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Research Article

Hepatoprotective Activity of *Argyreia pilosa* Wight and Arn

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Abstract

Objective: We have a shortage of trustworthy Hepatoprotective drugs in contemporary medication in order to avoid and treat drug-induced hepatotoxicity. The plant *Argyreia pilosa* belonging to family Convolulaceae is purported to have hepatoprotective effects. The present study aimed to identify and separate the phytoconstituents of *Argyreia pilosa* methanolic extract (MEAP) for its Hepatoprotective activity.

Materials and Methods: The dried plant of *Argyreia pilosa* had been extracted with methanol and partitioned with Petroleum ether, chloroform and ethyl acetate. The organic layer was fractionated through several stationary phases and determined by utilizing spectral analysis. MEAP (200 mg/kg, p.o.) has been evaluated for its Hepatoprotective activity in Paracetamol (PCM) induced liver toxicity in Rats. The Hepatoprotective activity had been analyzed through biochemical along with histopathological studies.

Results: Phytochemical analysis of the *Argyeia pilosa* (Convolvulacae) identified four phytoconstituents i.e. Stigmasterol (1), ß-Sitosterol (2), Rutin (3) and Quercetin (4). The structures of those separated phytoconstituents were elucidated using spectral analysis. Stigmasterol, Rutin, and Quercetin have been separated using this plant for the first time. The Paracetamol intoxication results in liver damage as evidenced histologically and biochemically. Treatment with MEAP significantly alleviated the acute liver injury induced by paracetamol, as reflected by lowered levels of SGOT, SGPT and ALP and partial liver architectural restoration.

Conclusion: The results showed that MEAP possessed a substantial protective impact towards Paracetamol induced liver injury due to Phytosterols i.e., Stigmasterol, ß-Sitosterol and Flavonoids i.e., Rutin and Quercetin.

Keywords: *Hepatoprotective; Rutin; Quercetin; Silymarin; Argyreia pilosa; β-Sitosterol*

Introduction

The liver is an essential metabolic organ that possesses secretory and excretory functions. It has a preeminent significance in the body because of its decisive functions like the removal of toxins associated with endogenous and exogenous substances like xenobiotics, viral infections, chronic alcoholism, bile secretion etc. All of the above can cause liver failure. Worldwide the fatality and morbidity of liver disorders raise every year. Almost 20,000 fatalities and 2,50,000 new cases identified every year. Liver injury or malfunction is usually associated with heaptocytes necrosis and increased levels of biochemical parameters including SGOT, SGPT, SALP and Total Bilirubin levels. Even though remarkable improvements in contemporary medication, there are hardly any reliable medicines which defend the liver from damage and/or assist in regeneration of hepatic cell. There are wide ranges of drugs and natural plants accessible in the commercial market for liver disorders. These products do not get rid of all of the liver conditions. For that reason, there is the urge to disclose the proper treatment [1]. Many plants that belong to family Convolvulaceae have been identified as Hepatoprotective like *Erycibe hainanesis* [2], *Ipomoea aquatica* [3], *Cuscuta chinensis* [4].

Argyreia pilosa is an ornamental, in addition to a medicinal plant. All components of these plants are usually well known being folklore medicine for the treatment of many ailments by the Indian conventional healer. The root is employed to remedy a variety of health issues such as sexually transmitted diseases viz., gonorrhea and syphilis, blood diseases. Traditionally, the paste from the leaves is placed on the neck region for any a cough, quinsy as well as utilized regarding itch, eczema along with other skin complications, antidiabetic, antiphlogistic, rheumatism, minimize burning perception and antidiabetic [5,6]. Young wines are combined along with rhizome of ginger and get spread around all over the body to alleviate from fever. The decoction of its root used for the treatment of diarrhea and cathartic [7,8]. An enormous variety of Phytochemical constituents continues to be isolated from the genus *Argyreia* i.e. Flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides and amino acids [9]. The genus *Argyreia* continues to be documented numerous pharmacological actions which includes nootropic, aphrodisiac, anti-ulcer, anti-oxidant, Hepatoprotective, immunomodulatory, anti-hyperglycaemic, anti-inflammatory, antimicrobial, anti-diarrheal, nematicidal, antiviral, analgesic, anti-convulsant, central nervous depressant activities and wound healing [7,9-12]. The pharmacological properties and phytochemistry of the plant is still poorly understood. As a result, the present exploration was sketched out to recognize and separate the chemical components of *Argyreia pilosa* (*A. pilosa*) methanolic extract for its Hepatoprotective effect in albino Wistar rats.

Materials and Methods

Plant material

Argyreia pilosa was acquired in the month of September 2015, from Sri Venkateswara University, Andhra Pradesh. It was established and identified by Dr. K. Madhavachetty, plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh and voucher specimen of the plant (No 1922) was deposited at the herbarium of Department of Pharmacognosy, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, for future reference.

Chemicals, Materials, Instrumentation, and Drugs

Each of the chemical substances employed for this investigation was of analytical grade. Paracetamol (E. Merck), Silymarin (Sigma Chemical Co.,) and thiobarbituric acid (Sigma Chemical Co.,). Silica gel for column chromatography (CC) had been performed on silica gel (Merck silica gel 60H, particle size 5 - 40 µm). TLC was executed on Merck aluminum-backed plates, precoated with silica (0.2 mm, 60F254). The ultraviolet (UV) spectra of the compounds in methanol were recorded on a Shimadzu UV-1800 spectrophotometer. The IR spectra were recorded using KBr discs on a NICOLET 380 FT-IR spectrometer (Thermo Fisher Scientific, France) in the range of 400 to 4000 nm. The mass spectrum in ESI mode was obtained using LCMS2010A (Shimadzu, Japan) having probes APCI and ESI. Nuclear magnetic resonance ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz, respectively on a Bruker DRX-400 spectrometer (Bruker Bio spin Co., Karlsruhe, Germany) in DMSO and $CDCl_3$ with tetramethylsilane as an internal standard. Melting points were determined using Royal Scientific RSW 138A melting point apparatus. Further elemental analysis of the compounds was performed on a Perkin Elmer 2400 elemental analyzer. Diagnostic sets for the evaluation of SGOT, SGPT, ALP, serum cholesterol and serum Bilirubin were produced by Ranbaxy Diagnostics Ltd., New Delhi, India. The standard orogastric cannula utilized for oral drug administration.

Test animals

The research was performed on Wistar albino rats (160 - 200g) of either sex (Mahaveer Enterprises, Hyderabad) and was maintained in an animal house in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru having CPCSEA registration number 1847/PO/Re/S/16/CPCSEA. These were permitted to get standard pellet food and water *ad libitum*. Prior to the test, the rats were held in standard environment circumstances at room temperature 25 - 27°C relative humidity (55 ± 5)% and 12h light/12h dark cycle for 7 days. All rats received humane care according to the "Guide for the Care and Use of Laboratory Animals" [13].

Preparation of Extracts and Solvent fractionation

The freshly obtained plant was shade dried and pulverized. The powder (1 Kg) was treated with petroleum ether to get rid of the fatty and waxy material. After that, it was air-dried and extracted with methanol by macerated, strained and concentrated at 45°C in

Buchi rotavapor. Methanol is used as solvent to extract both polar and non-polar compounds because of amphilic in nature. The weight of methanolic extract attained was 87g (8.7% w/w yield). The methanolic extract was revoked in distilled water within a separating funnel and partition eventually with petroleum ether, chloroform, ethyl acetate and n-Butanol to generate fractions. Later, the remaining residual aqueous fraction at the end was gathered. The solvents had been removed over rota-evaporator at reduced pressure to acquire dried fractions. Each one of these extracts was undergoing preliminary Phytochemical examination and the extracts were retained in the refrigerator at 4°C for further consumption [14].

Isolation of Constituents

Petroleum ether extract (PEE) was subjected to silica-gel column chromatography (elution rate of 2 ml min-1 flow having a total elution of 200 ml) and eluted with Petroleum ether and ethyl acetate in various ratios. The resulting fractions (Fr) were obtained and spotted over precoated silica gel F254 plates (20 × 20 cm, Merck, Germany). The best resolution had been attained using chloroform: ethyl acetate (5: 5 v/v) solvent system, as well as the plates, was dribbled using anisaldehyde–sulphuric acid reagent to visualize the spots. The chloroform portion was subjected to chromatography on silica gel (60 - 120 mesh, Merck) elided with chloroform: ethyl acetate (5:5) solvent system. Repeated chromatography to provide two main steroids i.e. PC-1 (β-Sitosterol) and PC-2 (Stigmasterol) (Rajput and Rajput, 2012). Soon after extraction, the aqueous portion was attained and leftover to stand in a cool place for 72 hours; a yellow colored product separated from the solution. The precipitate was filtered and washed with a combination of chloroform: ethyl acetate: ethanol (50:25:25). The undissolved portion of the precipitate was mixed in hot methanol strained, the filtrate was evaporated to dryness to provide 115 mg yellow powder i.e. PC-3 (Rutin), and its melting point had been determined (Hamad, 2012). The Ethyl acetate fraction was chromatographed utilizing the flash column on a Silica gel eluted with chloroform-methanol step-gradient (starting with 100:0 to 4:1); eluted fractions were put together on their TLC pattern to produce 8 fractions. The chloroform-methanol fraction (10:1) had been chromatographed on a Sephadex LH-20 column eluted with chloroform-methanol (1:1) to give PC-4 (Quercetin) [15].

Phytochemical Screening

The methanolic extract of *A. pilosa* was exposed to qualitative chemical evaluation by using standard techniques as follows.

The Phytochemical screening of carbohydrates was noticed by molisch's test; Proteins were recognized by utilizing two assessments particularly Biuret test and millon's test as well as amino acids through Ninhydrin's test; Steroids had been noticed by salkowski, Libermann- Burchards and Libermann's test; Alkaloids were recognized with freshly prepared Dragendroff's Mayer's, Hager's and Wagner's reagents and noticed for the existence of turbidity or precipitation. The Flavonoids were recognized by utilizing four assessments specifically Shinoda, sulfuric acid, aluminum chloride, lead acetate, and sodium hydroxides. Tannins were identified using four tests particularly gelatin, lead acetate, potassium dichromate and ferric chloride. The froth, emulsion, and lead acetate tests had been applied for the recognition of saponins. The steroids were recognized through (acetic anhydride with sulfuric acid) and (acetic chloride with sulfuric acid) tests. Sample extracted with chloroform was treated with sulfuric acid to examine the existence of terpenoids. Ammonia, as well as ferric chloride solutions, were utilized for the existence of anthraquinone [16-19].

Acute toxicity study

To assess the toxicity of *A. pilosa* extract, the acute toxicity study was executed based on OECD (Organization for Economic Cooperation and Development) 423 guidelines to the dosage of 2000 mg/Kg. The rats were witnessed for 1h constantly after which per hour for 4h and finally every 24h up to 14 days for any physical indications of the level of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or mortality. No animals perished. Therefore the LD50 is greater than 2000 mg/kg. Pre-screening exploration with 200 mg per body weight was carried out [13].

Evaluation of Hepatoprotective activity

Induction of Paracetamol-induced hepatotoxicity

Male Wistar rats with a weight 150 - 180g were intended for the research. Animals were separated into 4 groups of 5 animals each. All rats had been treated orally for 5 successive days [20].

Group I: (Normal control) received 0.5% tween80 (1 ml/kg b.wt. p.o.) for 5 days.

Group II: (Toxic group) received 0.5% tween80 (1 ml/kg b.wt. p.o.) for 5 days and PCM (2 g/kg b.wt. p.o) on the 5th day.

Group III: (Test group), received the methanolic extract of *A. pilosa* (200 mg/kg b. wt. p.o) for 5 days and PCM (2g/kg) was administered 1 hour after the last administration of crude extract.

Group IV: (Standard group), received the Silymarin (25 mg/kg b. wt. p.o) for 5 days and PCM (2 g/kg) was administered 1 hour after the last administration of crude extract.

Evaluation of liver function test

Animals were abandoned and blood was acquired instantly by the way of retro-orbital plexus. Serum was segregated following coagulating at 37°C for 30 min and centrifuged at 3000 rpm for 15 - 20 min. Serum was used for the estimation of biochemical parameters like SGPT, SGOT and ALP and the liver tissues collected were subjected to histopathology. SGOT, SGPT, and ALP were measured by using diagnostic kits [20].

Histopathological studies

Livers of diverse groups had been fixed in 10% buffered neutral formalin for 48h after which with a bovine solution for 6h. Paraffin sections had been attained at 5 mm thickness, prepared in alcohol-xylene sequence and had been stained with alum hematoxylin and eosin. The sections were analyzed microscopically for histopathological changes [13].

Statistical analysis

All values portrayed as mean ± SEM; the n = 5 rat in each group, by means of one-way ANOVA together with Tukey's Multiple Comparison Test making use of Graph Pad Prism-5 software. $P < 0.05$ was regarded as considerable [13].

Results

Acute Toxicity Studies

The methanolic extract of *A. pilosa*, when orally administered in the dose of 2000 mg/kg body wt. did not produce any significant changes in the autonomic or behavioral responses, including death during the observation period.

Phytochemical Screening

The Phytochemical assessment for different extracts viz., petroleum ether, chloroform, ethyl acetate, methanol, and water was executed and outcomes were shown in table 1.

Phytoconstituents	Method	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract
Flavonoids	Shinoda Test	-	+	-	+
	Zn. Hydrochloride test	-	+	-	+
	Lead acetate Test	-	+	-	+
Volatile oil	Stain test	-	-	-	-
Alkaloids	Wagner Test	-	-	+	+
	Hager's Test	-	-	+	+
Tannins and phenols	$FeCl_3$ Test	-	+	-	+
	Potassium dichromate test	-	-	-	+
Saponins	Foaming Test	-	-	-	-
Steroids	Salkowski test	+	-	+	+
Fixed oils and fats	Spot test	+	-	-	-
Carbohydrates	Molish test	-	-	-	+
Acid compounds	Litmus test	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	+
Proteins	Biuret	-	-	-	+

Table 1: *Phytochemical analysis of various extracts of Argyreia pilosa.*

"+": Present and "-": Absent.

Characterization of isolated Phytoconstituents

Stigmasterol

White powder, $C_{29}H_{48}O$, MW 412.69. UV λ_{max} ($CHCl_3$) nm: 257; IR (KBr) ν_{max} 3418 (-OH), 2934, 2866, 2339, 1602, 1566, 1461, 1409, 1383, 1251, 1191, 1154, 1109, 1089, 1053, 1020, 791 cm^{-1}; ESMS m/z (%): 409.2, 395.3, 335, 161, 144, 121.1, 105.1, 97.1, 85.1, 69, 67.2, 65, 50.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.25 (1H, s, OH-2), 5.34-5.35 (1H, d), 5.12-5.18 (1H, m), 4.99-5.05 (1H, m), 3.48-3.56 (1H, m), 2.18-2.31 (2H, m), 1.93-2.09 (3H, m), 1.82-1.87 (2H, m), 1.66-1.75 (1H, m), 1.37-1.54 (13H, m), 1.05-1.31 (m, 7H), 0.99-1.01 (m, 8H), 0.90-0.98 (m, 2H), 0.78-0.85 (m, 9H), 0.66-0.70 (3H, t); ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 140.85 (C-4), 138.31 (C-19), 129.40 (C-20), 121.72 (C-7), 77.34 (C-2), 71.86 (C-11), 56.95 (C-17), 56.09 (C-21), 51.29 (C-10), 50.29 (C-12), 42.41 (C-3), 42.30 (C-18), 40.46 (C-13), 39.77 (C-5), 37.35 (C-6), 36.59 (C-8), 32 (C-9), 31.96 (C-1), 31.91 (C-22), 31.77 (C-16), 28.91 (C-15), 25.41 (C-24), 24.41 (C-23), 21.24 (C-26), 21.14 (C-14), 21.06 (C-29), 19.42 (C-27), 19.03 (C-25), 12.23 (C-28). PC-01 was identified as Stigmasterol.

β-Sitosterol

White powder, $C_{29}H_{50}O$, MW 414.70; UV $\boldsymbol{\lambda}_{max}$ ($CHCl_3$) nm: 251; IR (KBr) $\boldsymbol{\nu}_{max}$ 3424, 2959, 2936, 2867, 1602, 1565, 1465, 1382, 1332, 1242, 1191, 1154, 1051, 779, 450, 432, 416 cm^{-1}; ESMS m/z (%): 411.2, 397.3, 383.3, 311.2, 161.1, 81.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.30 (1H, s), 5.34-5.35 (1H, d), 4.98-5.19 (1H, m), 3.47-3.55 (1H, m), 2.19-2.31 (2H, m), 1.03-1.30 (9H, m), 1.00 (4H, s), 0.90-0.98 (4H, m), 0.76-0.86 (9H, m), 0.68-0.69 (3H, d), 1.94-2.07 (2H, m), 1.79-1.88 (4H, m); ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 140.84 (C-4), 121.70 (C-7), 71.82 (C-2), 56.94 (C-11), 56.85 (C-17), 50.25 (C-10), 45.95 (C-21), 42.39 (C-7), 42.36 (C-3), 39.87 (C-13), 37.34 (C-5), 36.57 (C-6), 36.19 (C-18), 33.78(C-19), 32.15 (C-8), 31.99 (C-9), 31.97 (C-7), 30.39 (C-22), 26.28 (C-20), 25.90 (C-15), 25.40 (C-16), 24.40 (C-24), 23.2 (C-23), 21.17 (C-26), 21.06 (C-14) 21.06 (C-29), 19.32 (C-27), 19.34 (C-25), 12.11 (C-28). PC-02 was identified as β-Sitosterol.

Rutin

Yellow powder, $C_{27}H_{30}O_{16,}$ MW 610.52 ; UV $\boldsymbol{\lambda}_{max}$ (EtOH) nm: 203; IR (KBr) $\boldsymbol{\nu}_{max}$ 1001, 1013, 1065, 1092, 1150, 1166, 1203, 1295, 1362, 1458, 1504, 1566, 1601, 1649, 2340, 3422 cm^{-1}; ESMS m/z (%): 609.1 $(M-1)^-$, 610, 301; ^{1}H NMR (400 MHz, DMSO) δ ppm: 12.6 (1H, s), 10.84 (1H, s), 9.68 (1H, s), 9.18 (1H, s), 7.55-7.56 (1H, d), 7.54 (1H, s) 6.84-6.86 (1H, d), 6.39 (1H, d), 6.2 (1H, d), 5.34-5.36 (1H, t), 5.29 (1H, d), 5.11 (1H, s), 5.07-5.09 (1H, d), 4.53 (1H, s), 4.39 (2H, s). 4.35 (1H, s), 3.70-3.72 (1H, d), 3.21-3.32 (1H, m), 3.05-3.10 (2H, t); ^{13}C NMR (400 MHz, DMSO) δ ppm: 177.35 (C-4), 164.03 (C-7), 161.20 (C-5), 156.57 (C-8a), 156.40 (C-2), 148.37 (C-4'), 144.71 (C-5'), 133.31 (C-3), 121.56 (C-1'), 121.18 (C-2'), 116.26 (C-3'), 115.21 (C-6'), 103.96 (C-6'''), 101.19 (C-6''), 100.70 (C-4a), 98.65 (C-6), 93.55 (C-8), 76.46 (C-2''), 75.90 (C-4''), 74.06 (C-5''), 71.85 (C-2'''), 70.56 (C-5'''), 70.35 (C-3'''), 70.01 (C-4'''), 68.20 (C-3''), 66.97 (C-2a), 17.68 (C-2'''). PC-03 was identified as Rutin.

Quercetin

Yellow powder, $C_{15}H_{10}O_7$, MW 302.23; UV $\boldsymbol{\lambda}_{max}$ (EtOH) nm: 210; IR (KBr) $\boldsymbol{\nu}_{max}$ 3413, 2340, 1607, 1565, 1523, 1462, 1408, 1383, 1320, 1263, 1199, 1168, 1131, 1014, 959, 782, 465, 457, 441, 423 cm^{-1}; ESMS m/z (%): 301 $(M-H)^-$. 301.9, 300; ^{1}H NMR (400 MHz, DMSO) δ ppm:) 12.49 (1H, s), 10.77 (1H, s). 9.57 (1H, s), 9.29-9.33 (2H, d), 7.68-7.69 (1H, d), 7.53-7.69 (1H, m), 6.88-6.90 (1H, d), 6.41 (1H, d), 6.19 (1H, d); 13C NMR (400 MHz) 175.81 (C-7), 163.85 (C-1), 160.70 (C-3), 156.17 (C-5), 147.67 (C-9), 146.81 (C-15), 145.03 (C-8), 135.68 (C-11), 121.96 (C-12), 119.96 (C-13), 115.59 (C-16), 115.08 (C-4), 103.01 (C-2), 98.16 (C-6), 93.33. PC-04 was identified as Quercetin (Figure 1).

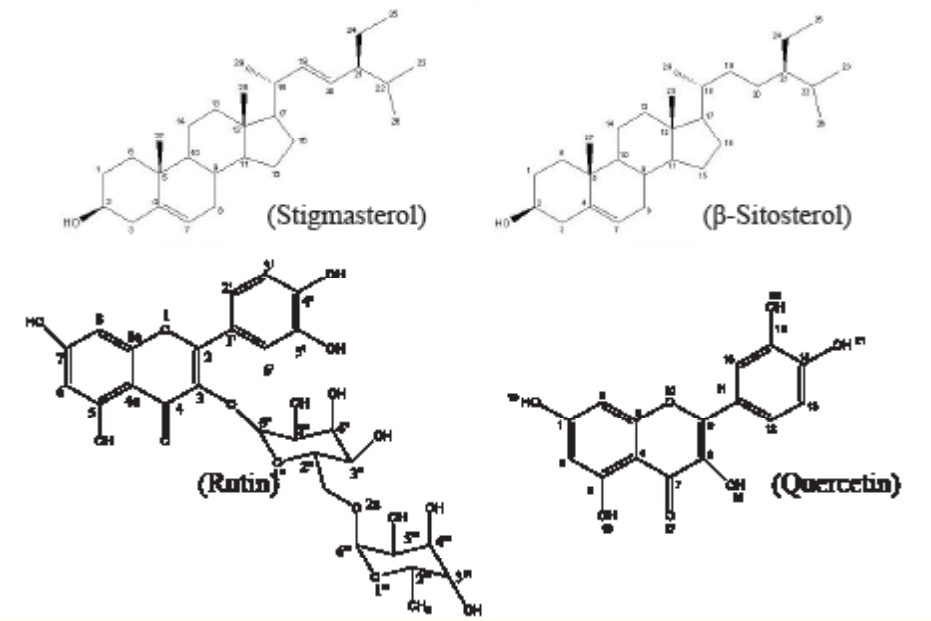

Figure 1: *Chemical Compounds Isolated from A. pilosa plant extract.*

Hepatoprotective activity

Serum biochemical parameters are shown in table 2. PCM administration induced destruction to hepatocytes confirmed by the raised level of liver enzymes (ALT, AST, and ALP), total Bilirubin and Bilirubin as compared to control. Increased levels of these enzymes are the sign of cellular damage and loss of functional integrity of hepatocytes. A single dose of PCM (2 g/kg) significantly higher ($P < 0.001$), increased the SGPT, SGOT, ALP, Bilirubin, cholesterol and protein levels (282.67, 190.67, 252, 0.5, 86.82 and 5.44 units/mL) when compared to the normal animals (156, 50, 276, 0.43, 38.08 and 8.11 units/mL) respectively indicating elevation in enzyme levels. Treatment of the rats with the MEAP (200 mg/Kg) has decreased the enzyme levels in the of 237 units/mL for SGPT, 167.33 units/mL for SGOT, 260.67 units/mL for ALP and 0.43 units/mL for Bilirubin, 65.53 units/mL for cholesterol and 6.06 units/mL for total proteins which were found to be comparable to the enzyme levels (SGPT, SGOT, ALP, Cholesterol and TP) elevated by PCM induced rats. Standard drug Silymarin also reduced the enzyme levels in the range of 215, 95.67, 282.67, 0.44, 44.68 and 7.15 units/mL, for SGPT, SGOT, and ALP, Bilirubin, cholesterol and total protein levels respectively. Results for histopathological examination are given in figures 2 and 3.

Silymarin is a well-established Hepatoprotective drug able to reduce the elevated levels of liver enzymes in various drug-induced hepatotoxicity. The administration of test compounds raised the reduced level of total protein in the level of 6.06 units/mL and also decreased the elevated values of other enzymes as compared to toxicity value induced (Table 2).

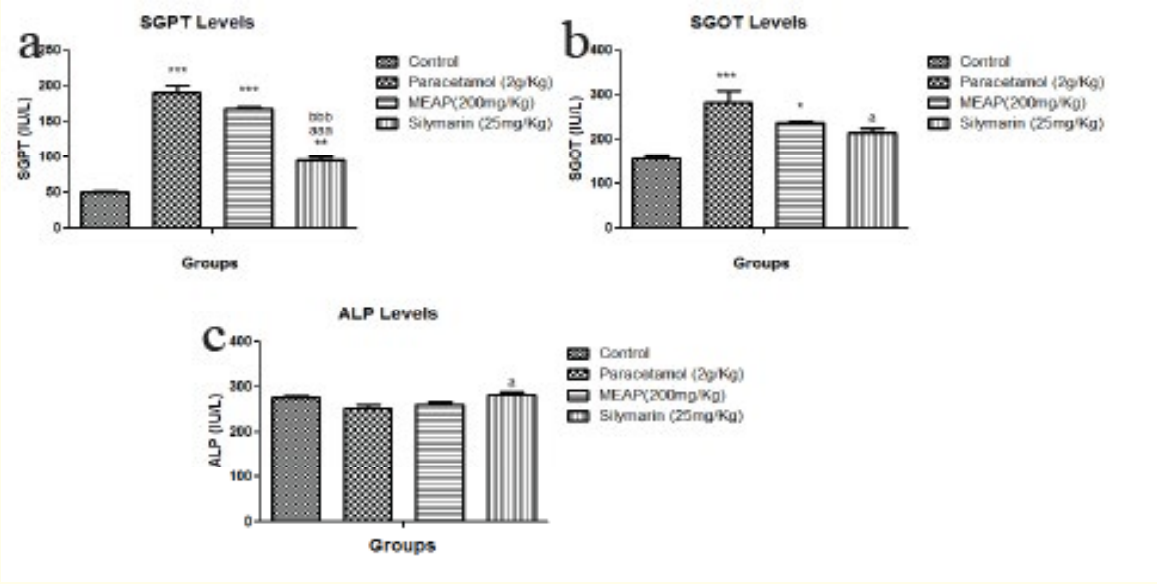

Figure 2: *Serum enzymes indices of liver toxicity in rats intoxicated with PCM and administered MEAP (200 mg/Kg) and Silymarin (25 mg/Kg). All values expressed as mean ± SEM; the n = 5 rat in each group, by one-way ANOVA followed by Tukey's Multiple Comparison Test. (a) Serum Glutamic Pyruvic transaminase activity in all groups. ***, p < 0.001 versus control, **, p < 0.01 versus control, aaa, p < 0.001 versus PCM, bbb, p < 0.001 versus MEAR (200 mg/Kg). (b) Serum glutamic oxaloacetic transaminase activity in all groups. ***, p < 0.001 versus control, *, p < 0.05 versus Control, a, p < 0.05 versus PCM. (c) Alkaline phosphatase activity in all groups *, p < 0.05 versus control.*

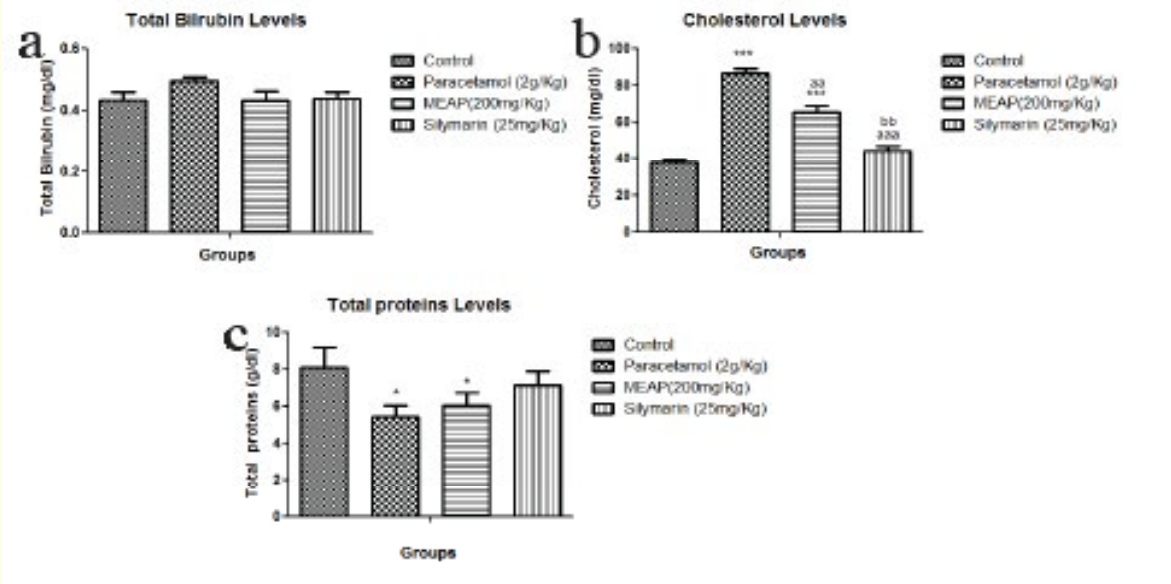

Figure 3: *Serum enzymes indices of liver toxicity in rats intoxicated with PCM and administered MEAP (200 mg/Kg) and Silymarin (25 mg/Kg). All values expressed as mean ± SEM; the n = 5 rat in each group, by one-way ANOVA followed by Tukey's Multiple Comparison Test. (a) Serum Bilirubin level in all groups. (b) Serum cholesterol level in all groups. ***, p < 0.001 versus control, aaa, p < 0.001 versus PCM, aa, p < 0.01 versus PCM, bb, p < 0.01 versus MEAR (200 mg/kg). (c) Protein level in all groups. *, p < 0.005 versus control.*

Treatment groups and liver-specific Variables	I (Normal Control: 0.5% Tween 80 1ml/kg b.wt)	II (Hepatotoxic Control: 0.5% Tween80 1 ml/kg b.wt+PCM (2 g/Kg b.wt)	III (MEAR 200 mg/kg b.wt + PCM 2 g/kg. b.wt)	IV (Silymarin 25 mg/kg b. wt. + PCM 2 g/kg b. wt.)
AST (U/L)	156 ± 10.8	282.67 ± 44.3	237 ± 5.6	215 ± 14
ALT (U/L)	50 ± 2.61	190.67 ± 15.7	167.33 ± 4.5	95.67 ± 7.5
ALP (U/L)	276.33 ± 6.8	252 ± 14.2	260.67 ± 9.1	282.67 ± 9.1
Total Bilirubin (mg/dl)	0.43 ± 0.045	0.5 ± 0.021	0.43 ± 0.05	0.44 ± 0.036
Cholesterol (mg/dl)	38.08 ± 2.32	86.82 ± 4.29	65.53 ± 6.05	44.68 ± 4.01
Total proteins (g/dl)	8.11 ± 1.86	5.44 ± 1.06	6.06 ± 1.19	7.15 ± 1.33

Table 2: *Effects of pretreatment with A. pilosa methanolic extract on the serum levels of AST, ALT, ALP, Bilirubin, cholesterol and total proteins in PCM induced hepatotoxicity in rat.*

All values expressed as mean ± SEM; a n = 5 rat in each group, by one-way ANOVA followed by Tukey's Multiple Comparison Test. **,* $p < 0.001^{a}$*,* $p < 0.01^{b}$*, p < 0.05 versus PCM treated group.*

Histopathology

The histological study of the liver section of the normal control group revealed the normal architecture of normal liver histology i.e., hepatic central vein and sinusoids (Figure 4A). The liver sections of rats given PCM alone confirmed notable hepatic cell necrosis (Figure 4B). The liver section of the rat given PCM and Silymarin-treated groups maintains the almost normal structure of hepatocytes (Figure 4D). In MEAP (200 mg/Kg) treated groups, showing areas of regeneration and minor hepatic damage (Figure 4C).

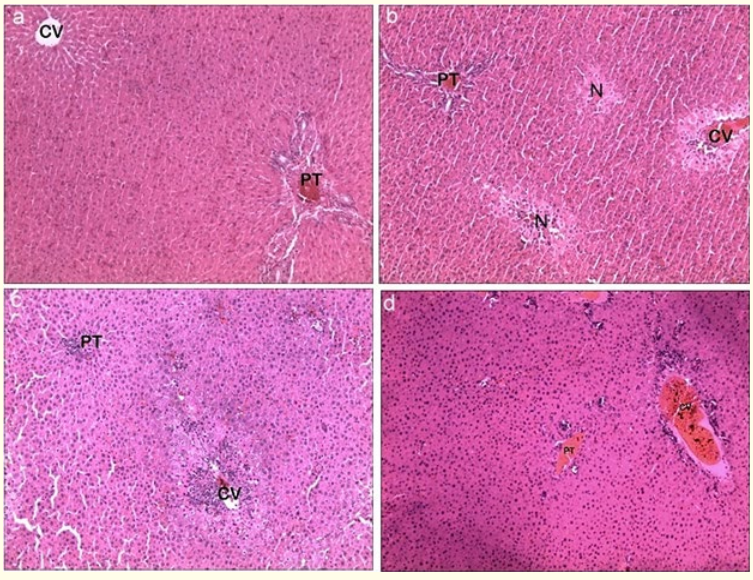

Figure 4: *Paraffin sections of liver stained with hematoxylin and eosin for histopathological changes. (A) Liver section of control group showing the normal architecture of normal liver histology. (B) Hepatotoxic liver after treatment of PCM (2 gm/Kg) (C) Liver section treated with PCM + MEAP (200 mg/kg) (D) Liver Section treated with PCM and Silymarin (25 mg/Kg).*

PT: Portal Triad; CV: Central Vein; N: Necrosis.

Discussion and Conclusion

Plant medicines play a significant role in their various formulations for the remedying of various diseases. A few are already examined and scientifically validated for their potentials. Here, we designed the experiments to examine the Hepatoprotective activity of MEAP for their development into safe natural drug candidates.

N-Acetylcysteine might certainly protect against the hepatotoxicity of paracetamol in a number of ways. N-acetvlcvsteine acts as a progenitor for glutathione synthesis. Glutathione has a crucial part in the protection against hepatic necrosis caused by paracetamol. Hepatocellular necrosis takes place only if the hepatic content of glutathione drops beneath a critical limit concentration. A stimulation of glutathione synthesis following the administration of N-acetylcysteine and therefore an increased availability of glutathione for the detoxification of the noxious paracetamol intermediate ought to protect against hepatic injury [21].

PCM is widely used as an antipyretic drug that is safe in therapeutic doses, however, could cause fatal hepatic damage in human beings and animal at higher toxic doses. Bioactivation of PCM by hepatic cytochrome P- 450 results in the formation of an extremely reactive and toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is generally detoxified through conjugation with reduced glutathione (GSH) to form the mercapturic acid that is eliminated by urine. The toxic overdose associated with PCM impoverishes hepatic GSH content so free NAPQI binds covalently to cellular mitochondrial proteins that inhibit mitochondrial fatty acid -oxidation and leads to significant necrosis and apoptosis of hepatocytes [13,22]. A distinct sign of hepatic injury is the leaking of cellular enzymes like ALT, AST, and ALP into plasma because of the disruption caused by the transport functions of hepatocytes. ALT is more specific to the liver, and it is a surpassing criterion for analyzing hepatic injury. Higher levels of AST signify the cellular exudation together with the diminished functional ability of cell membrane in the liver. Serum ALP is also elevated in liver injury. High concentration of ALP results in serious hepatic damage in PCM treated rats [23]. The liver is the key source of the majority of the serum proteins. Bilirubin is a product of home within the reticuloendothelial system; it's marked up in the bloodstream could be adduced to overproduction, increased hemolysis, decreased conjugation or impaired Bilirubin transport [24]. Bilirubin is a recueil which is used to assess the normal functioning of the liver rather than the extent of the hepatocellular injury. Phytoconstituents including Phytosterols and Flavonoids are well recognized for their antioxidant and Hepatoprotective activities. Phytochemical analysis of the methanolic extract of *A. pilosa* revealed the presence of Flavonoids, alkaloids, tannins, phenols, steroids, fixed oils, fats, acid compounds, glycosides, amino acids, and proteins.

In SGPT, PCM treated group demonstrated a significant increase ($P < 0.001$) in the values in comparison to control group. There was a significant change ($p > 0.05$) in the enzyme level of the treated group (MEAP) in comparison to PCM treated group.

In SGOT, PCM treated group exhibited a substantial increase ($P < 0.001$) in the values compared to control group. There was a tremendous change ($p > 0.05$) in the enzyme level reduction of the treated group (MEAP) compared to PCM treated group.

In ALP, PCM treated group exhibited a substantial increase ($P > 0.05$) in the values compared to control group. There was a tremendous change ($p > 0.05$) in the enzyme level reduction of the treated group (MEAP) compared to PCM treated group (Figure 2).

In total Bilirubin, PCM treated group exhibited a substantial increase ($p > 0.05$) in the values compared to control group. There was a significant declination ($p > 0.05$) in the enzyme level reduction of the treated group (MEAP) compared to PCM treated group.

In Cholesterol, PCM treated group exhibited a substantial increase ($p < 0.001$) in the values compared to control group. There was a significant reduction ($p < 0.01$) in the enzyme level reduction of the treated group (MEAP) compared to PCM treated group.

In the Total protein, PCM treated group exhibited a significant increase ($p < 0.05$) in the values in comparison to control group. There was a significant reduction ($p > 0.05$) in the enzyme level reduction of the treated group (MEAP) in comparison to PCM treated group (Figure 3).

Our results provided strong evidence that *A. pilosa* extracts significantly inhibited the acute liver toxicity induced by high doses of PCM in the rat, as shown by a decrease in serum liver enzyme activities (AST, ALT, and ALP) and Bilirubin concentrations (Table 2). Moreover, the liver morphology and histopathology findings confirm the protective activity of this extract against the PCM induced liver damage as it is evident by the reversal of centrilobular necrosis in hepatic parenchyma by *A. pilosa* administration. Thus, as shown in figure 4C, the extract restores the hepatocytes to normal architecture was observed. Despite the fact that *A. pilosa* extracts significantly reduced SGPT, SGOT, ALP, Bilirubin and Cholesterol levels in groups III completely restore these biochemical parameters to the normal values.

In conclusion, the results of the study demonstrate that methanolic extract of *A. pilosa* possesses Hepatoprotective activity against PCM induced liver injury in the rat. This property was attributed to the presence of Flavonoids i.e. Rutin, Quercetin which can normalize the disturbed antioxidant status possibly by maintaining the levels of glutathione through by inhibiting the production of malondialdehyde or might be because of inhibition of toxicant activation and the enhancement of body defense system. The hepatoprotection afforded by sterols i.e. β-Sitosterol and Stigmasterol were associated with the enhancement of mitochondrial glutathione redox status, possibly with the glutathione reductase-mediated improvement in mitochondrial glutathione redox cycling. Thus, these kinds of Flavonoids and Phytosterols serve as a potential mitohormetic agent for the prevention of oxidative stress evoked in the liver.

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Volume 5 Issue 2 November 2017