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Hepatoprotective activity of a benzopyrone from *Tephrosia purpurea* Pers.

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Abstract

Objective: To study hepatoprotective activity of a new benzopyrone derivative (TP) isolated from the alcoholic extract of aerial parts of *Tephrosia purpurea* Pers. Materials and methods: Compound TP was isolated from alcoholic extract of *T. purpurea* by normal phase column chromatography using toluene: ethyl acetate (70:30) as mobile phase and structure was elucidated by spectroscopic methods. Hepatoprotective activity of TP and the alcoholic extract was evaluated using carbon tetrachloride, paracetamol and rifampicin as toxicants. The potency of TP (100 mg/kg) and the alcoholic extract of aerial parts (200 mg/kg), was compared with silymarin (100 mg/kg). The SGOT, SGPT, total and direct bilirubin levels of blood were measured spectrophotometrically. Results: Compound TP was found to be 3-hydroxy, 6-methoxy, 2-oxy (3-butanone), 7 (dioxolane-4-one), 2, 3,-dihydrobenzopyrone. The alcoholic extract and TP caused significant fall in the enzyme levels in serum in rats. Conclusion: Alcoholic extract of *T. purpurea* and TP have shown significant hepatoprotective activity in rats against all the toxicants that were studied.

Key words: *Tephrosia purpurea*, Hepatoprotective effect, Carbon tetrachloride, Paracetamol Rifampicin, Silymarin.

1. Introduction

Tephrosia purpurea Pers. (Papilionaceae) is known as Sarphonka in Hindi. The drug possesses laxative, diuretic and it is traditionally used in jaundice [1]. Total aqueous extract [2] and the powder of the aerial parts [3] reported for its hepatoprotective activity. Ethanolic extract of the aerial parts was reported to be having anti-inflammatory [4,5], antioxidant [6] and hypoglycemic [7,8] activities. A new dihydro benzopyrone, TP, was isolated from the alcoholic extract of aerial parts of *T. purpurea.* Since the alcoholic extract was reported to be possessing various pharmacological actions including hepatoprotective activity also the compound TP was present in sufficient quantity (1.33% with respect to dried extract) and hence TP and the alcoholic extract were selected to study the hepatoprotective activity using carbon tetrachloride, paracetamol and rifampicin as toxicants.

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2. Materials and methods

2.1 Plant material

Aerial parts of *T. purpurea* were collected from the Botanical garden, Sardar Patel University, Vallabh Vidyanagar, in the month of March. The plant was authenticated and a voucher specimen was submitted to the Department of Pharmacognosy, A. R. College and G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar.

2.2 Isolation and characterization of TP

The plant material was shade dried and ground thoroughly in a mill to obtain coarse powder. The powdered material (3.5kg) was defatted with petroleum ether (40-60°C) followed by extraction with ethanol [95%] by cold maceration. The solvent was evaporated under reduced pressure. A portion of alcoholic extract was chromatographed in normal phase using a 125-cm column (ID 3.5cm), Silica gel (BDH) and toluene: ethyl acetate (70:30) as mobile phase and the elution was maintained at 35-38 drops/min and 70 fractions of 20 ml each were collected.

All the fractions were tested by TLC and those fractions which gave single blue spot at Rf 0.35, when observed under UV lamp at 366 nm on precoated TLC plates Silica gel 60 F_{254} (E.Merck) and toluene: ethyl acetate (70:30) mobile phase, were mixed together and filtered. The filtrate was evaporated under reduced pressure. The residue was recrystallized in methanol. The yield of crystals of TP was 1.33% with respect to dried extract.

Elemental analysis for TP was carried out using CHNS/O analyzer, (2400 Series, Perkin Elmer USA) and spectral analysis was done by UV (UV/Vis double beam spectrophotometer, Lambda 19 of Perkin Elmer, USA), FTIR, (Perkin Elmer, 16PC), ^{1}H-NMR (Brucker, DPX200, Version 3), and EIMS (Shimadzu, QP 508) at Sun Pharm Advanced Research Center, Vadodara.

2.3 Preparation of test solutions

TP and alcoholic extract were formulated as suspension in 4% gum acacia mucilage. Since, gum acacia has negligible effect on blood serum parameters. The strength of the suspension was according to dose administrated and expressed as weight on dried basis.

2.4 Preparation of Standard drug

Silymarin [9] was used as standard drug for evaluating hepatoprotective activity. Gift sample of silymarin was obtained from M/s Ranbaxy Laboratories, Dewas, and made into suspension using 4% gum acacia as suspending agent. The strength of suspension was adjusted to 50 mg/ml of silymarin. 4% w/v aqueous acacia mucilage was used as vehicle for all models.

2.5 Animals

Wistar albino rats of either sex (180-200 gm) were used for the study. The animals were housed and acclimatized under standard laboratory conditions and were supplied with standard laboratory feed and water *ad lib.* The animals were segregated into five groups of six animals each. In the entire three experimental models group 1 and 2 served as control and toxicant respectively while group 3, 4 and 5 received standard, alcoholic extract and TP respectively. The institutional animal ethical committee approved the experimental protocols.

2.6 Hepatoprotective activity

2.6.1 Carbon tetrachloride induced liver damage [10]

Carbon tetrachloride (1.25ml/kg, P.O.) was administered to the animals of group 2, 3, 4 and 5. This was followed by administration of vehicle, silymarin (100mg/kg, P.O.), alcoholic extract (200 mg/kg, P.O.), and TP (100mg/kg, P.O.) respectively at 12 h intervals for the period

of 36 h. Control animals (group 1) received 1ml/kg, P.O. of vehicle. After 48 h blood was collected from all the groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min. at room temperature. Serum was separated by centrifugation at 2500 rpm at 37°C for 10 min and analyzed for various biochemical parameters.

2.6.2 Paracetamol induced liver damage [11]

Suspension of silymarin (100mg/kg), alcoholic extract (200 mg/kg), and TP (100 mg/kg) were administered by gavage once daily for three days to group 3, 4 and 5. On the third day of treatment paracetamol (3 g/kg, P.O.) [9], was administered 30 minutes after administration of test suspension to group 2, 3, 4 and 5. Control animals received 1ml/kg, P.O. of vehicle. After 48 h of paracetamol administration, blood was collected from all the groups and biochemical parameters were analyzed as in CCl_4 induced liver damage.

2.6.3 Rifampicin induced liver damage [12]

Suspension of alcoholic extract (200 mg/kg, P.O.), TP (100mg/kg, P.O.) and silymarin (100mg/kg, P.O.) were administered four times at 12 h intervals for a period of 36 h to group 3, 4, and 5. Rifampicin (1g/kg, P.O.) [11] was administered 30 min after first dose of the suspension to group 2, 3, 4 and 5. Control animals received 1g/kg, P.O. of vehicle. After 48 h of rifampicin administration, blood was collected from all the groups and biochemical parameters were analyzed as in CCl_4 induced liver damage.

2.6.4 Assessment of liver functions

Biochemical parameters, i.e. serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) [13] were analyzed using SGOT and SGPT kits manufactured by Span diagnostics Pvt. Ltd., Surat, whereas total bilirubin (T Bil) and direct bilirubin (D Bil) were analyzed according to reported methods [14]. The mean value ± SEM was calculated for each parameter. Percent reduction against hepatotoxin by test sample was calculated by considering the enzyme level difference between the hepatotoxin treated and the control groups as 100% level of reduction and expressed by the formula:

$$H = \{1- (T_c – V / V_v – V)\} \times 100,$$

Where H is percentage hepatoprotective activity, T_c, V_c and V are the parameters measured in test drug + toxicant, vehicle + toxicant and vehicle treated animals respectively.

2.6.5 Statistical analysis

For determinations of significant inter group differences each parameter was analyzed separately and one-way analysis of variance was carried out. Dunnet's test was used for individual comparisons [15,16].

3. Results

Isolated crystals gave compound TP: yellowish brown crystals, yield; 1.33%, m.p.; 182-183°C, Elemental analysis, calculated for $C_{18}H_{20}O_8$,: Calcd. C, 59.34; H, 5.53; O, 35.13. Found C, 58.75; H, 5.65, O, 34.60. UV (MeOH): λ_{max}= 241nm ; FTIR (KBr): v_{max}, cm^{-1} = 3406 (-OH), 2928 (-CH_2), 1800-200 (overtone, Ar), 1723 (C=O), 1123 (C-O-C), 1274, 1075 (OCH_3); ^{1}H-NMR, (400 MHz, $CDCl_3$): δ_{ppm}= 0.9 (*q*, 3H, -CH_3), 1.2 (*s*, 2H, -C=CH_2), 2.5(*s*, 2H, -CH_2-C= C-), 3.3 (*s*, 2H, -O- CH_2-), 3.8 (*s*, 3H, Ar-OCH_3) 8.05 (*s*, 2H, Ar-H); EIMS: *m/z* (rel. int) = 365 (M^+, 2.27), 279 (18.83), 167 (44.62), 149 (100), 113 (16.35), 89 (11.11), 71 (29.71), 57 (41.46), 41 (39.88).

Table 1, 2 and 3 shows that activities of serum GPT, GOT, T Bil. and D. Bil were markedly elevated in CCl_4, paracetamol and rifampicin treated animals respectively compared to normal control rats, indicating the liver damage. Administration of alcoholic extract (200mg/kg, P.O.), TP (100 mg/kg, P.O.)

Table 1
Effect of TP and ethanolic extract of *T. purpurea* on CCl_4 induced hepatotoxicity in rats.

Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	T. Bil (mg/dl)	D. Bil (mg/dl)
1.	Control	100 ±2.4	10 ±0.4	0.98 ±0.04	0.22 ±0.01
2.	CCl_4	180 ±4.8[a]	120 ±0.61[a]	2.18 ±0.06[a]	1.55 ±0.05[a]
3.	Silymarin	70 ±2.5* (137.5)	10 ±1.6* (100)	1.0 ±0.08* (98.3)	0.21 ±0.02* (100.7)
4.	Eth. Ext	154 ±2.4*# (32.5)	59 ±1.5*# (55.4)	1.74 ±0.03*# (36.66)	0.91 ±0.07*# (48.12)
5.	TP	98 ±3.0*# (102.5)	51 ±2.2*# (62.72)	1.43 ±0.05*# (62.5)	0.82 ±0.08*# (54.88)

Values are expressed as mean ±S.E (n=6) and percentage hepatoprotective activity is expressed is expressed in brackets. [a]p< 0.001 significantly different from control; *p< 0.001 significantly different from CCl_4 ; #p<0.001 significantly different from Silymarin. One way ANOVA coupled with Dunnet's test: p value < 0.05 was taken as significant. SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate Transaminase; T. Bil: Total Bilirubin; D. Bil: direct bilirubin.

Table 2
Effect of TP and ethanolic extract of *T. purpurea* on paracetamol induced hepatotoxicity in rats.

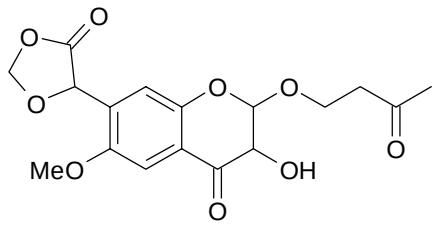
Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	T. Bil (mg/dl)	D. Bil (mg/dl)
1.	Control	122 ± 2.6	44 ± 0.8	1.10 ± 0.04	0.29 ± 0.01
2.	Paracetamol	284 ± 5.6[a]	198 ± 7.6[a]	2.64 ± 0.08[a]	0.98 ± 0.02[a]
3.	Silymarin	116 ± 3.5* (103.7)	48 ± 2.1* (97.5)	1.08 ± 0.01* (101.3)	0.42 ± 0.03* (81.2)
4.	Eth. Ext	214 ± 3.6*# (43.2)	115 ± 2.1*# (53.9)	2.02 ± 0.05*# (40.25)	0.78 ± 0.01*# (28.98)
5.	TP	120 ± 3.0* (101.2)	50 ± 2.4* (96.10)	1.6 ± 0.07*# (67.5)	0.46 ± 0.04*# (72.13)

Values are expressed as mean ± S.E (n=6) and percentage hepatoprotective activity is expressed is expressed in brackets. [a] p< 0.001 significantly different from control; *p< 0.001 significantly different from paracetamol ; # p<0.001 significantly different from Silymarin. One way ANOVA coupled with Dunnet's test: p value < 0.05 was taken as significant. SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate Transaminase; T. Bil: Total Bilirubin; D. Bil: direct bilirubin.

prevented CCl_4, paracetamol and rifampicin induced elevation of the serum parameters. These results were comparable with silymarin. The alcoholic extract of the plant was not as significant as TP in providing protection against toxicant induced liver damage.

4. Discussion

Structure elucidation of TP suggest the structure to be 3-hydroxy, 6-methoxy, 2-oxy (3-butanone), 7(dioxolane-4-one), 2, 3,-dihydrobenzopyrone. And was found to be present in sufficient quantity for easy isolation.



TP

Table 3
Effect of TP and ethanolic extract of *T. purpurea* on rifampicin induced hepatotoxicity in rats.

Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	T. Bil (mg/dl)	D. Bil (mg/dl)
1.	Control	122 ± 2.6	44 ± 0.8	1.10 ± 0.04	0.29 ± 0.01
2.	Rifampicin	304 ± 5.3[a]	162 ± 6.5[a]	3.26 ± 0.08[a]	1.45 ± 0.06[a]
3.	Silymarin	158 ± 2.4* (80.2)	54 ± 1.5* (91.5)	1.29 ± 0.05* (91.2)	1.0 ± 0.03* (38.8)
4.	Eth. Ext	198 ± 2.5*# (35.60)	125 ± 3.0*# (55.4)	2.63 ± 0.07*# (29.2)	1.26 ± 0.03*# (16.4)
5.	TP	131 ± 2.6* (92)	78 ± 2.7* (81.3)	1.7 ± 0.05*# (72.2)	0.44 ± 0.08*# (87.1)

Values are expressed as mean ± S.E (n=6) and percentage hepatoprotective activity is expressed is expressed in brackets. [a] $p < 0.001$ significantly different from control; *$p < 0.001$ significantly different from rifampicin; # $p<0.001$ significantly different from Silymarin. One way ANOVA coupled with Dunnet's test: p value < 0.05 was taken as significant. SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate Transaminase; T. Bil: Total Bilirubin; D. Bil: direct bilirubin.

The carbon tetrachloride hepatotoxicity is supposed to be produced by enhanced production of active metabolites by ethanol activated mixed functional oxidase system [17].

This eventually leads to hepatocellular necrosis and is reflected by marked changes in various enzymatic and non-enzymatic parameters of carbon tetrachloride treated rats. The most serious delayed toxic effects of CCl_4 results from its hepatotoxic and nephrotoxic action [18]. Liver dysfunction begins soon after ingestion of CCl_4 and maximum malfunction occurs within 48-72 h [19]. The alcohol extract and TP were effective in reversing the effects of CCl_4 as shown by decreasing the elevated levels of the parameters.

However the injury produced by CCl_4 seems to be mediated by a reactive metabolite trichloro methyl free radical (CCl_3) formed by hemolytic cleavage of CCl_4 or by an even more reactive species trichloro methyl peroxy free radical (Cl_3COO) formed by the reaction of CCl_3 with O_2 [18]. The hepatoprotective properties of the test samples might be related to scavenging of free radicals generated by microsomal reduction of CCl_4 [20].

Paracetamol produces hepatic necrosis in high doses [21, 22] by covalent binding of its toxic metabolite N-acetyl-p-benzoquinone imine to sulphhydryl groups of proteins resulting in cell necrosis through lipid peroxidation induced by decreasing glutathione in the liver [23]. Rifampicin is largely metabolized to desacetyl rifampicin, which actively and specifically binds to RNA polymerase, it inhibits the synthesis of all forms of RNA. Thus inhibiting nucleic acid and protein synthesis it induces fatty liver and finally cirrhosis. This causes fatal liver damage and acute hepatic failure, which is accompanied by increase in the activity of some serum enzymes [24]. The alcoholic extract and TP reduced the increasing levels of serum GOT, GPT, total bilirubin and direct bilirubin. TP is proved to be more potent than the alcoholic extract.

5. Conclusion

The present study reveals that the alcoholic extract and compound TP alleviates the symptoms of the liver damage as evident by biochemical assays. *T. purpurea* is a rich source of flavanoids [25-28], which are

benzopyrone derivatives and also flavanoids have been shown to possess various biological properties related to antioxidant mechanisms [29, 30]. Its ethanolic extract has been reported to possess significant free radical scavenging activity and also inhibit *in vitro* lipid peroxidation [6], which could be the possible mechanism for hepatoprotective activity shown by the extract and TP.

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