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IN VIVO AND *IN VITRO* HEPATOPROTECTIVE STUDIES OF VARIOUS EXTRACTS OF *APHNAMIXIS POLYSTATCHYA*

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ABSTRACT

The Alcoholic extract of bark and leaf of *Aphanamixis polystachya* (Wall) Parker for its anti hepatotoxic activity against Isonizid (INH) and Rifampicin (RIF) induced hepatic damage in rats. The activity was evaluated by using biochemical parameters such as total Bilirubin, total protein, Alkaline phosphatase (ALP), Asiartate Transaminase (AST), Alanine Transaminase (ALT), glutamate transpeptidase (GGTP). The histopathalogical changes of liver sample were compared with respective control. The extract showed remarkable hepatoprotective effect.

Key words : *Aphanamixis polystachya,* Hepatoprotective, INH & RIF, Biochemical parameters, Histopathological studies.

INTRODUCTION

The liver is the key organ of metabolism and detoxification.Continuous exposure to a variety of environmental toxicagents enhances hepatic injury (Garner *et al* 2004). A growing interest hasemerged around the globe in rediscovering medicinal plants as useful therapeutic agents for the prevention of such injury. Successful liver therapy owes much to the identification of pathogenesis and elaboration of suitable models of hepaticinjury, comparable to those encountered in clinical practice. In the present study, a liver disease model was produced with a potent hepatotoxin, carbon tetra chloride (CCl_4), toevaluate the sequence of pathophysiological events andbiochemical pharmacology. The Meliaceae plant family has long been used in India for its medicinal properties. *Aphanamixis*, a genus of this family, has been used in this study. The crude ethanolic stem bark extract of *Aphanamixis polystachya* has shown hepatoprotective activities (Sharma 2004; Sodhi *et al* 1997; Shankar *et al* 2005; Pal *et al* 2003; Tasdug *et al* 2005) . In thisinvestigation, crude ethanolic leaf extract has been used to determine its efficacy against liver damage, using the activities of marker enzymes such as plasma aspartateaminotransferase (ASAT), alanine aminotransferase (ALAT),alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH), and the plasma concentrations of bilirubin and albumin.

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Tuberculosis is one of the most common infectious diseases. In India pulmonary tuberculosis is one of major causes for adult deaths (Garner *et al* 2004). Isoniazid (INH) and Rifampicin (RIF) the first fine drugs used for tuberculosis chemotherapy, are associated with hepatotoxcity (Tasdug *et al* 2005). The rate of hepatotoxcity has been reported to be much higher in developing countries. The India (8 to 30%) compared to that in advanced countries (2 to 3%) with a similar dose schedule (Sharma 2004). Oxidative stress as one of the mechanism for INH + RIF induced hepatic injury (Sodhi *et al* 1997). Majority of normally formed free radicals are removed by the action of reduced glutathione.

In circumstances where there is a reduction in glutathione results in the nitration of lipid per oxidation (LPO) resulting in tissue injury (Shanker *et al* 2005).

Hepatotoxicity can affect hundreds of millions of people world wide. It is the common-non-neoplastic cause of death among hepatobiliary and digestive disorders.

Serious side effects, the cost of the modern medicine and improper channel of treatment and competitive efficacy of natural products made the persons through the world to look for classic plant drugs for the treatment of hepatotoxicity. In view of the biological properties and chemical constituent of the plant from *Aphnamixis polystatchya* it was decided to study the plant *(Aphnamixis polystachaya)* which is widely used in folk medicine.

MATERIALS AND METHODS

PLANT MATERIAL AND PREPARATION OF EXTRACTS

Bark and leaves of *Aphanamixis polystachya* (Wall) Parker (Sanskrit Name : Rohituka, Tamil Name : Malampuluvan) were collected from hilly areas in an around Tirunelveli District in the month of December 2008 and authenticated by Dr. Michael Jayaraj Siddha Medicine Practionor, Papanasam, Tamil Nadu, India. The plant materials were dried in shade and pulverized. The powder was treated with petroleum ether for defatting as well as to remove chlorophyll. 5 Kg of each powder material of the above mentioned two plants were soaked in ethanol for 48 hours in four separate glass jars and extracts were filtered and solvent was evaporated using water bath and vacuum desiccators.

In vivo HEPATROPROTECTIVE STUDY

ANIMALS

Male Wister rats weighing 150 – 200 gm body weight were used in this study. The protocol was approved by the institutes animal ethical committee.

Body weights of these rats were monitored sequentially in control and experimental animals for a period of 28 days.

Animals were divided in to 5 groups as control (n=6) INH + RIF (n=6), INH + RIF + Ethanolic extract of *Aphnamixis polystatchya* bark. INH + RIF + Ethanolic extract of *Aphnamixis polystatchya* leaf, INH + RIF + Silymarin (n=6) where n was the number of animals included in this study.

For hepatotoxic model, 50 mg/kg per day of INH and RIF each was used in this study (Pal *et al* 2003).

INH + RIF solutions were prepared separately in sterile distilled water. INH + RIF were administered orally for 21 days. Both extracts were administered orally at dose of 100 mg/kg for 21 days. Liver transaminases, Total protein, GGTP (Gamma glutamyl – 3- carboxy -4- nitro anilide) and total bilirubin were estimated on 21 days in both control and experimental animals.

TREATMENT PROTOCOL

Group I-Normalcontrol (Received lml of 1% CMC Carboxymethyl cellulose)

Group II-Hepatotoxic Control(Received 50 mg/Kg INH + RIF for 21 days) orally

Group III-Treatment group were given INH + RIF + ethanolic extract of *Aphnamixi spolystatchya* Bark(100mg/Kg suspended with 1% CMC)

Group IV-Treatment group were given INH + RIF + ethanolic extract of *Aphnamixi spolystatchya* leaf(100mg/Kg suspended with 1% CMC)

Group V-Positive control were given INH + RIF + Silymarin 70mg/Kg orally for 21 days.

METHODS

Rats were treated as per the protocol. The protocol was approved by the IAEC. Body weight of these rats were monitored sellentially in control and experimental animals for a period of 21 days. They were scarificed 1 hour after administration drug on day 21. The blood was collected by retro orbital artery bleeding. Blood samples were kept for 30 minutes without any disturbance in clot activator sample tubes. Then blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum.

BROCHEMICAL MARKERS

Aspartate transaminase, Alanine transaminase, Alkaline phosphate, total bilirubin, total protein and Gamma Glutamyl -3- Carboxy 4 nitro anilide levels

were estimated from the serum samples using auto analyzer. Results of biochemical analysis are given in table No.1.

STATISTICS

The results were expressed as mean ± SEM (Standard error mean) statistical analysis was carried out by using ONE WAY ANOVA followed by Newman Kevi's multiple range test.

TABLE - 1

Effect of various extract in different biochemical parameters in INH + RIF induced hepatotoxic rats.

Group	Total Bilirubin	Total Protein	ALP (Alkaline Phosphatase)	AST Aspartate Transaminase	ALT Alanine Trans aminase	GGTP
Group – I Normal Control	0.64 ± 0.03	0.56 ± 0.04	128.20 ± 4.53	124.4± 3.6	38.4± 3.6	96.6± 1.56
Group – II Hepatotoxic Control	2.08 ±[a] 0.16	0.29 ±[a] 0.05	352.20 ±[a] 6.65	442.9 ±[a] 8.92	152.4 ±[a] 3.06	190.4 ±[a] 3.01
Group – III Treatment group	1.16 ± 0.18	0.34 ± 0.05	228.4 ± 7.01	298.4 ± 5.06	103.4 ± 2.06	142.4 ± 4.16
Group-IV Treatment group	1.28 ± 0.12	0.36 ± 0.09	233.6 ± 5.23	303.9 ± 6.05	110.1 ± 2.16	148.6 ± 3.16
Group – V Positive control	0.78 ±[b] 0.02	0.51 ±[b] 0.11	148.2 ±[b] 4.28	182.4 ±[b] 2.06	58.6 ±[b] 1.68	103.6 ±[b] 1.90

- Values are expressed as Mean ± SEM
- Values were found out by using ONE WAY ANOVA followed by Newman Kevi's multiple range test.

a-Values are significantly different from hepatotoxic control at P <0.01.
b-Values are significantly different from hepatotoxic control at P <0.01.

RESULT

BIOCHEMICAL PARAMETERS

Control Group (Group – I)

The basal levels of liver enzymes (ALP, AST, ALT & GGTP) in control were 128 20 ± 4.53, 124.4 ± 3.6, 38.4 ± 1.11 and 96.6 ± 1.56 respectively. Total bilirubin and Total protein levels were 0.64 ± 0.03 and 0.56 ± 0.04 respectively.

Hepatotoxic Control (Group – II)

There was significant increase in total bilirubin (2.08 ± 0.16) accompanied by significant decrease in level of total protein (0.29 ± 0.05) and also significant increase in ALP (352.20 ± 6.65) AST (442.9 ± 8.92) ALT (152.4 ± 3.06) & GGTP (190.4 ± 3.01) as compared to the control.

ETHANOLIC EXTRACT OF BARK OF *Apahnamixis polystatchya* TREATED GROUP (GROUP - III)

There was no significant decrease in total bilirubin (1.16 ± 0.18) accompanied by no significant increase in level of total protein (0.34 ± 0.05) and also there is no significant decrease in ALP (228.4 ± 7.01) AST (298.4 ± 5.06) ALT (103.4 ± 2.06) GGTP (142.4 ± 4.16) as compared to the toxic control.

ETHANOLIC EXTRACT OF LEAF OF *Aphnamixis polystachya* TREATED GROUP (GROUP - IV)

There was no significant decrease in total bilirubin (1.28 ± 0.12) accompanied by no significant increase in level of total protein (0.36 ± 0.09) and also there is no significant decrease in ALP (233.6 ± 5.23) AST (303.9 ± 6.05) ALT (110.1 ± 2.16) GGTP (148.6 ± 3.16) as compared to the hepatotoxic control.

POSITIVE CONTROL GROUP

There was no significant decrease in total bilirubin (0.78 ± 0.02) accompanied by significant increase in level of total protein (0.51 ± 0.11) and also significant decrease in ALP 148.2 ± 4.28) AST (182.4 ± 2.06) ALT (58.6 ± 1.68) GGTP (103.6 ± 1.90) as compared to the toxic control.

DISCUSSION

Although *Aphnamixis polystatchya* is reported to possess varied medicinal properties. But there is no previous clinical data about the hepatoprotective activity of these both plants. The present investigation reports the hepatoprotective effect of ethanolic extracts of both these plants.

In the present study hepatotxic model in wistar rat was successfully produced by administering INH & RIF (50mg/kg/day) orally. During the metabolism of INH, hydrazine in produced directly (from INH) or indirectly (from acetyl hydrazine) from earlier study, it is evident that hydrazine plays a role in INH Induced liver damage in rats, which is consistent with the report by Tasdug *et al* (2005).

The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation and cytochrome P450 was thought to be involved in the synergistic effect of RIF on INH.[8] However, its role in INH – induced hepatotoxcity is unclassified as INH itself is an inducer of cyp $2E_1$ (Shakun and Shman 1985).

A small retrospective analysis of patients who developed hepatic dysfunction whilst on Antituberculosis drugs, hospitalized in a unit between 1st Jan 1991 to 31st Dec 1992 was recently under taken. Out of 1, 118 patients who received RIF & INH with (or without) pyraznamide and other drugs, 142 developed clinically symptomatic dysfunction. An Assumption that the vast majority hepatic dysfunction episodes should have occurred within 2 months of commencement of antituberculosis chemotherapy was made as generally reported (Girling 1982). The previous report also says that there did not seem to be clear evidence that isoniazid proves much more injuries than RIF and in this connection, they consider that it is the combination of these 2 drugs that confer the additive (or) even synergistic, potential of liver toxicity than either agent alone as conjectured (Yasuda *et al* 1990; Wu *et al* 1990; Srele *et al* 1991). INH is metabolised in Liver primarily by acetylation and hydrolysis and these acetylated metabolites that are thought to be hepatotoxins previous report in rat suggest that hydrazine metabolite of INH and its subsequent effect on cyp $2E_1$, induction is involved in the developed INH induced hepatotoxcity and also oxidative stress as one of the mechanism for INH + RIF induced hepatic injury (Peretti *et al* 1987).

In this study, the result suggest that the statistically significant difference in biochemical parameters in toxic control group (Group - II) indicate that hepatic damage has been induced by INH + RIF. Following treatment with Ethanolic extract of Bark and leaf of *Aphnamixis polystatchya* dose (100mg/Kg) and silymarin all the parameters were reduced and total protein restored to normal value.

But in Group - III and Group - IV rats do not significantly reduce and restore the all parameters when compared to hepatotoxic control (Group - II).

The estimation of GGTP (Gamma glutamyl transpeptidase) level is a valuable screening test with high negative predictive value for liver disease (Nemesaaszky 1996).

IN VIVO AND *IN VITRO* HEPATOPROTECTIVE STUDIES OF VARIOUS EXTRACTS OF *APHNAMIXIS POLYSTATCHYA*

A number of drugs and chemicals are known to increase GGTP activity by the induction of hepatic microsomal enzymes.

Comparatively in Ethanolic extract of bark of *Aphanamixis polystachya* treated groups significant changes occurred in total bilirubin and GGTP levels this suggest that the extracts have hepatoprotective effect.

However, treatment with these extracts could not completely reversed the hepatic injury. But had resulted in early hepatic damage.

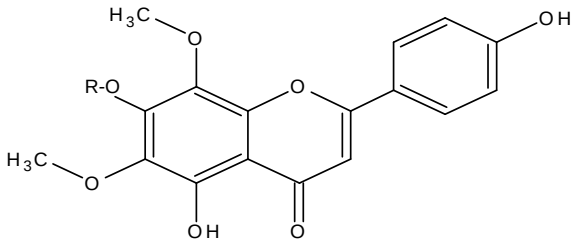
The reason for hepatoprotective effect of the extracts may be that *Aphanamixis polystachya* contains flavonoids which might have scavenged the free redical offering hepatoprotection.

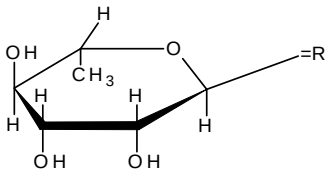
Purification of extracts and indentification of active principle flavonoids may yield a good hepatoprotective drugs.

In vitro HEPATOPROTECTIVE STUDY

Hepatoprotective effect of the compound I (5, 4′-dihydroxy 6,8-dimethoxy flavone) which was isolated from the bark of *Aphanamixis polystachya* in freshly isolated rat hepatocytes is studied as given below.

Compound I :5, 4′-Dihydroxy 6,8-dimethoxy 7-O-rhamnosyl flavone





CHEMICALS

All chemicals were obtained from SD Fine Chemicals, Mumbai. Collagenase, dexamethasone, insulin, HEPES buffer (Seglen 1994), minimum essential medium (MEM), trypan blue (Freshney 2000), Ham's F12 medium and antibiotics were purchased from Sigma Chemical Co., St. Loius, MO, USA.

ISOLATION AND CULTURE OF HEPATOCYTES

Liver cells were isolated by a modified procedure of Seglen (1994). The calcium-free HEPES buffer and collagenase solutions were warmed in a water bath (37 °C). The abdomen of the rat was opened under phenobarbital sodium (35mg/kg body weight) anesthesia. A midline incision was made and a loosely tied ligature was placed around the portal vein approximately 5mm from the liver and the cannula was inserted up to the liver and then the ligature was tightened and heparin was injected into the femoral vein (1000 IU) (Kind and King 1954). The inferior venacava was cut below the renal vein. Perfusion was performed for 20 min (37 °C) with calcium free HEPES buffer, which contained 1% bovine serum albumin fraction V at a flow rate of 30 ml/min. The liver swells during this time, slowly changing its color from dark red to grayish white. The swollen liver was then perfused with TPVG solution (50 ml) followed by perfusion with calcium free HEPES buffer (Mabry *et al* 1970), which contained additional collagenase solution (0.075%) and calcium chloride (4mM) at a flow rate of 15 ml/min for 20 min.

After the perfusion, the lobes were removed and transferred into a sterile petri dish containing calcium-free HEPES buffer and dispersed gently (Faini *et al* 1982). It was transferred into a sterile conical flask and the crude cell suspension was stirred with the help of a magnetic stirrer for 5 min to release hepatocytes into the solution. The cell suspension was filtered through a nylon mesh (250m) and the preparation was centrifuged at 1000 rpm for 15 min. The supernatant was aspirated off and the loosely packed pellet of cells was gently re-suspended in calcium free HEPES buffer. This washing procedure was repeated three times. Cell viability was determined by the Trypan blue dye exclusion method (Agarwal 1989 ; Freshney, 2000). These isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf serum, antibiotics, 10-6M dexamethasone and 10-8 bovine insulin (Loo *et al* 1986). The cell suspension was incubated at 37 °C for 30 min in a humidified incubator under 5% CO_2.

In vitro EXPERIMENTS

About 1ml of hepatocyte suspension was incubated for 24 h following the protocol given below.

Control : 0.1 ml of hepatocyte suspension + 0.1 ml vehicle (distilled water) + 0.1 ml PBS (phosphate buffer saline, pH 7.4.)

Toxicant : 0.1 ml of hepatocyte suspension + 0.1ml of toxicant (Thioacetamide 20 µg/ml in distilled water) + 0.8 ml PBS.

Standard : 0.1 ml of hepatocyte suspension + 0.1 ml of toxicant (Thioacetamide 20 µg/ml in distilled water) + 0.1 ml silymarin (100 µg/ml, suspended in distilled water) + 0.7 ml PBS.

Test substance: 0.1 ml of hepatocyte suspension + 0.1 ml of toxicant (Thioacetamide 20 µg/ml in distilled water) + 0.1 ml Compound I (1000, 100, 10 µg/ml, suspended in <1% DMSO + 0.7 ml PBS.

Each set contained three samples.

TABLE - 2

Effect of different concentrations of compound I on % viability and biochemical parameters of rat hepatocytes intoxicated with Thioacetamide

Treatment	Concentration (µg/ml)	% Viability	AST U/L	ALT U/L	ALP U/L
Control	-	95.12 ± 1.43	115.3 ± 1.06	82.24 ± 1.78	226.12 ± 2.104
Thioacetamide	20	24.67 ± 1.19 [a]	325.6 ± 3.67[a]	214.8 ± 1.15 [a]	405.27 ± 3.35 [a]
Silymarin	100	82.72 ± 1.23 [a,c]	158.6 ± 1.18[a,c]	108.5 ± 1.27 [b,c]	214.65 ± 2.16 [c]
Compound-I	10	34.78 ± 1.08 [a,d]	280.5 ± 2.24 [a,c]	180.5 ± 2.36 [a,c]	325.7 ± 2.83 [a,c]
	100	49.28 ± 1.85 [a,c]	206.7 ± 1.45 [a,c]	152.4 ± 1.67, [a,c]	297.5 ± 1.83 [a,c]
	1000	70.14 ± 1.26 [a,c]	170.5 ± 1.10 [a,c]	119.5 ± 1.21 [a,c]	253.10 ± 2.27[a,c]

Values (% viability) are mean ±S.E.M of three readings in each group
[a]P<0.001; [b]P<0.01 Vs Control
[c]P<0.001; [d]P<0.01 Vs Thioacetamide
Data were analysed by one way ANOVA followed by Tukey multiple comparison test

BIOCHEMICAL INVESTIGATIONAL PROCEDURE

ALANINE AMINO TRANSFERASE

Reagents

Phosphate buffer: 0.1M, pH 7.4

Substance: Dissolved 2.66 g of DL – aspartic acid and 38 mg of α – ketoglutaric acid in 20.0 ml 0.1N sodium hydroxide with gentle heating. This was made up to 100 ml with water.

2, 4 - dinitrophenyl hydrazine (DNPH) reagent: 1.0 mM dinitrophenyl hydrazine in 2.N hydrochloric acid.

Sodium hydroxide 0.4 N.

Standard pyruvic acid: 10 mg of sodium pyruvate was dissolved in 10 ml of phosphate buffer 0.1M, pH 7.4.

Procedure

In different tubes, 1.0 ml of the buffered substrate was added to 0.1 ml of serum and incubated at 37^0 C for one hour. Then 1.0 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1ml of enzyme was added only after the addition of DNPH reagent. The tubes were kept aside for 15 minutes, then 10 ml of 0.4N Sodium hydroxide was added and read at 520 nm in a UV spectrophotometer.

The enzyme activity is expressed as IU/ litre of serum.

ASPARTATE AMINO TRANSFERASE

The reagents and methods used were the same as those used for the assay of serum glutamate oxaloacetate transferase but substrate solution was different and the incubation time was reduced to 30 minutes.

Substrate

1.78 g of DL- alanine and 38mg of α-Ketoglutarate were dissolved in buffer. 0.5 ml of sodium hydroxide was added and the volume was made up to 100 ml with buffer.

The enzyme activity is expressed as IU / litre of serum.

ALKALINE PHOSPHATASE

Alkaline phosphatase was assayed by using disodium phenyl phosphate as the substrate.

Reagents

Carbonate – bicarbonate buffer-0.1M, pH 10.0

Substrate: 0.01M disodium phenyl phosphate solution

Folin's phenol reagent:

Sodium carbonate 15%

Magnesium chloride 0.1M.

Standard phenol solution: A solution of distilled crystalline phenol in water containing 5.0 µg in 0.1ml was prepared.

Procedure

The incubation mixture contained the following compounds in a final volume of 3.0 ml of carbonate-bicarbonate buffer: 1.0ml of substrate and 0.1ml magnesium chloride and requisite amount of the enzyme source (0.2ml of serum). The reaction mixture was incubated at 37^0 C for 15 minutes. The reaction was terminated by the addition of 1.0 ml Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme source were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. Then 1.0 ml of 15% sodium carbonate solution was added and incubated further for 10 minutes at 37^0 C. The blue colour developed was read at 640nm against blank. The standards were also treated similarly.

The activity of the enzyme is expressed as IU / litre of serum.

ASSESSMENT OF ANTIHEPATOTOXIC ACTIVITY

CELL VIABILITY ASSAY

Cell viability was evaluated by trypan blue dye exclusion test (2). Aliquot of cell suspension (2 ml) was combined with 0.08% trypan blue (2 ml) for 3 min. Then, 400 µl of the mixture were counted for cells using a hemocytometer. Cell viability was defined by the following formula:

$$\text{Cell viability} = \frac{\text{cells excluding trypan blue}}{\text{total cells}} \times 100$$

DETERMINATION OF AST/ALT/ALP LEVEL

The hepatocyte suspension after drug treatment was centrifuged at 5000 rpm for 10 min and the supernatant obtained was used for estimation of AST , ALT and ALP levels (Sharma 2004; Shanker *et al* 2005).

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test. P values
< 0.05 were considered as significant.

RESULT DISCUSSION

The effect of the compound I on freshly isolated rat hepatocytes intoxicated with Thioacetamide are recorded in Table - 2. The hepatoprotective activities of **CPDs** of **I** were assessed by measuring their effects on the release of AST, ALT, ALP and cell viability from primary cultures of rat hepatocytes injured with thioacetamide.

Incubation of hepatocytes with TA and Compound **I** resulted in 34.78, 9.28 & 70.14 % increase in the % viability on concentration dependent manner. The maximum increases in the % viability (70.14 %) was obtained at concentration of 1000 µg/ml against TA induced toxicity.

A significant increase in the levels of AST, ALT and ALP ($P<0.001$) and a significant decreased in cell viability ($P< 0.001$) were observed in hepatocytes exposed to TA when compared to control (Table 1). These cells, when treated along with the CPDs of I, showed a significant restoration of the altered biochemical parameters towards the normal ($P<0.001$) when compared to thioacetamide treated group and is dose dependent. However, the hepatoprotective effect of CPD of compound I was observed at very low concentrations (10 µg/ml). The decrease in the levels of AST, ALT, and ALP in freshly isolated hepatocytes treated with CPD at 1000 µg/ml was significant ($P<0.001$), when compared to control and TA on dose dependent manner.

Thioacetamide leads to induction of toxicity by altering semi permeable character of cell membrane of hepatocytes resulting in increased influx of Ca_2^+ and finally death (Shakun and Shman 1985). Induction of toxicities are indicated by a significant decrease in % viability of hepatocytes. The protective effect was indicated by restoration of marked changes. The maximum protective effect was observed in concentrations of 1000 µg/ml and 100 µg/ml against Thioacetamide induced toxicities.

Treatment with the compound of *Aphanamixis polystachya* exhibited significant restoration of the altered biochemical parameters and cell viability towards normal in thioacetamide intoxicated rat hepatocytes. These effects were comparable to silymarin, which was used as a positive control. The present study has shown that compound have a significant hepatoprotective effect on TAA-induced cytotoxicity in primary cultured rat hepatocytes.

So, it is clear that compound I of shows prominent hepatoprotective activity as compare to standard. As such, compound I might hold significant therapeutic value in the prevention or treatment of liver disease. The isolated compound as well as extract of bark of *Aphanamixis polystachya* shows hepatoprotective effect (Gallgher *et al* 1956). Hence, the compound may be responsible for hepatoprotective activity of bark of *Aphanamixis polystachya.*

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REFERENCES

1. Agarwal, P.K. 1989. 13C NMR of flavonoids. Elsevier, Amsterdam, pp 289.
2. Faini, F.A. ,Castillo, M., and Torres, M.P. 1982. Flavonoids of *Baccharis incarum*. *J Nat Prod* 45:501–502.
3. Freshney, I. Cytotoxicity. *Culture of Animal Cells: A Manual of Basic Technique*. 4th Edition, Wiley-Liss, New York, 2000, pp. 331–332.
4. Gallgher, C.H., Judah, J.D. and Rees, K.R.1956. Mechanism of thioacetamide toxicity. *Journal of Pathology of Bacteria* 72: 193–201.
5. Garner, P., Holmes, A., and Ziganshina, L.2004. *Tuberculosis Clin Evid* 11:1081-1093.
6. Girling, D.J. 1982. Adverse effect of Antituberculosis Drugs, *Drugs* 23:56-61.
7. Kind, P.R. and King, E.J.1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin. *J Clin Path.,7* :322- 326.
8. Loo, P.V., De, Bruyn A. and Budesinsky, M.1986. Reinvestigation of the structural assignment of signals in the 1H and 13C NMR spectra of the flavone apigenin. *Mag Reson Chem*., 24: 879–882.
9. Mabry, T.J., Markham, K.R. and Thomas, M.B.1970.The systemic identification of flavonoid. Springer, Berlin Heidelberg New York, pp 268.
10. Nemesaaszky, E. 1996. Enzyme test in hepatobiliary disease in Donald W Moss & Sidney B Rosark, editor's Enzyme test in diagnosis, New York, Oxford university Press, p. 25-29.
11. Pal, R., Vajphei, K., Singh, K. and Rana, S.V. 2003. Garlic confers hepatoprotection in isoniazid rigampicin induced hepatic injury. *Ind .J Gastro* 22(Suppl.1) : A100.
12. Peretti, E., Kavlagnis, G. and Lauterburg, B.H.1987. Acetylating of Acetyl hydrazine, the toxic metabolite of isoniazid, in numans inhibition by concomitant administration of isoniazide. *J. Pharmacol Exp Ther,* 243:686 – 689.

13. Ramaiah, S.K., Apte, U. and Mehendale, H.I.M. 2001. Cytochrome P4502E_1 induction increases thioacetamide liver injury in diet restricted rats. *Drug metab Dispos* 29:1088-95.

14. Reitman, S. and Frankel, S. 1957.A colorimetric method for determination of serum glutamate oxaloaectate and glutamic pyruvate transaminase. *Amer J Clin Path.,* 28: 56-58.

15. Seglen, P.O. Culture of specific cell types: Epithelial cells – Isolation of Hepatocytes. *Cell Biology: A Laboratory Handbook*, Julio E, Celis, ed., Vol. 1. Academic Press, 1994, pp. 96–102.

16. Shakun, N.P. and Shman, Ko V.V. 1985. Synergistic effect of rifampicin on hepatotoxicity of isoniazid. *Antibiot Med Bio tekhnol* 30:185-189.

17. Sharma, S.K.2004. Antituberculosis drugs and hepatotoxicity. *Infect Genet Evol* , 4:167-170.

18. Shanker, G., Syversan, T., Aschner, J.L. and Aschner, M. 2005 . Modulatory effect of glutathione status and antioxidants on methyl mercury – induced free radical formation in primary cultures of cerebral astrocytes.*Mol Brain Res* 137:11-22.

19. Sodhi, C.P, Rana, S.V, Mehta, S.K., Vaiphei, K., Attaris, S. and Mehta, S.1997. Study of oxidative stress in isoniazid rifampicin induced hepatic injury in young rats, *Drug Chem Toxicol,* 20:255-269.

20. Srele, M.A, Burk, R.F, Des, and Prez, R.M. 1991. Toxic hepatitis with isoniazid and rifampicin in a metanalysis. *Chest* 99: 465 – 471.

21. Tasdug, S.A., Peerzada, K., Koul, S., Bhat, R., and Johni, R K.2005. Biochemical manifestations of anti tuberculosis drugs induced hepatotoxicity and the effect of silymarin. *Hepatol Res* 31:132 – 135.

22. Tasdug, S.A., Peerzada, K., Kovl, S., Bhat, R., and Johri, R.K. 2005 . Biochemical Manifestation of antituberculosis drug induced hepatotoxicity and the effect of Silymarin. *siepatol Res* 31:132 – 135.

23. Yasuda, K., Sato, A., and Chida, K. 1990. Pulmonary tuberculosis with chemotherapy related liver dysfunction. *Kekkadv* 65:407-413.

24. Wu, J.W., Leev, S.D., and Yeh,P.F. 1990. Isoniazid – Rifampicin induced hepatitis in hepatitis B cumers, *Gastroenterology* 98:502-504.