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In vitro antioxidant and anti-hepatotoxic activities of roots of *Potentilla fulgens* Wall: An Ethno Medicinal Plant of North Eastern India

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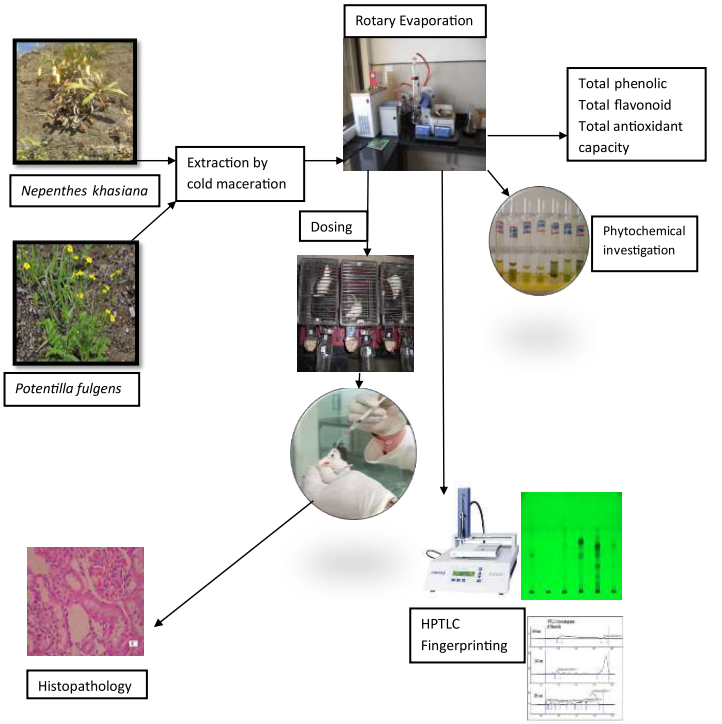
Graphical Abstract



Abstract

Aim: To evaluate the *in vitro* antioxidant and hepatoprotective activity of *Potentilla fulgens* root extracts against ethanol-induced hepatotoxicity.

Materials and methods:In vitro antioxidant activity for the methanolic and aqueous root extracts of *Potentilla fulgens* was carried out by DPPH, superoxide anion and hydroxyl radical scavenging methods. For hepatoprotective effect, the hepatic markers such as alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin and total proteins were estimated using standard kits. The histopathological studies of the liver sections excised from rats was also done.

Findings: Preliminary phytochemical test reveals the presence of carbohydrate, flavonoids, alkaloids, tannins and phenolic compounds. *In vitro* antioxidant activity shows that the methanolic root extract of *Potentilla fulgens* exhibited strong antioxidant capacity with IC50 values of 60.27µg/ml (DPPH) , 73.12µg/ml (Superoxide anion radical) and 80.55µg/ml (Hydroxyl radical) respectively in comparison to the aqueous extract. Hence, the methanolic extract was selected for hepatoprotective studies. The results showed that administration of methanolic root extract of *Potentilla fulgens* inhibits the liver damage due to alcohol as evident from histopathological studies of the liver. The HPTLC fingerprinting analysis of the methanolic root extract of *Potentilla fulgens* at different wavelengths showed numerous peaks indicating the presence of many compounds which may provide basic information regarding the isolation, purification, characterization and identification of marker chemical compounds of the plant species.

Conclusion: From our study, the methanolic root extract of *Potentilla fulgens* shows potent hepatoprotective effectsin a dose dependant manner, hence it can be used as a natural protecting agent against liver damage.

Keywords: Potentilla fulgens; DPPH; ethanol- induced liver injury; hepatic markers.

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Introduction

Liver is the largest and important glandular organ in the body which performs multiple critical functions to keep the body pure of toxins and harmful substances and can synthesize useful principles(Shanani,1999). It plays an important role in maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways associated with growth, immunity, nutrition, energy provision and reproduction(Ward *et al*,1999). Due to this unique metabolizing property and relationship to the gastrointestinal tract, liver is the main target for toxicity produced by drugs, xenobiotics and oxidative stress. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Subramonium *et al*,1999). Liver disease is still a worldwide health problem and are mainly caused by drugs like acetaminophen (in large doses), toxic chemicals, and excess consumption of alcohol, infections and autoimmune disorders. Alcohol abuse and alcoholism are serious current health and socioeconomic problems throughout the world (World Health Organization, 2014). Because liver is primarily responsible for alcohol metabolism it is especially vulnerable to alcohol related injury leading to a global disease burden. It affects the liver in both nutritional disturbances and damaging cells, leading to life-threatening liver diseases ranging from fatty liver (steatosis) to alcoholic hepatitis and cirrhosis (Bouneva *et al*,2003) . There is an acute necessity of reliable hepatoprotective drugs in modern medical practice to prevent and treat drug-induced liver damage. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al*, 1992). Even with the progress in modern medicine; there is no effective drug available that could stimulates liver function, offers protection to liver from damage or help to regenerate hepatic cells (Chattopadhya,2003) . A number of natural remedies from medicinal plants are recommended to be effective and safe alternative treatments for the treatment of liver disorders (Chatterjee, 2000) and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs.

Potentilla fulgens (Rosaceae) commonly known in English as *'Himalayan Cinquefoil'*, *'Bajradanti'* in Assamese and Hindi, *'Ganephul'* in Nepali and *'Lynniangbru* or *lynniangkynthei'* in Meghalaya, 'Roishing' in Arunachal, have been widely used as a vital medicinal plant of the Himalayan region (Laloo *et al*, 2013) . In their natural habitat they are commonly found in the higher altitudes (1500–2000 MSL) of Meghalaya (Khasi and Jaintia Hills),Sikkim, Assam, Manipur, Arunachal Pradesh, Nagaland, Himachal Pradesh, and Uttarakhand states in India. More than three hundred species of genus Potentilla are used in Ayurvedic, Unani, Siddha, Chinese and Tibetan systems of medicines (Tomczyk *et al*, 2009) due to high polyphenol content in their aerial and underground parts. This plant has been known since ancient times for its curative properties.

The aerial and/or underground parts have been used in the treatment of inflammations, wounds, certain forms of cancer, jaundice, infections due to bacteria, fungi and viruses, diarrhoea, diabetes mellitus and other ailments (Anonymous,1969). In Nepal and Bhutan, plant juice is taken for the treatment of stomach problems, cough, cold and respiratory complaints. In Meghalaya, it has been used as folk remedy for a variety of ailments, including diabetes mellitus. The root- stock and whole herb is utilized as astringent and tonic curing gum and tooth ailments (pyorrhea, toothache and caries). In medieval ages, the plant extracts (water, milk, honey and alcoholic) were used for curing mouth ulcers, dysentery, haemostatic and wound healing (Kumar,1998)Earlier experimental studies on this plant have revealed that its root extract possesses hypoglycaemic, anti-hyperglycemic, anti-hypolipidemic and antioxidant activity(Syiem *et al*,2002; Syiem *et al*, 2009; Syiem *et al*, 2009) antitumor (Rosangkima *et al*, 2004), gastroprotective(Laloo *et al*,2013), anthelmintic(Roy *et al*,2010)activities.The root of the plant is used as one of the ingredient in the formulation of 'ViccoVajradanti tooth powder and toothpaste' manufactured by Vicco Laboratories, India (Farooqui *et al*, 2001).

Materials and Methods

Plant material

The roots of *Potentilla fulgens* werepurchased fromIewduh (Bara bazar), a local market in Shillong, Meghalaya. The botanical identification of the plant material was done by Dr. B. K. Sinha Scientist-E & H.O, at the Herbarium of the Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya where a voucher of the specimen was deposited.

Preparation of extracts

The roots were washed, dried under shade, cut into small pieces and coarsely powdered. Two hundred grams of the dried powdered material was extracted by cold maceration separately with methanol and water under room temperature for seven days with occasional shaking and stirring. The solvent was completely evaporated under reduced pressure using IKA Rotary Evaporator at 40° C. The concentrated mass obtained was kept in hot air oven at 40° C until it is completely dried, finally the product is packed in airtight container for further studies.

Test Animals

Adult Wistar albino rats weighing 150-200 g of either sex were used in the experimental work. The animals were housed in groups of six comprising of six animals each and maintained under standard conditions of temperature, humidity and 12/12 h light/dark cycles, fed with standard rat diet and clean drinking water *ad libitum* throughout the experimental schedule. The experimental design was approved by the Institutional Animal

Ethics Committee (IAEC) of KLE College of Pharmacy, Belgaum, Karnataka (Reg. No. 221/CPCSEA; 16th June 2000), protocol approval resolution number KLECOP/IAEC/res.17-31/08/2013.

Chemicals

Folin-Ciocalteu reagent , gallic acid, quercetin, aluminium chloride, trichloroacetic acid (TCA) , 2-deoxy-2-ribose, trisHCl, ascorbic acid, thiobarbituric acid (TBA), ferric chloride, ammonium molybdate, nitrobluetetrazolium, methanol were procured from Hi-Media, Merck and Rankem . 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldirch. Silymarin (10gms) was received as a gift sample from ZydusCadilla, Ahmedabad. Diagnostic Kits of ERBA Diagnostic Mannheim GmbH, Germany was used for biochemical parameters. All chemicals and solvents used for the experimental work were of analytical grade.

Phytochemical screening

Preliminary phytochemical screening of the prepared plant extracts was performed as per the standard method (Kokate, 2005, Trease and Evans, 2002) to identify the presence of various phytoconstituents.

HPTLC fingerprinting analysis

The HPTLC fingerprinting analysis of the methanolic root extract of *Potentilla fulgens* was developed whereby a sample volume of 5µl were spotted in the form of bands of width 8mm with a 100 µl syringe with the help of Linomat 5 applicator. The mobile phase for developing the chromatogram was composed of toluene: chloroform: ethanol mixture in the ratio 4:4:1 (v/v/v). The study was carried out using a CAMAG-HPTLC instrumentation equipped with a CAMAG TLC scanner 3, CAMAG TLC visualizer and WINCATS software for data interpretation. The Rf values were recorded and the developed plate was screened and photo-documented at three different wavelength (λ max) of 240 nm, 366 nm and 540nm respectively.

Estimation of total phenolic content

Total phenolic content of the methanolic and aqueous root extract of *Potentilla fulgens* were determined by Folin–Ciocalteau assay (Ainsworth,2007).1.0 ml of the extract solution in a test tube was added to 0.2 ml of Folin- Ciocalteu reagent (1:1 in distilled water) and 2.0 ml of sodium carbonate (20%) was added. The contents were vortexed and kept in the dark for 40 minutess and the absorbance was measured at 725 nm. The total phenolic content in the extract was determined by using gallic acid as a standard and the values were expressed as mg gallic acid equivalent (GAE) per gram of the dry sample.

Estimation of total flavonoid content

Total flavonoid content of the extracts was determined by Aluminum chloride method as described by Zhischen et al (Zhischen *et al*,1999). Different concentrations of the methanolic and aqueous root extracts of *Potentilla fulgens* were prepared. Each sample was diluted with distilled water. 3ml of 5% sodium nitrite and 0.3ml of 10% aluminium chloride was added to the samples. After 6 minutes, 2ml of 1M sodium hydroxide were added to the mixture. The absorbance was measured at 510nm. Quercetin was used as a standard to calculate the concentration of flavonoid content and the values were expressed as mg quercetin equivalents (QE) per gram of the dry sample.

Estimation of total antioxidant content

The method described by Prieto *et al* (Prieto *et al*, 1999) was used for the determination of total antioxidant capacity. The reaction mixture consists of 0.1ml of plant extract (100 µg) solution and 1.0 ml of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated in a water bath at 95°C for 90 min. After cooling the absorbance was measured at 695 nm. The antioxidant capacity was calculated in terms of mg of gallic acid equivalent (GAE) per gram of dry extract.

Free radical scavenging assays

DPPH radical-scavenging activity (Brand-Williams *et al*,1995)

DPPH was prepared in methanol and the reaction mixture consist of 2ml of 0.1 mM DPPH solution added to the plant extracts of varying concentrations . The mixture was vortexed and allowed to stand for 30 minutes at room temperature. The absorbance was measured at 517nm against a blank. The percentage of antioxidant activity was calculated by the following equation:

% inhibition = [(Absorbance of control-Absorbance of sample)/Absorbance of control] ×100

Superoxide radical scavenging activity(Hsu *et al*, 2007)

For measuring superoxide anion scavenging activity the reaction mixture consist of 0.5 ml of NBT (0.3 mM), 0.5 ml NADH solution (0.936 mM), 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8) . The reaction was initiated by the addition of 0.5 ml PMS solution, incubated at 25°C for 5 minutes and the absorbance was measured at 560 nm. Quercetin was used as a standard. The percentage of inhibition was calculated using the following formula:

% inhibition= [(Absorbance of control-Absorbance of sample)/Absorbance of control]*100

Hydoxyl radical scavenging activity

The hydroxyl radical scavenging activity was assayed by the Deoxyribose method(Samak *et al*,2009).The reaction mixture contained 3ml of phosphate buffer (0.1M, pH 7.4), 1 ml of 2-deoxyribose (10mM) , 0.5ml of EDTA (20 mM) , 0.5ml of ferric chloride (20 mM) , 3.8 ml of distilled water, various concentrations of plant extracts ,1 ml of Hydrogen peroxide (10 mM) and 0.1ml of ascorbic acid (1mM). The above mixture was

incubated at 37ºc for 1hr. Thereafter 5 ml of trichloro acetic acid (TCA) (2.8%; w/v) and 5 ml of 1% aqueous thiobarbituric acid (TBA) was added to the reaction mixture. It was heated for 15 minutes on a boiling water bath until a pink color has developed. After cooling the absorbance was taken at 532 nm against a blank. The scavenging activity on hydroxyl radical was calculated as follows:

% inhibition= [(Absorbance of control-Absorbance of sample)/Absorbance of control]*100

Experimental procedure

Rats were divided into six experimental groups (n=6). The test group animals were pre-treated with the methanolic root extract of *Potentillafulgens* (100, 200 and 400 mg/kg/day p. o) for seven days. Thereafter,all the groups except the normal group received ethanol at a dose of 5 g/kg b.w, 30% w/v once daily per oral and 2 hour apart. The normal group received normal saline (1ml/kg b.w) in addition to their normal diet. The duration of the experiment was 21days.

The animals were then anesthetized using anaesthetic ether24 h after the last treatment and blood was collected by retro orbital puncture. The blood was allowed to clot and serum was separated at 3000 rpm for 15 min for biochemical investigations. The level of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin and total protein were determined by using commercially available kits (ERBA Diagnostic Mannheim GmbH,Germany) following the manufacturer's standard procedure. The animals were sacrificed immediately after blood collection and liver was dissected out, washed and their wet weight was determined. The livers were washed in normal saline and fixed in 10% formalin and later dehydrated with alcohol. The liver tissue were embedded in paraffin and 3-5 μ thickness microtome sections were made. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

Statistical analysis

The data for various biochemical parameters were analyzed by one way ANOVA followed by Tukey's multiple comparison tests using statistical soft wares. P values <0.01 were considered as statistically significant.

Results

Phytochemical investigation of the crude extract

The percentage yield of the methanolic and aqueous root extract of *Potentilla fulgens* (PFM and PFA) after cold maceration was found to be 13.84%w/w and 16.27%w/v of the plant material. Preliminary phytochemical screening reveals the presence of carbohydrates, flavonoid, alkaloid, tannins and phenolic compounds as shown in Table 1.

Table 1: Preliminary phytochemical analysis of the methanolic and aqueous root extracts of *Potentillafulgens*

Phytoconstituents	Test performed	PFM	PFA
Carbohydrates	Molisch's test, Fehling's test, Barfoed's test, Benedict's test	+ve	+ve
Flavonoids	Shinoda test, Lead acetate test	+ve	+ve
Steroids	LibermannBurchard test, Libermann's test	- ve	- ve
Alkaloids	Dragendorff's test ,Mayer test,Hager's test , Wagner's test	+ ve	+ve
Tannins and phenolics	5% Ferric Chloride, dilute iodine solution, dilute $KMnO_4$	+ ve	+ ve

+ve: indicates the presence of phytoconstituents , -ve: indicates the absence of phytoconstituents

The total phenolic content of PFM and PFA were found to be 189.66 ± 1.36 and 172.13± 1.75mg/g (gallic acid equivalent per gram of the dry extract), respectively .The total flavonoid content were found to be 91.41±9.30mg/g and 22.6±2.16 mg/g (quercetin equivalent per gram of the dry extract), respectively. The total antioxidant content were found to be 226.95±2.27mg/g and 215.2 ±2.32mg/g (gallic acid equivalent per gram of the dry extract), respectively as shown in Table 2.

HPTLC fingerprinting analysis of the methanolic root extract of *Potentilla fulgens* revealed numerous peaks as shown in Fig 1, 2 & 3. The spots of the entire chromatogram were visualised at 254, 366 and 540 nm and the different Rf values were recorded in Table 4.

Table 2: Total phenolic, total flavonoid, and total antioxidant content of the root extracts of *Potentillafulgens*

S.No	Name of extracts	Total phenolic (mg GAE /g) of dry extract	Total flavono id (mg QE /g) of dry extract	Antioxidant capacity (mg GAE /g) of dry extract
1	*Potentilla fulgens* (Met)		91.41±9.3	226.95±2.27
2	*Potentilla fulgens* (Aq)	172.13±1.75	22.6±2.16	215.2±2.32

The average values of three calculations are presented as mean ± SEM (n=3)

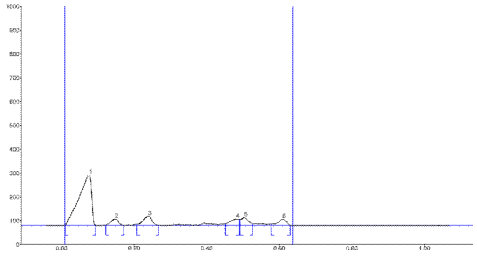

Fig 1: HPTLC chromatograms of the methanolic root extract of *Potentilla fulgens* showing six different peaks of phytoconstituents at 254nm

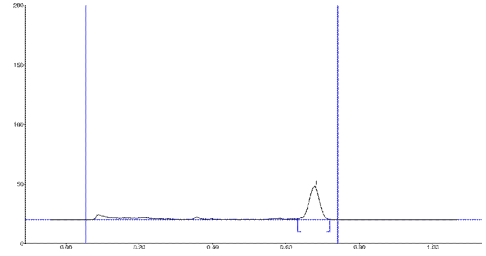

Fig 2: HPTLC chromatograms of the methanolic root extract of *Potentilla fulgens* showing a single peak of phytoconstituent at 366nm

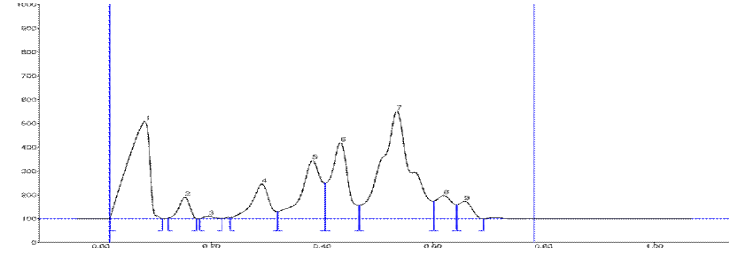

Fig 3: HPTLC chromatograms of the methanolic root extract of *Potentilla fulgens* showing nine different peaks of phytoconstituents at 540nm

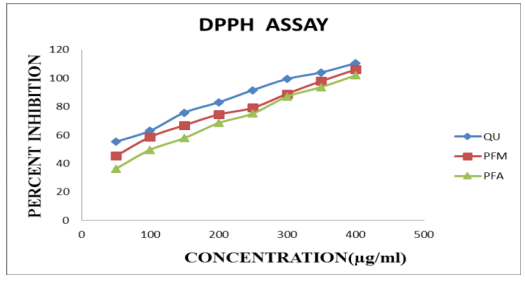

Fig 4. DPPH radical scavenging activity of the root extracts of *Potentilla fulgens*.

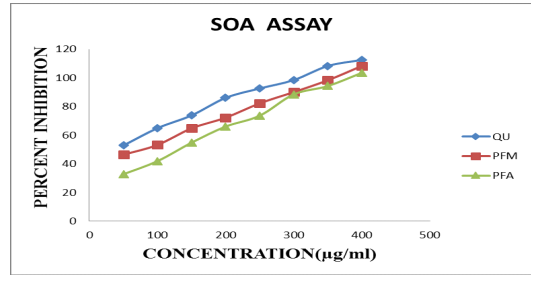

Fig 5. Superoxide anion radical scavenging activity of the root extracts of *Potentilla fulgens*.

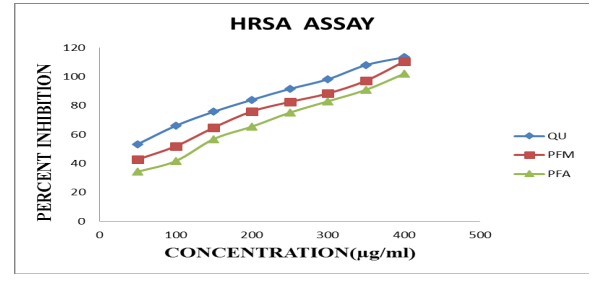

Fig 6. Hydroxyl radical scavenging activity of the root extracts of *Potentilla fulgens*.

*QU; Quercetin, PFM; methanolic root extract of *Potentilla fulgens*, PKA; aqueous root extract of *Potentilla fulgens*.

Free radical scavenging activity

In the DPPH scavenging assay the IC50 (the concentration required to scavenge 50% of radical) values of Quercetin, PFM and PFA were 3.907µg/mL,60.27 µg/mL and 109.74 µg/mL respectively. In the superoxide anion scavenging assay the IC50 values of Quercetin, PFM and PFA were 12.73 µg/mL, 73.12µg/mL and 130.82µg/mL respectively. In the hydroxyl radical scavenging assay the IC50 values of Quercetin, PFM and PFA were 9.19 µg/mL, 80.55 µg/mL and 128.10 µg/mL, respectively as shown in Fig 4, 5 & 6.

Hepatoprotective activity

Ethanol intoxicated rats developed a significant hepatic damage which is shown by the elevated serum levels of hepato specific enzymes like ALT, ASTT, ALP and total bilirubin with a significant decrease in total protein levels as compared to the normal group. However, the amount of these enzymes reduced after treatment with varying doses of the methanolic root extract of *Potentilla fulgens*. The levels of the above enzymes were significantly reversed after treatment in a dose dependant manner. A significant decrease on the levels of ALT,AST, ALP ,TB and an increase in the total protein level was found to be the greatest on treatment with a higher dose (400mg/kg bw) as shown in Table 3.

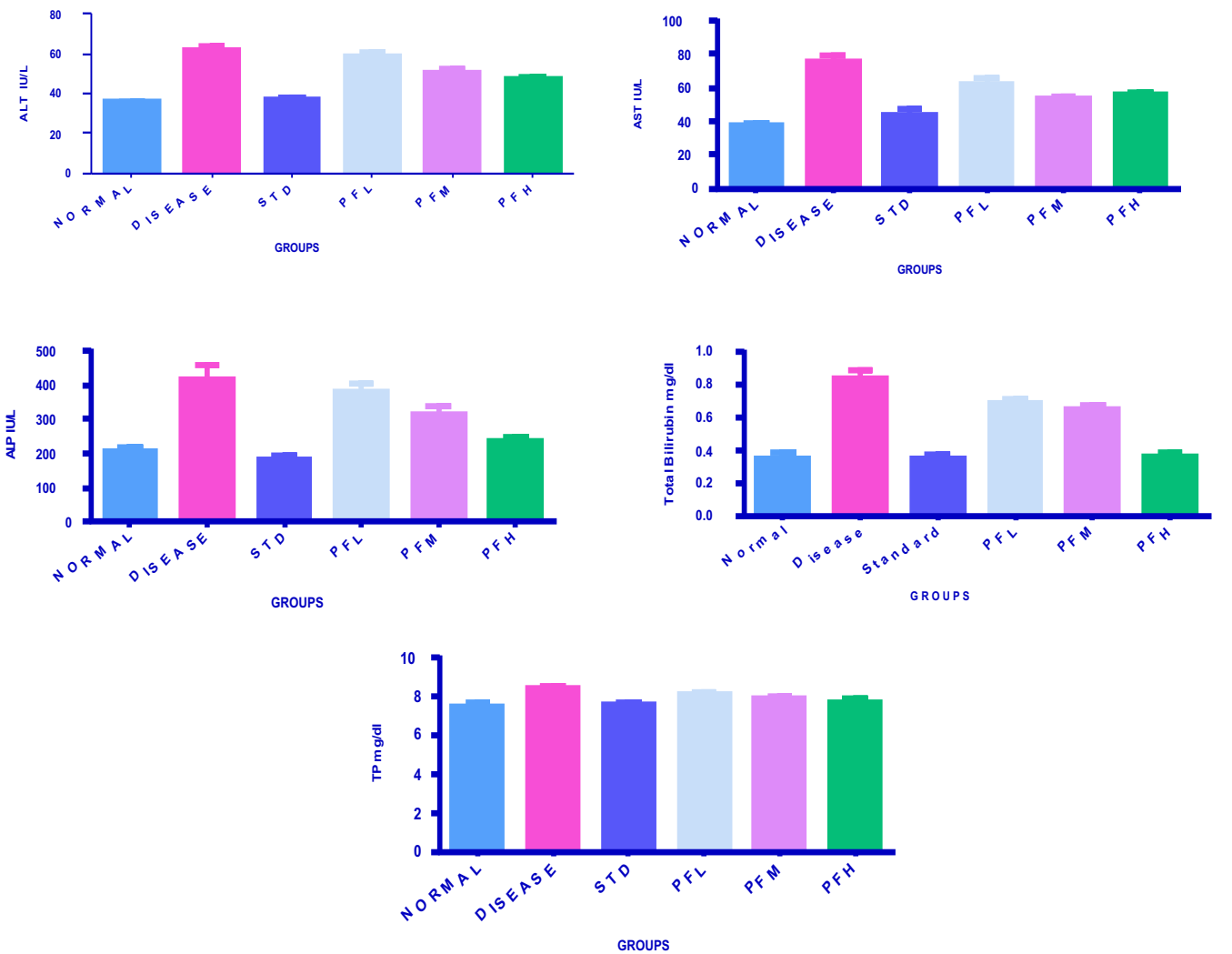

Fig 7: Effect of the methanolic root extract of *Potentilla .fulgens* on serum biochemical parameters against ethanol (5 g/kg bw, 30% w/v) induced liver injury. (A) Representation of alanine aminotransferase. (B) Representation of aspartate aminotransferase. (C) Representation of alkaline phosphatase. (D) Representation of total bilirubin. (E) Representation of total proteins. Values are expressed as mean ±S.E.M with six animals in each group. * indicates statistical significance ***p<0.0001, **p<0.001, *p<0.01 vs. ethanol intoxicated group, # p<0.0001 vs normal group.PFL: *Potentilla fulgens* low dose, PFM: *Potentilla fulgens* medium dose and PFH: *Potentilla fulgens* high dose.

Table 3:Effect of themethanolic root extract of *Potentillafulgens* on hepatic markers in the serum of normal and ethanol-administered rats

GROUP	ALT (IU/L)	AST(IU/L)	Alk .P (IU/L)	TB (mg/dl)	TP (mg/dl)
Normal		37.50 ± 1.176	203.0 ± 12.51	0.3500 ± 0.04282	7.500 ± 0.1265
Alcohol induced (5 g/kg/day)	61.83 ± 2.301 #	75.67 ± 3.383 #	418.5 ± 41.73 #	0.8358 ± 0.04972 #	8.383 ± 0.1400 #
Silymarin (50mg/kg b.w) and 30% v/v ethanol	36.83 ± 0.9098***	43.83 ± 3.842***	184.3 ± 10.37***	0.3500 ± 0.02236***	7.583 ± 0.09458***
P.fulgens extract (100 mg/kg/day) and 30% v/v ethanol	58.17 ± 2.358	62.33 ± 3.283*	378.7 ± 25.30	0.6853 ± 0.03074*	8.067 0.07149
P.fulge ns extract (200 mg/kg/day) and 30% v/v ethanol	50.00 ± 2.160**	52.83 ± 1.400***	317.0 ± 21.16*	0.6513 ± 0.02608**	7.833 ± 0.1054*
P.fulgens extract (400 mg/kg/day) and 30% v/v ethanol	47.50 ± 1.232***	55.33 ± 1.453***	234.7 ± 11.57***	0.3680 ± 0.02120***	7.700 ± 0.1461**

N=6, Values are expressed as mean ±S.E.M with six animals in each group.* indicates statistical significance ***p<0.0001, **p<0.001, *p<0.01 vs ethanol intoxicated group, # p<0.0001 vs normal group

Table 4: Data pertaining to HPTLC fingerprinting analysis of the methanolic root extract of *Potentillafulgens*

S. No	Wavelength(nm)	No. of peaks	Rf Value	Percent area (%)
1	254	6	0.09,0.17,0.27,0.49, 0.53,0.63	67.36,5.36,8.84,6.17,6.49, 5.77
2	366	1	0.72	100.00
3	540	9	0.11,0.17,0.22,0.32, 0.41,0.47,0.60,0.64, 0.69	22.86,2.54,0.30,6.55,12.72, 14.02,34.34,4.16,2.52

Histopathological examination

On analysis of the normal groups that received no treatment, there were no pathological abnormalities and the liver showed normal structure of the hepatocytes that were polyhedral in shape (Fig 8a). Histopathological changes were observed in rats that received alcohol only and the extract treated groups. In the ethanol treated group, histological changes observed were more pronounced. The rats showed severe hydropic degeneration of hepatocytes and the portal area showed lymphocytic infiltration (Fig. 8b).The liver exhibited normal appearing hepatocytes arranged in cords radiating towards the periphery from the central vein in case of Silymarin treated group (Fig 8c). However, there was significant improvement on the histological changes in the extract treated rats. In the liver section of those that received higher dose (400 mg/kg bw) of the extract after ethanol induction, showed signs of protection against toxicity evidenced from reduced fatty degeneration and infiltration (Fig. 8f). In the groups treated with lower doses (100mg/kg bw& 200mg /kg bw) of the extract, mild portal triaditis with inflammatory portal tract infiltration are well marked. Hepatic cells showed fatty accumulation and central nucleus with fat filled cytoplasm/ foamy cytoplasm were observed (Fig 8d & 8e)

Discussion

Alcohol-induced tissue damage results from associated nutritional deficiency and the direct toxic effects during alcohol breakdown primarily in the liver .This leads to impaired digestion, reduced absorption and impaired utilization of nutrients (Charles, 2003). Excessive alcohol consumption or long term use of alcohol promotes the deposition of dietary fat in the liver leading to the most common and initial histopathological changes in the liver in the form of fatty liver which may sensitize the cells to further injury. Alcoholic liver disease development is caused by excess production of reactive oxygen species (superoxide, hydroxyl and hydroxyl ethyl radicals) which are generated during ethanol metabolism (Gramenzi *et al*, 2006). Alcohol can be eliminated from the body by various metabolic mechanisms. The major pathway for its metabolism involves the enzyme alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). ADH catalyzes reversible oxidation of ethanol to acetaldehyde a highly reactive and toxic substance which can lead to tissue damage even in low concentrations. Then the enzyme aldehyde dehydrogenase (ALDH) further metabolized acetaldehyde to acetate, which is then metabolized into carbon dioxide and water for easy elimination (Jacquelyn, 1997).

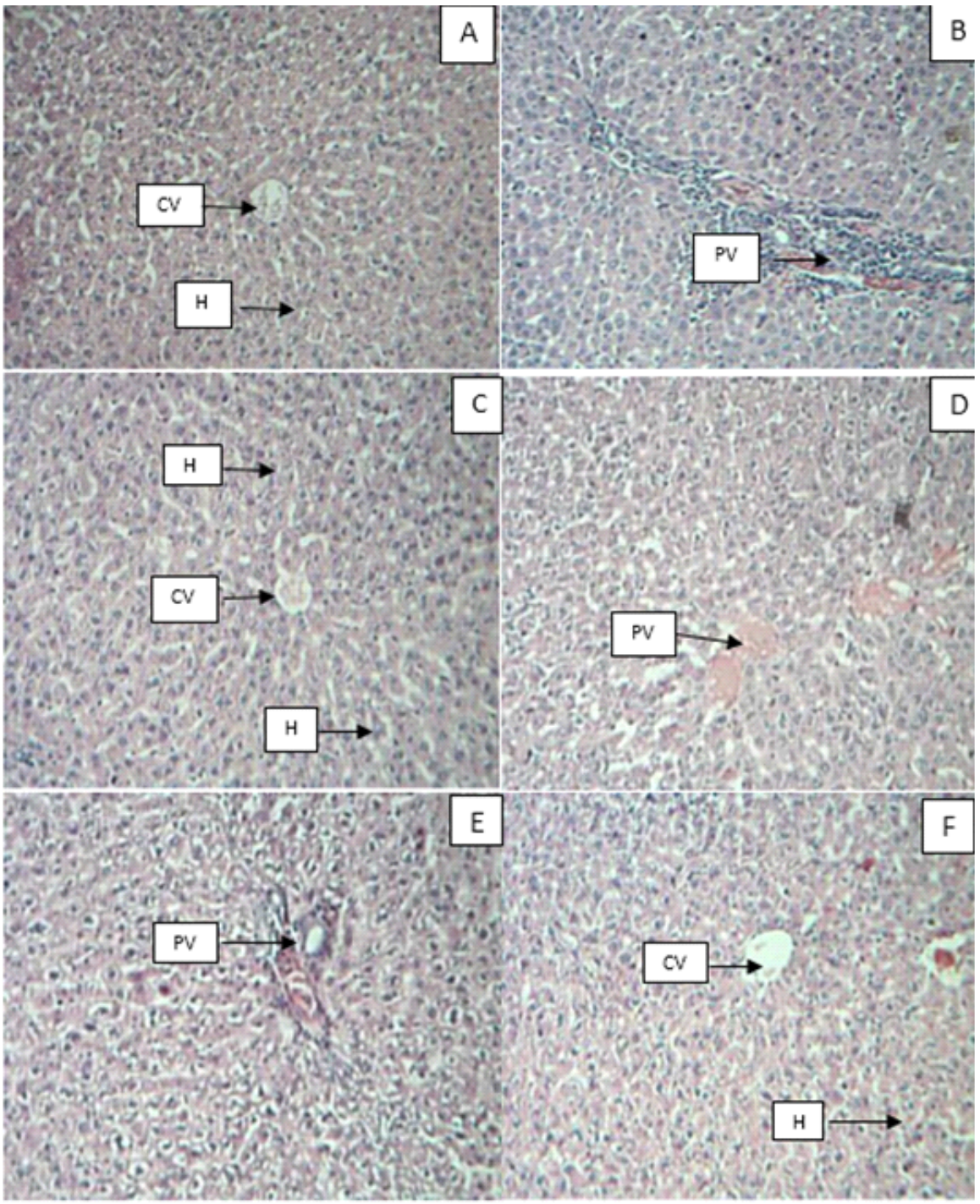

Fig 8: Histopathalogical monograph of methanolic root extract of *Potentillafulgens*and standard (40× magnifications).

A. Liver section of rat administered with normal saline. B. Liver section of rat administered with ethanol and non- extract treated (5 g/kg, 30% w/v) .C. Liver section of rat administered with ethanol and Silymarin treated (50mg/kg + ethanol 5 g/kg, 30% w/v). D. Liver section of rat administered with ethanol and PFM extract treated (100mg/kg + ethanol 5 g/kg, 30% w/v) E.Liver section of rat administered with ethanol and PFMextract treated (200mg/kg + ethanol 5 g/kg, 30% w/v) F.Liver section of rat administered with ethanol and PFMextract treated (400mg/kg + ethanol 5 g/kg, 30% w/v). (PFM-Methanolic root extract of *Potentillafulgens*, CV-Central vein, H-Hepatocytes, PV-Portal vein)

Numerous studies have indicated that excessive ethanol intake induces the mass production of free radicals in the body, which are considered to be associated with alcoholic liver disease (Dahiru *et al*,2007). The aminotransferases (transaminases) are sensitive indicators of liver injury and are most helpful in recognizing acute hepatocellular diseases. During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations (Deb,1998). The results of our study were in agreement with the previous studies which reveals that ethanol ingestion in rats significantly increased the hepatic enzyme activities which is attributed to damaged structural integrity of the liver leading to an enhanced permeability of the liver cell membrane, injury , damage and /or necrosis of hepatocytes (Ighodaro *et al*,2012).On administration of ethanol for 21 days, it has been observed that there is a significant elevation in all these serum enzymes, and on oral administration of the methanolic root extract of *Potentilla fulgens* a significant decrease in the levels of these marker enzymes in a dose dependant manner was witnessed. This indicates that the extract provides protection against liver and ultimately preserving the structural integrity of the liver from the toxic effects of ethanol.

Serum bilirubin provides clues to the severity of hepatocellular damage and its accumulation in the serum is a measure of liver injury. In the present study, the amelioration of liver toxicity is evidenced by a decrease in the levels of serum bilirubin after treatment with the methanolic root extract of *Potentilla fulgens* indicating its protective effect against liver injury due to alcohol.

The decrease in the level of total protein observed in the ethanol intoxicated group suggested that there is destruction in the number of hepatic cells which may result in decrease of hepatic capacity to synthesize proteins and accumulation of triglycerides leading to fatty liver (Kiran *et al*, 2012). Treatment with the methanolic root extract of *Potentilla fulgens* leads to stabilization of serum protein level which is further a clear indication of the improvement of the functional status of the liver cells.

The biochemical tests can further be supported by histopathological observations of the liver sections. In the ethanol treated group, histological changes observed were more pronounced. The rats showed severe hydropic degeneration of hepatocytes and the portal area showed lymphocytic infiltration. Section of the livers treated with methanolic root extract of *Potentilla fulgens* at 100mg and 200mg showed mild portal triaditis with inflammatory portal tract infiltration and the protective effect was not significant. Treatment with 400 mg/kg bw inhibits these histological changes which is evidenced from reduced fatty degeneration and infiltration when compared to ethanol intoxicated group.

In conclusion, ethanol-induced toxicity led to liver dysfunction which was dramatically reversed by treatment with the methanolic root extract of *Potentilla fulgens*. The extract restored the hepatic activates of ALT, AST, ALP, TB and TP possibly leading to its preventive activity. However, although protective action of the methanolic root extract of *Potentilla fulgens* was clearly beneficial for ethanol-treated rats, the exact mechanism at the pharmacological level still needs to be investigated for its effective components.

Conflict of interest

The authors declares no conflict of interest in the content and writing of this manuscript.

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