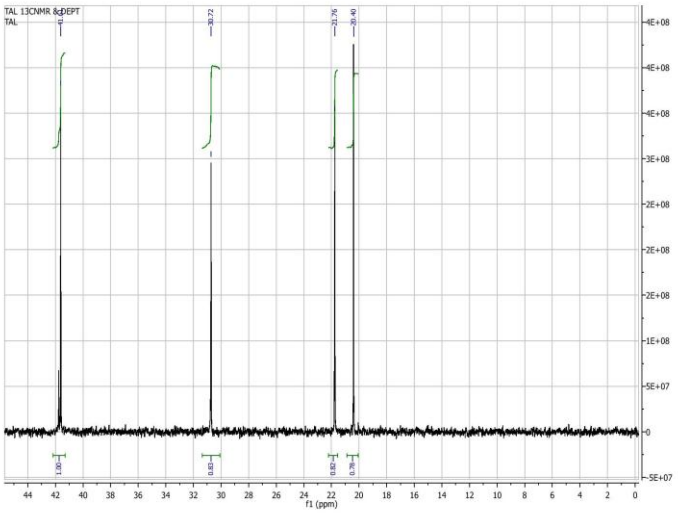

Figure 6. DEPT *[13]C NMR* spectrum of 2-piperidone.

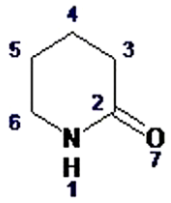

Figure 7. Structure of piperidine-2-one or 2-piperidone.

Acute toxicity testing

The animals were overnight fasted prior to the experiment. Different doses (50–3000 mg/kg, orally) of the hydroalcoholic and acetone extracts of the plant and 2-piperidone were administered to groups of rats. The animals were observed continuously for 1 h, next half-hourly intervals for 4 h for any gross changes in their behavior and then up to 24 h for any mortality as per the Organization for Economic Cooperation and Development (OECD) guidelines 425 (Vani et al., 2017; OECD 2008).

Hepatoprotective activity by carbon tetrachloride induced liver toxicity in rats on biochemical parameters ALT, AST and ALP

Wistar rats (100–150 g), fifty-four in number were equally divided into nine groups containing six animals in each to assess the hepatoprotective potential of plant extracts and isolated phytoconstituents. The animals from Group I served as control, received the vehicle olive oil *p.o.* at a dose of 1 mL/kg body weight (b.w.). Group II served as positive control, received the vehicle carbon tetrachloride (CCl_4) in olive oil (1:1) 2 mL/kg orally twice a week. Group III received standard drug silymarin at a dose of (17.5 mg/kg) *p.o* twice a day. Test groups IV and V received *Talinum portulacifolium* hydroalcoholic extract (TPHA) at doses of 250 and 500 mg/kg. Test groups VI and VII received acetone extract (TPAE) at doses of 250 and 500 mg/kg). Test groups VIII and IX received isolated compound 2-piperidone (TPC) at doses of 0.25 and 0.5 mg/kg by gavage twice daily. The study duration was 28 days for all the treated groups. All the anesthetized animals were exsanguinated at the end (28th day) of the experiment. On day 28, the blood samples were collected separately through the caval vein, by carotid bleeding into a sterilized dry centrifuge tube and allowed to coagulate at 37°C for 30 min. The clear serum was separated and investigated for biochemical marker enzyme levels such as alanine aminotransferase (ALT/SGPT) or serum glutamic pyruvic transaminase, aspartate aminotransferase (AST/SGOT) or serum glutamic oxaloacetic transaminase and alkaline phosphatase (ALP) to assess the liver function (Table 1).

Histopathological studies

Livers were carefully collected, examined, rinsed with a solution of 10% NaCl, weighed and preserved in 10% formalin. Liver pieces (3-5) weighing about 1 g were fixed in formalin and carnoy solution. They were embedded, cut, stained with azocarmine aniline blue (AZAN), and observed under trinocular microscope (ESAW TRINO 5MP, Ambala Cantt, Haryana, India) for histopathological study (400x magnification, 5.0 mp Cmos camera) (Bickel et al., 1990; Khan et al., 2012) (Fig. 8A-I).

Statistical analysis

The results were expressed as mean ± SEM (standard error mean) of 6 animals from each group. The data was evaluated by One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Differences among groups were found significant at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, respectively (Table 1).

RESULTS

Isolation of phytoconstituents

The chloroform fraction yielded (0.42%) an oily brown compound characterized as 2-piperidone.

Spectral data of 2-piperidone

The obtained compound was brown liquid with characteristic little pungent smell, bp 253°C, soluble in water, acetone and dichloromethane. It showed UV absorbance was found to be at λ max 322 nm. The IR spectrum showed characteristic band at 3311.43 cm^{-1} (N-H stretching), which indicates the presence of amine moiety. The other characteristic band at 1621.67 cm^{-1} indicates the presence of carbonyl (C=O, stretching) group. The bands at 1494.38 and 1409.04 correspond to C-N stretching. The bands at 2948.65 and 2872.65 cm^{-1} correspond to C-H stretching (Fig. 2). The mass spectrum showed molecular ion peak at m/z 199.1, which corresponds to the molecular weight and molecular formula (C_5H_9NO) of 2-piperidone (Fig. 3). ^1H NMR, (in ppm); δ=4.79 (S, 1H, NH); δ=3.27 (t, J=5.7 Hz, 2H, H-3); δ=2.30 (t, J=6.4 Hz,

2H, H-6); δ=1.76 (m, 4H, H-4 & H-5) (Fig 4). In proton NMR spectrum (Fig. 4), the compound showed a singlet at δ= 4.79 ppm (S, 1H, NH), indicates an amine group proton. The presence of triplet at δ =3.27 ppm, (t, J=5.7Hz, 2H, H-3); indicates two protons of C-3. The presence of triplet at δ =2.30 ppm (t, J=6.4 Hz, 2H, H-6) indicates two protons of C-6. A multiplet at δ= 1.76 ppm (m, 4H, H-4 & *H-5)* indicates four methylene protons of C-4 and C-5. The ^{13}CNMR spectrum showed carbonyl carbon at δ= 173 ppm, at δ= 41.61, 30.72, 21.76 and 20.40 ppm showed methylene carbons of C-2, C-5, C-3, and C-4 (Fig. 5). DEPT^{13} CNMR (Distortion less enhancement by polarization transfer) spectrum showed four methylene groups at δ= 41.61, 30.72, 21.76, 20.40 ppm for C-3, C-6, C-4 and C-5 respectively (Fig. 6). The compound passes test with Dragendorff's reagent, tests for carbonyl and amide functional groups. The results of spectroscopy agree with data reported for 2-piperidone (Shanmugam et al., 2005), hence the obtained compound was confirmed to be 2-piperidone (Fig. 7).

Acute toxicity studies

Acute toxicity studies revealed that both plant extracts and TPC (2-piperidone) did not produce any toxic symptoms when administered orally to rats at doses of 100-3000 mg/kg. The experiment recorded no toxic symptoms and death of the animals during the study.

Effect of hepatoprotective activity by carbon tetrachloride-induced liver toxicity in rats on biochemical markers ALT, AST and ALP

The animal group treated with CCl_4 in olive oil (1:1) showed significant increase in the levels of marker enzymes ALT 279, AST 275, ALP 315 U/L due to CCl_4 induced hepatotoxicity.

TPHA treated group decreased the enzyme levels ALT 198 ($p<0.05$), AST 182 ($p<0.01$), ALP 260 U/L at 250 mg/kg and ALT 149 ($p<0.001$), AST 136 ($p<0.001$) and ALP 216 ($p<0.01$)U/L at 500 mg/kg.

TPAE treated group reduced the enzyme levels ALT 173 ($p<0.01$), AST 167 ($p<0.01$), and ALP 232 ($p<0.0$) U/L at 250 mg/kg and ALT 145 ($p<0.001$), AST 122 ($p<0.001$), ALP 202 ($p<0.001$) U/L at 500 mg/kg.

2-piperidone (TPC) treated group displayed reduction in enzyme levels ALT 186 ($p<0.01$), AST 167 ($p<0.01$), ALP 280 ($p<0.01$) U/L at 0.25 mg/kg and ALT 138 ($p<0.001$), AST 111 ($p<0.001$), ALP 210 ($p<0.001$)U/L at 0.5 mg/kg.

Standard drug silymarin at 17.5 mg/kg exhibited ALT 131($p<0.001$), AST 106 ($p<0.001$) and ALP 207 ($p<0.001$) U/L reduction.

Plant extracts and standard drug silymarin demonstrated ability to counteract the CCl_4 induced hepatotoxicity by decreasing the raised marker enzyme levels ALT, AST and ALP at $p<0.05$, $p<0.01$ and $p<0.001$ levels of significance compared to CCl_4 group.

TPC treated group at 0.5 mg/kg showed better reduction in the raised levels of (ALT 138 and AST 111 U/L) compared to CCl_4 (ALT 279 and AST 275 U/L) treated group.

TPAE treated group at 500 mg/kg prominently reduced the raised levels of (ALP 202 U/L) than TPC 0.5 mg/kg (ALP 210 U/L) when compared to CCl_4 treated group (ALP 315 U/L).

TPAE treated group at 500 mg/kg b.w. (ALT 145, AST 122 and ALP 202 U/L) and TPC treated group at 0.5 mg/kg b.w. (ALT 138, AST 111, ALP 210 U/L) have exhibited better reduction in the raised levels of enzymes than other groups when compared to CCl_4 treated group ($p<0.001$) (Table 1).

Effect of histopathological studies for hepatoprotective activity by carbon tetrachloride-induced liver toxicity

Observation of the changes of liver histopathological study from the normal control group showed normal hepatic cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Fig. 8A).

Table 1. Hepatoprotective effects of *T. portulacifolium* extracts and 2-piperidone for serum marker enzymes in CCl4-induced toxicity.

Group	Treatment	Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)
1	Control/normal	1 mL/kg	127.7 ± 0.57	102.1 ± 0.4	199.2 ± 1.2
2	CCl_4 in olive oil (1:1)	2 mL/kg	279.2 ± 0.90	275.2 ± 0.7	315.1 ± 2.1
3	Silymarin	17.50	131.0 ± 0.80***	106.0 ± 0.6***	207.0 ± 0.5***
4	TPHA	250	198.6 ± 0.70*	182.7 ± 0.6**	260.0 ± 1.6
5		500	149.2 ± 0.48***	136.1 ± 1.2***	216.0 ± 1.9**
6	TPAE	250	173.0 ± 0.90**	167.0 ± 1.7**	232.0 ± 1.6**
7		500	145.0 ± 0.40***	122.0 ± 0.8***	202.0 ± 1.9***
8	TPC	0.25	186.0 ± 1.18**	167.0 ± 2.3**	280.0 ± 2.8***
9		0.50	138.0 ± 0.95***	111.0 ± 1.2***	210.0 ± 1.1***

Values are Mean ± S.E.M. (n= 6 rats per each group). *p <0.05, **p<0.01, ***p<0.001 significantly different from the group treated with $CC1_4$, One-way ANOVA, Dunnett's multiple comparison test. TPHA: *T. portulacifolium* hydroalcoholic extract; TPAE: *T. portulacifolium* acetone extract; TPC: 2-piperidone; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; ALP: Alkaline phosphatase. Control group was administered with olive oil.

The CCl_4 intoxicated treatment group showed severe hepatocellular degeneration such as necrosis and fatty changes (Fig. 8B). Prominent damage to central lobular region appeared in the liver. The histopathological studies of liver sections in the standard group have shown normal cellular architecture, cytoplasm and visible central veins (Fig. 8C). TPHA treated group at 250 and 500 mg/kg has shown moderate recovery and protection of hepatocytes degradation (Fig. D-E). TPAE treated group at 250 mg/kg has shown moderate recovery (Fig. 8F), but at the dose of 500 mg/kg offered normal hepatocytes recovery and protection of hepatocytes degradation (Fig. 8G). TPC treated group at 0.25 mg/kg and 0.5 mg/kg restored the structural damages (Fig. 8H-I) induced by CCl_4. Treatment with TPHA, TPAE and TPC decreased the abnormal liver architecture induced by CCl_4 (Fig. 8D-I) and restored the altered histopathological changes. TPAE treated group at 500 mg/kg (Fig. 8G) and TPC treated group at 0.5 mg/kg (Fig. 8I) exhibited better regeneration of hepatocytes when compared to control. TPC treated group showed better recovery of hepatocytes than plant extracts compared to control. The results show that plant extracts exhibited hepatoprotective activity, which may due to the presence of 2-piperidone, which has been isolated from this plant species *T. portulacifolium*. Therefore, both plant extracts and 2-piperidone could be used to treat hepatic ailments.

DISCUSSION

CCl_4 used in the study as a toxicant to liver cells causes damage by peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. Cytochrome P450 2E1 (CYP2E1) is a key enzyme in the metabolic activation of many low molecular weight toxicants, an important contributor to oxidative stress. The changes associated with CCl_4 are biotransformed by the cytochrome P-450 system to produce CCl_3 a free radical, that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum and finally result in cell death (Okuno et al., 1986; Recknagel et al., 1989). Cytosol releases a variety of enzymes release into the bloodstream in liver cell plasma membrane damage.

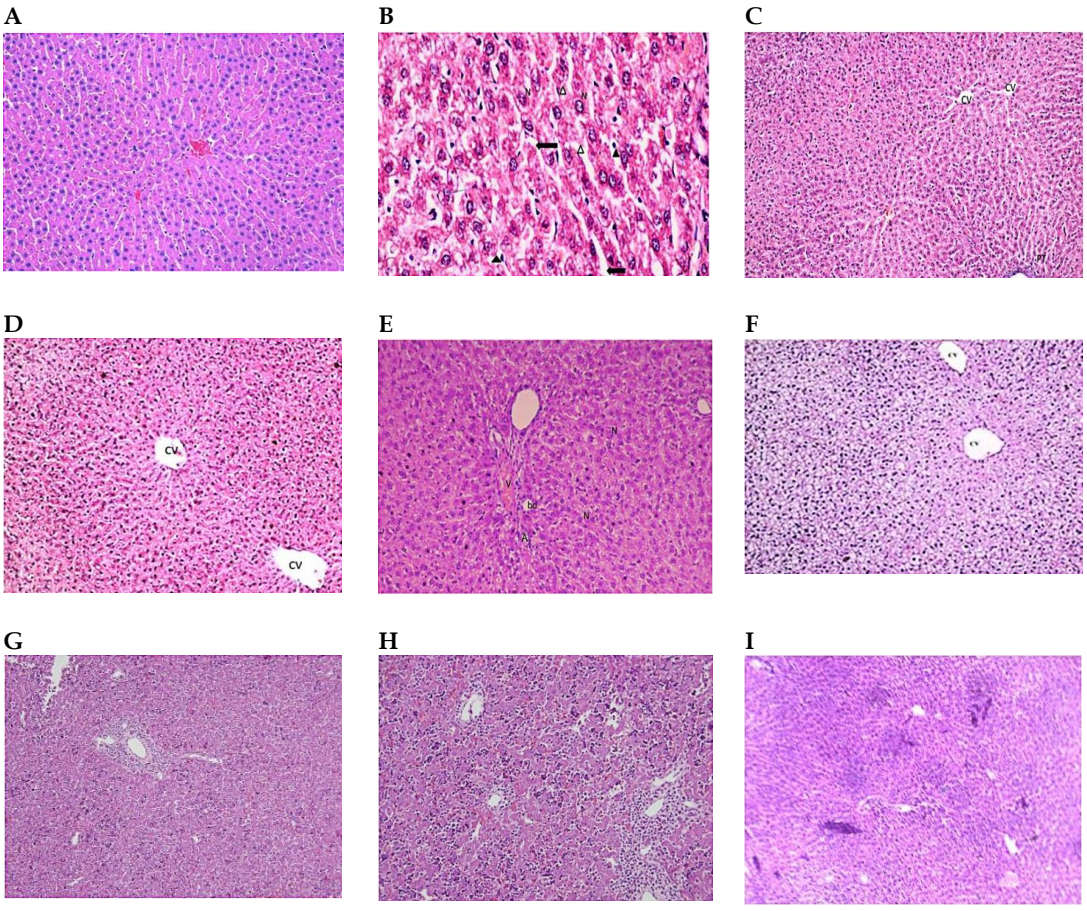

Figure 8. Histopathological section of the liver tissue of **(A)** control/normal rat showing normal architecture of hepatocytes at portal triad; **(B)** CCl_4 treated rat showing severe necrosis (N), fibrosis (Arrow), vascularization (dark arrow head), and fatty degeneration (plane arrow head); **(C)** standard silymarin treated rat showing normal hepatocytes. CV indicates central vein and PT indicates portal triad; **(D)** TPHA 250 mg/kg treated rat showing regeneration of hepatocytes with moderate sized nucleus and central vein (CV); **(E)** TPHA 500 mg/kg treated rat showing hepatocytes reverting to regeneration with moderate sized nucleus (N), bile duct (bd), hepatic artery (A) and hepatic vein (V); **(F)** TPAE 250 mg/kg treated rat showing hepatocytes reverting to regeneration with moderate sized nucleus, central vein (CV) and portal triad; **(G)** TPAE 500 mg/kg treated rat showing almost regeneration of normal hepatocyte architecture; **(H)** 2-piperidone (TPC 0.25 mg/kg) treated rat showing normal hepatocytes with central vein and portal triad; **(I)** 2-piperidone (TPC 0.5 mg/kg) treated rat showing complete regeneration and almost normal architecture of hepatocytes with normal sized nucleus and portal triad. Liver sections were stained with azocarmine aniline blue, viewed under 400x magnification.

The increased levels of ALT, AST and ALP show cellular leakage and loss of functional integrity of the cell membrane as a result of hepatic damage (Saraswat et al., 1993; Shuid et al., 2011). Their estimation in the serum is a useful measure for determining the hepatocellular damage (Shuid et al., 2011). The abnormally high levels of marker enzymes ALT, AST and ALP (Table 1) observed in the CCl_4 treated group are the consequences of CCl_4 induced liver dysfunction and denote the damage to the hepatic cells. In the present study, the plant extracts and 2-piperidone reduced higher levels of serum marker enzymes. TPAE at 500 mg/kg b.w. and 2-piperidone at 0.5 mg/kg b.w. have exhibited an equipotent reduction in the

higher levels of enzymes when compared to standard silymarin (Table 1).

The elevated CYP2E1 expression and activity is commonly found associated in chemical-induced toxicities and stress-related diseases. 2-piperidone acts as a potential endogenous CYP2E1 substrate, metabolic phenotype because of the structural similarity. CYP2E1 plays a catalytic role in the biotransformation of 2-piperidone to its main metabolite 6-hydroxy-2-piperidone through 2-piperidone 6-hydroxylase, excreted in urine. It was reported that cadaverine, decarboxylated product of lysine is metabolized to 2-piperidone in humans and excreted in urine. Polyphenol rich food, which contains valerolactone an analogue of 2-piperidone also contributes to increased urinary output of 2-piperidone. It was stated that content of urinary 2-piperidone was inversely correlated to CYP2E1 expression and found to be a potential endogenous biomarker for CYP2E1 activity (Jie et al., 2013). Therefore, it can be assumed that both 2-piperidone as well as TPAE could offer protection against CCl_4 induced liver injury and keep up the functional integrity of hepatic cells by interfering with CYP2E1.

A comparative histological study of liver from the study groups further corroborated the hepatoprotective effect of *T. portulacifolium* extracts and 2-piperidone. Histological profile from normal control group showed prominent central vein and normal arrangement of hepatic cells (Fig. 8A). Histopathological examination of CCl_4 treated liver section showed higher degrees of pathological changes centrilobular necrosis of hepatic cells, vacuolization and fatty regeneration (Fig. 8B). The animals treated with plant extracts (TPHA 250 and 500 mg/kg) showed moderate recovery where TPAE at 500 mg/kg and 2-piperidone at 0.5 mg/kg showed more prominent recovery of hepatic cell damage. It was clear that necrosis was absent, normal hepatic cords and lesser fatty infiltration were present (Fig. 8G-I). The observations indicate that acetone extract exhibited hepatoprotective response due to the presence of 2-piperidone. Phytochemical screening also indicated the presence of 2-piperidone from chloroform fraction of acetone extract (Vani et al., 2017). The compound 2-piperidone demonstrated more effective functional improvement of hepatocytes than plant extracts. The plant extracts justified the ethnobotanical claims (Seetharami Reddy et al., 2004). The results of the histopathological study also support the results of biochemical trials.

Moreover, the results of the current study are supported by earlier reports on the plant species. The alcoholic extracts of whole plant lowered high blood glucose (Nageswara Rao et al., 2007), plasma cholesterol, triglycerides, LDL (low-density lipoprotein), HDL (high-density lipoproteins) (Hima Bindu et al., 2014), SGOT, SGPT, glutathione, catalase, and lipid peroxidase levels (Nageswara Rao et al., 2008) The plant hydroalcoholic and acetone extracts block inflammatory mediators like histamine, bradykinin (Gundamaraju et al., 2014; Vani et al., 2017).

However, the phytochemicals isolated from the plant species luteolin, kaempferol (Sunil et al., 2010) and quercetin (Adithya et al., 2012) were studied for hepatoprotective property. Kaempferol acts by expression of CYP2E1 and enhances the protective role of anti-oxidative defense system (Wang et al., 2015). Luteolin acts by the antioxidant, anti-inflammatory and immune modulating mechanisms (Papiya and Singh, 2015). Quercetin acts by calcium channel blocking and antioxidative mechanisms (Anwar et al., 1997).

In the current study, 2-piperidone has displayed profound hepatoprotective activity than plant extracts. Comparison of results indicates that 2-piperidone exhibited higher hepatoprotective activity than luteolin, kaempferol, and quercetin. This fact can be attributed to previous reports on 2-piperidone. Using various chromatographic methods, a new piperidinone alkaloid, (3*S*)-3-{4-[(1*E*)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenoxy}piperidin-2-one from the roots of *Heracleum dissectum* screened for anti-inflammatory activity *in vitro* showed significant inhibitory activity on nitric oxide production in RAW 264.7 cells (Hai et al., 2017). Moreover, 2-piperidone as a biomarker was used to monitor cytochrome 2E1 activity (Jie et al., 2013). In the current study 2-piperidone alone showed equipo-

tent results to silymarin for the first time against CCl_4 induced liver toxicity. The results obtained in the current study are concurrent with literature reports on hepatoprotective activity studies (Tao et al., 2008; Sanjay et al., 2009). Therefore, from the current research findings and in correlation to the available literature reports, the possible mechanism of action of *T. portulacifolium* acetone extracts and 2-piperidone could be by inhibiting CYP2E1 activation and ROS (reactive oxygen species) production. The study indicates that hepatoprotective activity of plant acetone extracts might be due to the presence of 2-piperidone. Therefore, *T. portulacifolium leaf* acetone extracts and 2-piperidone can be used to treat hepatic disorders as they controlled the liver enzymes, which was established through histological findings.

CONCLUSIONS

Since, the results of the study showed a significant decrease in the raised levels of serum enzymes and regeneration of hepatocytes, equipotent to standard drug silymarin against CCl_4 induced hepatotoxicity, 2-piperidone could be explored towards further studies on mechanism of action, formulation, and clinical trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Mamillapalli V	Shaik AR	Avula PR
Concepts or ideas	x	x	
Design	x		
Definition of intellectual content	x		
Literature search	x		
Experimental studies	x	x	x
Data acquisition	x		
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x		
Manuscript editing	x	x	x
Manuscript review	x	x	x

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