IJPSR (2015), Vol. 6, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 June, 2014; received in revised form, 10 October, 2014; accepted, 20 September, 2014; published 01 January, 2015

STRUCTURE ELUCIDATION AND ANTIHEPATOTOXIC ACTIVITY OF *CIS* AND *TRANS* ISOMERS OF PIPERINE ISOLATED FROM DRIED FRUITS OF *PIPER LONGUM*

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Keywords:

Antihepatotoxi**c** activity, *Piper longum*, *cis*-piperine, *trans*-piperine, AST, ALT

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ABSTRACT: The two pure phytochemicals characterized as *cis*-piperine and *trans*-piperine isolated from the dried fruits of *Piper longum* were tested for antihepatotoxic activity at the dose of 50 mg/kg orally respectively, against silymarin as a standard reference. The toxicity was induced by CCl_4, and then various biological parameters such as AST, ALT, ALP, SOD, GPx, GSH, total protein, total albumin and total bilirubin were measured at 134.24 IU/L 71.96 IU/L, 375.02 IU/L, 37.02 U/g, 17.02 U/g, 29.12 U/g, 1.95 g/dl, 1.09 g/dl, 4.16 g/dl respectively. The phytochemicals *cis*-piperine and *trans*-piperine showed significant antihepatotoxic activity. Both *cis*-piperine and *trans*-piperine exhibited antihepatotoxic activity by reducing the increased levels of serum AST by 90.14%, 91.19% ALT by 82.65%, 58.12% and ALP by 97.37%, 92.23% when compared with standard drug silymarin that have decreased AST by 91.64%, ALT by 89.92%, ALP by 94.54%, respectively, and by elevation in the antioxidants SOD, GPx, and GSH) respectively, and total albumin, total protein. The overall experimental results have suggested that the both the isomers of piperine possessed significant antihepatotoxic activity as well as antioxidant effects on the experimental albino male rats of Wistar strain.

INTRODUCTION: The liver is the largest glandular organ in the body, and has more functions than any other human organ [1]. Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites. Additionally, it is also managing the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(1).251-58

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(1).251-58

Liver disease is a global problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects [3].

The fruits of *Piper longum* Linn (Piperaceae) have been used traditionally for the treatment of diseases like jaundice and allergies [4]. The dried fruit of Piper longum Linn taste is bitter, hot and pungent and a tonic to the liver [5]. *Piper longum* Linn (Piperaceae), known in India, as "Indian Long Pepper" and pippali. The word "Pepper" is derived from the Sanskrit word [6]. *P. longum* Linn is an indigenous plant of North East India [7]. The plant grows in hotter parts of the country and found wild as well as cultivated in Assam, evergreen forests of Western Ghats, southern States, along lower hills of Bengal and west coast of the nation [8]. *P. longum Linn is* a slender aromatic climber with red fruits. This fruit when dried turns grayish black with

pungent smell [9]. *P. longum* Linn isa vital component of Indian traditional medicine reported to be used as a remedy for the treatment in gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infection, chronic gut-related pain and arthritic conditions [10]. Moreover, analgesic and diuretic effects, relaxation of muscle tension, alleviation of anxiety and immunomodulatory and antitumor activity are also reported [6, 11].

The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure liver diseases, scientific evidence in terms of modern medicine is lacking in most cases. However today it is necessary to provide scientific proof as whether to justify the use of plant or its active principles [12].

Piperine was the first amide isolated from piper species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity. Piperine is a potent inhibitor of the mixed function oxygenase system and non-specific inhibition of P450 isoenzymes [11].

The literature survey has revealed that antihepatotoxic effect mediated to antioxidant effect along with nine different biological parameters (various serum marker enzyme level and antioxidant activity and other biological parameters namely AST, ALT, ALP, SOD, GPx, GSH, total protein, total albumin and total bilirubin) has not yet been measured so far with respect to these nine parameters. We have, therefore, conducted a thorough study for the first time of *cis* and *trans* isomers of piperine.

MATERIAL AND METHODS:

Collection of Plant Material and Other Organic Solvents:

The dried fruits of *Piper longum* collected from the market Kharibawli; Chandni Chawk, Delhi and authenticated at the Department of Botany, Jamia Hamdard University, New Delhi. All the organic solvents used in experiments were of analytical grade, Silica gel for column chromatography and purchased from Merck, Germany.

Preparation of extracts and different fractions:

The dried fruits (5.0 kg) of *Piper longum* were crushed to coarse powder and extracted with ethanol using cold percolation method and concentrated on water bath under reduced pressure. The ethanolic extract was dried (600 g.) and then successively fractionated into three fractions: 1. Petroleum ether (60-80^{0}C) fraction (150 g.); 2. Ethyl acetate fraction (200 gm); 3. Methanol fraction (250 g.) all the fractions were showed different TLC pattern therefore they were subjected individually for column chromatography.

Spectroscopic Analysis:

Isolated compounds were analyzed by ^{1}H-NMR, ^{13}C NMR, DEPT, COSY, FTIR, ESI-MS spectra. ESI-MS spectra were recorded on Max. 3.9e8 cps; Infrared spectra were recorded on Perkin Elmer Spectrum Version 10.03.05; ^{1}H-NMR, ^{13}C NMR, DEPT, COSY, were recorded on Bruker 400 spectrophotometer in $CdCl_3$ using TMS as an internal standard.

Isolation of Chemical Components:

Compound PL-1:

Compound **1** was eluted from methanol fraction from column chromatography at polarity of petroleum ether-chloroform (20:80) yielded 100 grams as fluorescent yellow crystals. Crystalline solid, m. p. 130^{o}C; R_f value: 0.56 ($CHCl_3$ + EtOAc = 8:2); IR ν_{max}cm^{-1}(KBr): 3067(OCH_3), 3009(C-H),1747(C=O), 1634(N-H); MS (70ev): *m/z* 285 [M]$^+$(100%), 272(10%), 251(10%), 180(5%).

Compound PL-10:

Compound **2** was eluted from ethyl acetate fraction from column chromatography at polarity of petroleum ether-chloroform (10:90) yielded 50 grams as fluorescent green crystals. Crystalline solid, m. p. 131^{o}C-135^{o}C; R_f value: 0.58 ($CHCl_3$ + EtOAc = 7:3 v/v)IR ν_{max}cm^{-1}(KBr): 3067 (OCH_3), 3009(C-H), 1700(C-O), 1628 (C=C), 1565 (C=C aromatic stretching), 1485 (CH_2bending), 1362(CH_3 bending), 1128(C-O); MS (70ev): *m/z* 285 [M]$^+$ (100%), 272(10%), 251(10%), 180(5%).

Experimental animals:

Albino male rats of Wistar strain (150 ± 10 g), 4–6-week-old, were obtained from Central Animal House of Hamdard University, New Delhi. They

were housed in polypropylene cages in groups of 5 rats per cage and kept in a room maintained at 25±2 °C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd, Pune, India) and water *ad libitum*. Approval to perform the animal experiment was obtained from Institutional Animal Ethics Committee (IAEC) registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA).

Dosing and Grouping of Animals:

Rats were equally divided into 5 groups with 5 animals in each group (**Table 1**). The time duration of experiment was 15 days. The treatment of cis and trans-piperine were administered orally for 15 days.

TABLE1: DOSING AND GROUPING OF EXPERIMENTAL ANIMALS

Group 1	Control group: Received only vehicle i.e. olive oil at the dose of 1.5 ml/kg of rat body weight orally
Group 2	Toxic group: Hepatotoxicity was induced in rats by an injection of CCl_4 at the dose of 2 ml/kg body weight, 1:1 with olive oil i.p.
Group 3	Standard group: Received silymarin (silbyon-70) at the dose of 50 mg/kg Body weight was given orally after hepatotoxicity caused by CCl_4.
Group 4	Cis-piperine group: Received cis-piperine at the oral dose of 50 mg/kg of body weight after the hepatic injury was induced.
Group 5	Trans-piperine group: Received trans-piperine at the oral dose of 50 mg/kg of body weight after the hepatic injury was induced.

Blood Collection:

Each animal was anaesthetized with diethyl ether. Heart puncture was done with 5 ml disposable syringe and 2 ml blood was drawn very gently and slowly. The blood collected was shifted immediately to clean dried centrifugation tubes, allowed to clot and serum was separated by centrifugation at 4000 rpm for 10 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis. Biochemical estimations were made the following day.

Biochemical Assessment of liver function:

Biochemical parameters like AST and ALT were determined by (Reitman and Frankel, 1957)[13]; ALP was determined by (Kind and King, 1954)[14]; SOD (Dhindsa et al, 1981)[15]; GPx (Wheeler et al, 1990)[16]; GSH (Jollow et al, 1974) [17]; total protein (Lowry et al, 1951)[18]; total albumin (Dumas, 1981)[19]; TBL (Rajendran et al, 2009)[20] were determined by reported methods.

Histopathological study of liver:

Liver sections taken immediately after dissection from the liver, and then fixed in 10% buffered formalin (Luna, 1986) [21], dehydrated in gradual ethanol (50 to 100%), cleared in xylene, and embedded in paraffin. Sections (4 to 5 µm thick) were prepared and then stained with Haemotoxylin and Eosin (H and E) dye for photomircoscopic observations like cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of Kupffer cells and lymphocytes under power 40X and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan).

Statistical Analysis:

All the values are expressed as mean ± S.E.M. The statistical comparisons were done by using analysis of variance (ANOVA) using Prism graph pad software. Values $P < 0.05$ were considered as significant.

RESULT &DISCUSSION:

Compound PL-10 was obtained as fluorescent green crystals gave as single spot on TLC and exhibited a molecular formula $C_{17}H_{19}NO_3$ as established on the basis of FAB-MS showed a molecular ion peak at *m/z* 285.34 $[M]^+$, ^{13}C-NMR and DEPT spectra. The IR spectrum showed a sharp peak at ν_{max} 1565 cm^{-1} for aromatic double bond, 1485 cm^{-1} for aromatic bending, 1128 cm^{-1} is due to C-O phenolic groups, and 1362 cm^{-1}, shows the presence of methyl group, 1700 cm^{-1} is due to aldehyde conjugation, 3009 cm^{-1} for aromatic double bond.

The ^{13}C-NMR and DEPT spectra showed seventeen carbon atoms including one carbonyl, three

quaternary, six methylene and seven methine carbons (in total $C_{17}H_{19}O$). The ^{1}H-NMR spectrum exhibited three aromatic signals at δ_H 6.69 (1H, *d*, J= 8.4 Hz, H-5'), 6.71 (1H, *d*, J= 8.4 Hz, H-6'), and 6.94 (1H, *d*, J= 1.2Hz, H-2') attributable to H-5', H-6' and H-2' positions of ring-A respectively.

A two proton singlet displayed at δ 5.91 and also gave -CH_2- carbon signal at δc 102.0 in ^{13}C-NMR and DEPT spectra which could be attributed to methylene group attached to oxygen atoms and thus was assigned at position 7', four signals of one proton each were exhibited at δ 6.45 (1H, *d*, J= 14.8 Hz, H-2, δc 121.1), 7.42 (1H, *dddd*, J= 1.6, 9.6 Hz, H-3, δc142.3), 6.8 (1H, *dd*, J= 1.6, 9.6 Hz, H-4, δc125.2) and 6.73 (1H, *dd*, J= 3.2, 16.0 Hz, H-5, δc122.1), which were attributed at positions 2, 3, 4 and 5 respectively. The other carbon signals in the aromatic region exhibited signals at δc 105.1 (C-2'), 125.2 (C-6'), 105.2 (C-5') due to methine carbon atoms of the aromatic ring, in addition carbon signals at δc138.0 (C-1'), 148.08 (C-4'), and 148.01 (C-3') were assigned to quaternary carbon atoms of the aromatic ring. ^{1}H-NMR also displayed broad signals at δ 3.45 (4H, *bs*) attributed to CH_2-2″, δ3.61 (4H, *bs*) due to CH_2-6″, δ1.56 (4H, *m*) attributable to CH_2-3″ and CH_2-5″ of the piperidine ring-B.

These assignments were also substantiated further by correlating the proton peaks in COSY experiment and have been presented in Table-2. The high coupling constant of H-5 (*dd*, J= 3.2, 16.0 Hz) indicated that this is in *trans* position to H-3 rather than *cis* as has been seen in case of PL-1. Other spectral data of PL-10 are same as in case of PL-1. Thus, on the basis of above observations the structure of PL-10 was elucidated as *trans*-5-Benzo-[1, 3]-dioxol-5-yl-1-piperidin-1-yl-penta-2,4-dien-1-one, and has been designated as *trans*-piperine.

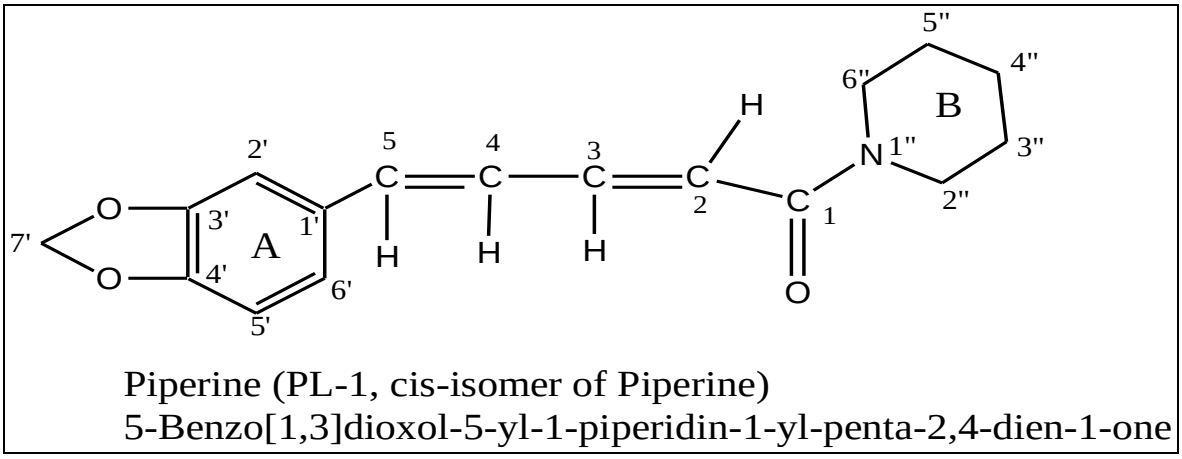

FIGURE 1: *cis*-PIPERINE

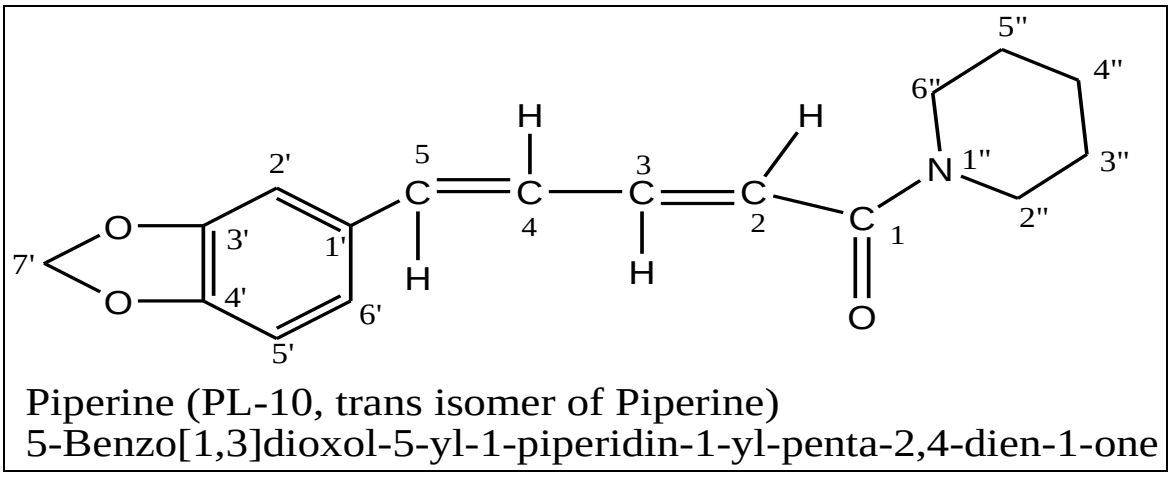

FIGURE 2: *trans*-PIPERINE

TABLE 2: 1D AND 2D NMR OF PL-10

Position	^{1}H-NMR	^{13}C-NMR	DEPT-135	Conclusion	COSY
1	-----	165.4	No peak	C	--
2	6.457-6.420d (J= 14.8 Hz)	121.1	Positive peak	CH	H-3
3	7.422-7.360 dddd (J= 1.6,9.6 Hz)	142.3	Positive peak	CH	H-2, H-4
4	6.853-6.829dd (J= 1.6,16.0 Hz)	125.2	Positive peak	CH	H-3, H-5
5	6.761-6.726 d (J= 3.2, 12.8 Hz)	122.1	Positive peak	CH	H-5
1′	-----	138.0	No peak	C	--
2′	6.94d (J= 1.2 Hz)	105.1	Positive peak	CH	--
3′	-----	148.01	No peak	C	--
4′	-----	148.08	No peak	C	--
5′	6.699d (J= 8.4 Hz)	105.2	Positive peak	CH	H-6′
6′	6.718 d (J= 8.0 Hz)	125.2	Positive peak	CH	H-5′
7′	5.91s	102.0	Positive peak	CH_2	--
N-1″	------	------	-----	N	--
2″	3.45bs	46.7	Negative peak	CH_2	H-3″
3″	δ: 1.56m	26.6	Negative peak	CH_2	H-2″, H-4″
4″	-------	25.5	Negative peak	CH_2	H-3″, H-5″
5″	δ: 1.56m	24.5	Negative peak	CH_2	H-4″, H-6″
6″	3.61bs	43.0	Negative peak	CH_2	H-5″

Antihepatotoxic Activity:

TABLE 4: EFFECT OF *cis*-PIPERINE AND *trans*-PIPERINE SUPPLEMENTATION ON SERUM MARKER ENZYMES

Group	Treatment	Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
G1	Control	---------	48.28±1.21	28.12±1.21	145.62±6.23
G2	Toxic group (CCl_4)	2ml/kg (p. o.)	134.24±2.05[a]	71.96±2.88 [a]	375.02±8.13 [a]
G3	Standard group(silymarin)	50 mg/kg (p. o.)	55.46±1.96[b] (91.64%)	32.54±3.01 [b] (89.92%)	158.14±4.53 [b] (94.54%)
G4	cis-piperine	50 mg/kg (p. o.)	63.22±1.59 [b] (90.14%)	39.38±2.34 [b] (82.65%)	157.02±2.43 [b] (92.23%)
G5	trans-piperine	50 mg/kg (p. o.)	62.40±1.62 [b] (91.19%)	49.05±1.87 [b] (58.12%)	163.84±194 [b] (97.37%)

Values are expressed as mean ± S.E.M. (n=5) using ANOVA. CCl_4 group showed significant increase in AST, ALT and ALP levels compared to the control group (a, P<0.05 CCl_4 vs. control group): *P. longum* supplementation significantly decreased AST, ALT, and ALP levels in the CCl_4+ *P. longum* groups compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ *P. longum* vs. CCl_4 group). Values given in parentheses are percent decrease in enzyme activity with respect to toxic group (G-2). The results of various biochemical parameters revealed the elevation of serum enzyme level and decreased the level of antioxidants and total protein and total albumin in CCl_4 treated group, indicating that CCl_4 induces damage to the liver. A significant reduction was observed in AST, ALT and ALP levels in the groups treated with both of isolated chemical components of *P. longum* and silymarin (**Table 4**).

The results obtained for serum enzyme level have suggested that the groups treated withcis- piperine and trans-piperine of *P. longum* are comparable with silymarin (Silbyon-70), the standard

hepatoprotective drug and our results are also supported with percent decrease of enzyme activity values given in parentheses.

TABLE 5: EFFECT OF *cis*-PIPERINE AND *trans*-PIPERINE SUPPLEMENTATION ON ANTIOXIDANT ENZYMES SUCH AS SOD, GP_X AND GSH LEVEL

Group	Treatment	Dose	SOD (U/g)	GPx (U/g)	GSH (U/g)
G1	Control	----------	84.62±1.25	42.62±1.16	78.16±1.14
G2	Toxic group	2ml/kg (p. o.)	37.02±2.51[a]	17.02±3.61[a]	29.12±1.32[a]
G3	Standard group (silymarin)	50 mg/kg (p. o.)	78.14±3.22[b]	39.15±1.12[b]	70.53±2.92[b]
G4	cis-piperine	50 mg/kg (p. o.)	57.02±2.88[b]	37.02±1.43[b]	57.72±2.31[b]
G5	trans-piperine	50 mg/kg (p. o.)	63.84±0.22[b]	39.84±3.41[b]	59.56±1.84[b]

Values are expressed as mean ± S.E.M. (n=5) ANOVA. CCl_4 hepatotoxic group showed significant decrease in SOD activity compared to the control group (a, P<0.05 CCl_4 vs. control group): *P. longum* supplementation significantly increased SOD activity in the CCl_4+ *P. longum* groups compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ *P. longum* vs. CCl_4 group).

Effect of *P. longum* treatment on GPx activity. CCl_4 hepatotoxic group showed significant decrease in GPx activity as compared to the control group (a, P<0.05 CCl_4 vs. control group). *P. longum* treatment significantly increased GPx activity in the CCl_4+ *P. longum* groups compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ *P. longum* vs. CCl_4 group). Effect of *P. longum* treatment on GSH activity: CCl_4 hepatotoxic group showed significant decrease in GSH activity as compared to the control group (a, P<0.05 CCl_4 vs. control group). *P. longum* treatment significantly increased GSH activity in CCl_4+ *P. longum* groups compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ *P. longum* vs. CCl_4 group).

The antioxidant enzymes SOD, GPx, and GSH can be used to predict the severity of CCl_4 induced hepatotoxicity because these enzymes such as SOD, GPx, and GSH dependently act in the metabolic pathways involved in free radicals; as we see in the Table-5 there are a significant lowering in the group treated with CCl_4. The cis-piperine and trans-piperine showed significant elevation in SOD, GPx and GSH in some of the groups treated with the isolated isomers of piperine from the dried fruits of *P. longum* Linn (**Table 5**).

TABLE 6: EFFECT OF *cis*-PIPERINE AND *trans*-PIPERINE SUPPLEMENTATION ON TOTAL PROTEIN, TOTAL ALBUMIN, AND TOTAL BILIRUBIN LEVELS

Group	Treatment	Dose	Total Protein (g/dl)	Total Albumin (g/dl)	Total Bilirubin (mg/dl)
G1	Control	----------	6.32±1.23	4.12±1.43	0.74±2.34
G2	Toxic group	2.0 ml/kg (p. o.)	1.95±0.36[a]	1.09±3.41[a]	4.16±1.53[a]
G3	Standard group (Silymarin)	50 mg/kg (p. o.)	6.76±1.06[b]	4.04±0.63[b]	0.98±1.26[b]
G4	cis-piperine	50 mg/kg (p. o.)	6.98±1.12[b]	3.75±0.97[b]	2.09±2.11[b]
G5	trans-piperine	50 mg/kg (p. o.)	6.21±1.98[b]	3.55±0.23[b]	1.89±1.91[b]

Values are expressed as mean ± S.E.M. (n=5) using ANOVA. CCl_4 group showed significant decrease in total protein, total albumin, and increase in total bilirubin levels compared to the control group (a, P<0.05 CCl_4 vs. control group). *P. longum* supplementation significantly increased total protein, total albumin, levels and decreased total bilirubin level in the CCl_4+ *P. longum* groups compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ *P. longum* vs. CCl_4 group).

As we can see in **Table 6** there is a marked decrease values of total protein, total albumin and marked elevation in total bilirubin in group 2 treated with CCl_4. The cis-piperine and trans-piperine treated groups showed significant elevation of total protein, total albumin and significant decreased value of total bilirubin levels.

Histopathological Examination:

The histopathological examination of the liver section of the male albino Wistar rats treated with CCl_4 showed hepatocytic necrosis and evident vacuolation of hepatocytes. The rats treated with silymarin and cis and trans- piperinealong with toxicant showed sign of protection against toxicants

to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vacuoles *cis*-piperine and trans-piperine showed a remarkable recovery of hepatic cells, disappearance of necrosis, a mild vacuolation with almost normal central vein (CV) and portal triad (PT) indicating a potent antihepatotoxic activity and supporting the data obtained from the analysis of biochemical parameters (**Table7, Fig. 3 -7**).

TABLE 7: HISTOPATHOLOGICAL OBSERVATIONS OF THE EXPERIMENTAL ANIMALS

Groups	Treatment	Histopathological observations
1.	Normal	Liver histopathology showed normal hepatic cells with distinct nucleus and sinusoidal architecture.
2.	Toxic	Liver histopathology showed vacuolation of hepatocytes and focal necrosis in the centrizonal area with enlarged portal triad (PT) and central vein (CV).
3	Standard	Liver histopathology showed mild sinusoidal dilatation in the centrizonal area PT portal triad and CV central vein was clearly visible.
4	cis-piperine	Liver histopathology showed mild vacuolation with almost normal central vein (CV) and portal triad (PT)
5	trans-piperine	Liver histopathology showed mild vacuolation with the disappearance of necrosis.

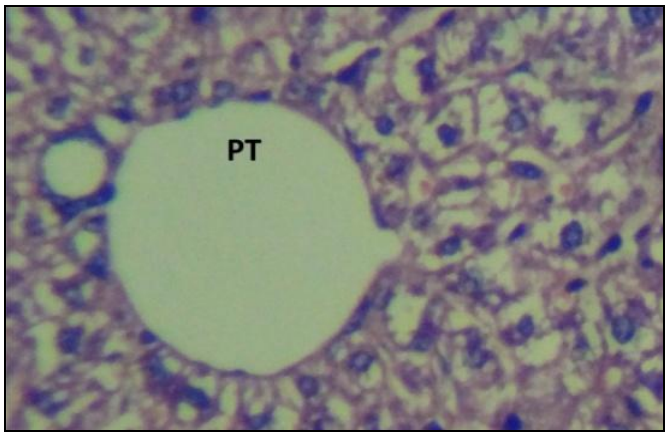

FIGURE 3: LIVER HISTOPATHOLOGY OF NORMAL ANIMAL AT 40X MAGNIFICATION: LIVER HISTOPATHOLOGY SHOWING NORMAL HEPATIC CELLS WITH DISTINCT NUCLEUS AND SINUSOIDAL ARCHITECTURE WITHOUT ANY NECROSIS.

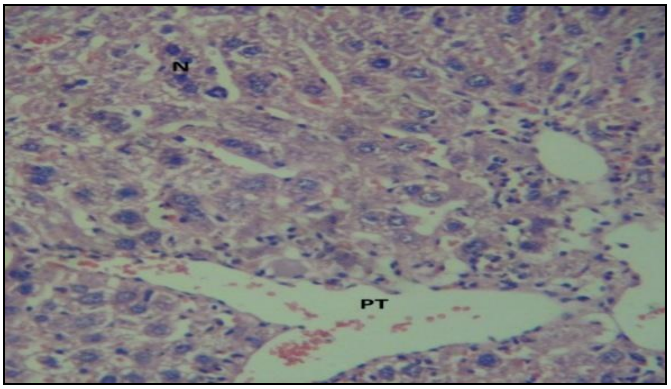

FIGURE 4: LIVER HISTOPATHOLOGY OF ANIMAL TREATED WITH CCL_4 AT 40X MAGNIFICATION: SHOWING VACUOLATION OF HEPATOCYTES AND FOCAL NECROSIS IN THE CENTRIZONAL AREA, PT PORTAL TRIAD AND CV CENTRAL VEIN, HEPATOCYTIC NECROSIS (N) AND EVIDENT VACUOLATION OF HEPATOCYTES.

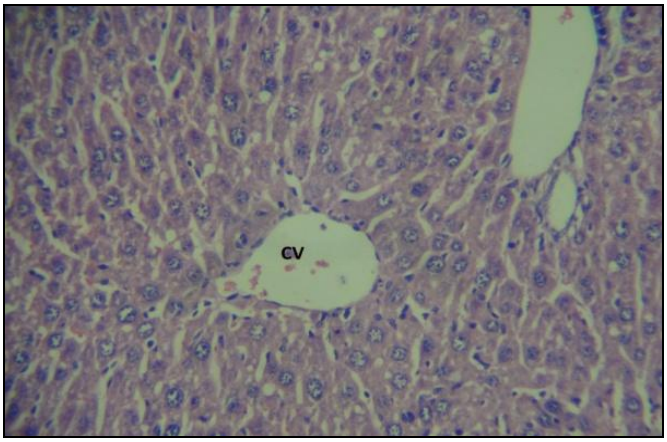

FIGURE 5: LIVER HISTOPATHOLOGY OF ANIMAL TREATED WITH CCL_4 AND SILYMARINAT 40X MAGNIFICATION LIVER HISTOPATHOLOGY SHOWING MILD SINUSOIDAL DILATATION IN THE CENTRIZONAL AREA, PT PORTAL TRIAD AND CV CENTRAL VEIN WAS CLEARLY VISIBLE

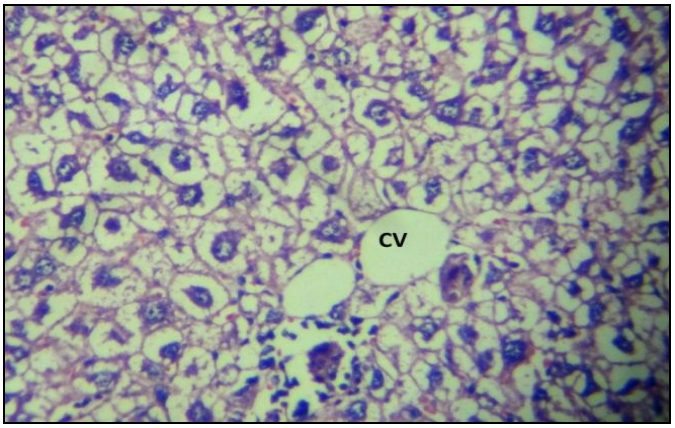

FIGURE 6: LIVER HISTOPATHOLOGY OF ANIMAL TREATED WITH CCL_4 AND *cis*-PIPERINE AT 40X MAGNIFICATION SHOWING MILD VACUOLATION, NECROSIS AND INFLAMMATION AND CV IS NORMAL.

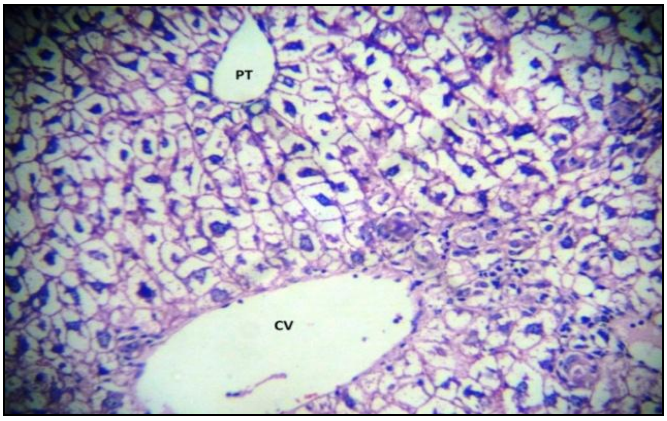

FIGURE 7: LIVER HISTOPATHOLOGY OF ANIMAL TREATED WITH CCL_4 AND *trans*-PIPERINE AT 40 X MAGNIFICATIONS SHOWING MILD VACUOLATION, MODERATE DEGREE OF INFLAMMATION AND NECROSIS AND CV IS NORMAL.

CONCLUSION: Thus, from the above investigation we can say that the *cis*-piperine and trans-piperine exhibited significant antihepatotoxic activity as well as antioxidant actvity against CCl_4 induced hepatotoxicity. This significant antihepatotoxic effect is probably mediated through its significant antioxidant activity. These studies place this medicinal plant a novel agent for bio-prospection and drug development for the treatment of liver disorders using modern methods. Attempts are also bring made to characterize other chemical components of the plant, which are responsible for the antihepatotoxic efficacy as well as antioxidant efficacy of this valuable medicinal plant. Further studies are required to explain exact mechanism of action in neutralizing the toxic effects.

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How to cite this article:
Tripathi K, Pandey ND and Ahmed B: Structure elucidation and antihepatotoxic activity of *cis* and *trans* isomers of piperine isolated from dried fruits if *Piper Longum*. Int J Pharm Sci Res 2015; 6(1): 251-58.doi: 10.13040/IJPSR.0975-8232.6 (1).251-58.