Hepatoprotective Activity methanolic extract of *Argyreia cymosa* and *Cappparis brevispina*

Venkateswarlu G *[1], Raja sundrarajan[2]
[1]Department of Pharmacognosy and Phytochemistry,A. M. Reddy Memorial College of Pharmacy, India.
[2]Department of Chemistry, Gitam University, India

Abstract:

Introduction: Indian traditional medicinal system is konowone the best alternative system of medicine In the present research article I tried to explore the Hepatoprotctive activity of two plant spcies which are well known to the people near to Tirumalhills.Materials and methods: In the present investigation methanol extract of *Arygreiacymosa and Cepparіasbravespina*elavulted for its hepatoprotective activity using Paraetamol induced hepatoprotective Rat model. Results and discussion: All the result values of different biologicalparameters were compared with the Standrad natural drug Sylimarin.also the histo-pathological studies performed compared all the histomorhographic .Conclusion:By the comparisonthe resultant values showed that the methanolic extract of both plant acting for its hepatoprotective activity as like standard sylimarin natural product .Hence the these both plants can be used as herbal medicine for the treatment of various liver diseases after conducting the further molecular studies.

Keywords:Hepatoprotective,Paracetamol,Sylimarin, AST,ALT ALP and PCM

Introduction to Liver

The largest and growing glandular organ in the body is liver. The body's homeostasis is a key organ of critical importance.is the principal site of the body and it plays an important role in management of physiological processes for several fundamental functions including strong metabolism and excretion. It plays an important role in bile development, bilirubin excretion, cholesterol, hormones and medicinal products. It is responsible for starch, protein and fat metabolism.

Several hepatotoxins, such as viruses, bacteria, chemical substances, free radicals, tobacco, xenobiotics, food additives and contaminants are vulnerable to liver injury and are the major risk factors that lead to a variety of hepatitis, cirrhosis and liver diseases. Within the human body, free radicals are generated continuously by exposure to either exogenous chemicals or endogenous hepatotoxins in the environment. Hepatic instability is also caused during biochemical reactions by the production of free radicals. Hepatotoxicity is one of the common conditions leading to severe outcomes from metabolic disorder to death. This is one of the major causes of morbidity and mortality in all fatal diseases today. There is a need to protect the liver (Habbu, P et al., 2008)

Role of hepatoxicants in liver damage

Paracetamol (PCM), the most widely prescribed analgesic and antipyretic medication in this respect, is also known as acetaminophen. Paracetamol causes serious hepatotoxicity at higher doses and contributes to genius hepatic and renal tubular necrosis. The most popular and commonly used experimental model for researching therapeutic hepatoprotective activity is hepatotoxicity triggered by Paracetamol. D-Galactosamine is the best hepatotoxicity induced agent among many laboratory hepatitis models. Therefore, hepatotoxicity caused by D-Galactosamine was also used as the test model (Swarnalatha L et al., 2012).

Alcohol (ethanol) has been considered the world's most used psychoactive substance for thousands of years. Alcohol has important medicinal value in small doses. But if alcohol is absorbed in excess, it causes hepatic damage, since 80% of the alcohol consumed is metabolized in the liver. ALD is one of the big serious health problems .Thus, hepatotoxicity caused by ethanol is used to test hepatoprotective activity of curing drugs(Roy SP et al., 2015).

In many metabolic processes, free radicals are formed and the membrane lipids are targeted. It leads to a number of serious conditions in pathophysiology. Antioxidants in plants form the basis for the prevention and treatment of a disease and serve as free radicals and as antioxidants, which scavenge the free radicals in plants and act as hepatoprotective agents. Antioxidants are the main line of protection and the most active universal components amongst body's free radical neutralisingsystems In the last three decades, natural plant - based antioxidants have played a major role in preserving human health for hepatic disorders (Sha Li, et al.,2015)

Experimental work

For calculating the hepatoprotective activity, different methods are used, because the Paracetamol - induced hepatotoxicity process is one of the most commonly employed processes.For research purposes, Wistar male rats weighing 150-180 g both rats were orally treated for 5 days after Paracetamol, extracts and std. Silymarin(Anbarasu C et al., 2011 andSingh AK et al., 2012)

Experimental protocol

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

Group II: (toxic group) received PCM (2 g/kg b.wt. p.o) on the 5^{th} day for 5 days.

Group III: (test group), The MEAC (200 mg / kg b.wt. p.o) was obtained for 5 days and the PCM (2g / kg) was administered 1 hour after the last crude extract administration.

Group IV: (test group), MEAC (400 mg / kg b.wt. p.o.) was obtained for five days, and PCM (2g / kg) was given 1 hour after crude extract had been last given.

Group V: (test group), MECB was administered for 5 days (200 mg / kg b. wt. p.o) and PCM (2 g / kg) was administered 1 hour after last crude extract administration.

Group VI: (test group), The MECB (400 mg / kg b. wt. p.o.) was given over 5 days and the PCM (2g / kg) was provided 1 hour after last crude extract administration.

Group VII: (Standard group), Received 5 days of Silymarin (25 mg / kg b. wt. p.o.) and 1 hour after the final administration of the raw extract, PCM (2g / kg) was administered.

Biochemical Parameters:

Animals were killed, and retro-orbital plexus obtained blood afterwards. During coagulation, serum was separated at 37 ° C for 30 minutes and centrifuged at 3000 to 20 minutes. Serum has been used to measure biochemical parameters including AST, ALT and ALP from tissues collected. Diagnostic kits were used to check the above.

Histopathological Study:

Livers of different groups were set for 48 h to 10 % buffered neutral formalin and then for 6 h to bovine solution. Paraffin parts were made of alcohol-xylene sequence at a thickness of 5 mm, and aluminum hematoxylin and eosin were painted. The sections were examined for histo- pathological changes microscopically.

Tablexxxx shows plasma biochemical parameters. Administration of paracetamol (PCM), dependent damage of hepatocytes, evidence of increased levels of liver enzymes (ALT, AST and ALP), total bilirubin and regulated cholesterol. High levels of these enzymes indicate cell damage and loss of hepatocyte's functional integrity. The PCM dosage (2 g / kg) was slightly higher than the normal (42, 48, 108, 0.37 units / ml) level, as compared to normal animals (42, 48, 108, 0.37 units / ml), and the PCA (2 g / kg) dose was significantly higher. Treatment of MEAC rats (200mg / kg, 400mg / kg) and MECBs (200mg / kg, 400mg / kg) have lowered their amounts of ALT enzymes by 49-68 units / ml, AST by 52-64 units / ml, ALP by 108-143 units / ml and PCM-induced rats by 0.40-0.69 units / ml, which have been reported as comparable with the PCM mediated enzyme levels (AST, ALT, ALP, TP). The standard drug Silymarin has lowered the enzyme levels of ALT , AST, ALP, bilirubin and cholesterol respectively, to 36.52, 46.23, 102 and 0.34 units / ml. Figures xxxx show the results for histopathological examination.

Regarding particular medication-inducing hepatotoxicities, silymarin is a well-established hepatoprotective drug capable of reducing high levels of liver enzymes. The administration of test compounds has increased the total protein level by 4.89 to 5.51 units per ml and lowered the elevated ALT, AST, ALP, gross bilirubin and cholesterol levels when opposed to the caused toxicity value.

Histopathology

Histological examination of the typical control group’s liver section showed the normal layout of normal liver histology, i.e. central hepatic vein and sinusoids (Figure 4.6A).

There was significant hepatic cell necrosis in the liver sections of rats treated with PCM alone. (Show 4.6B). The liver section of rats treated with PCM and the group treated with silymarine maintains the almost usual hepatocyte structure . Measured groups of MEAC, and MECB (200, 400 mg / kg) show liver restoration with little liverdamage (Figure **1** and **Table**

Treatment groups	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**

1)

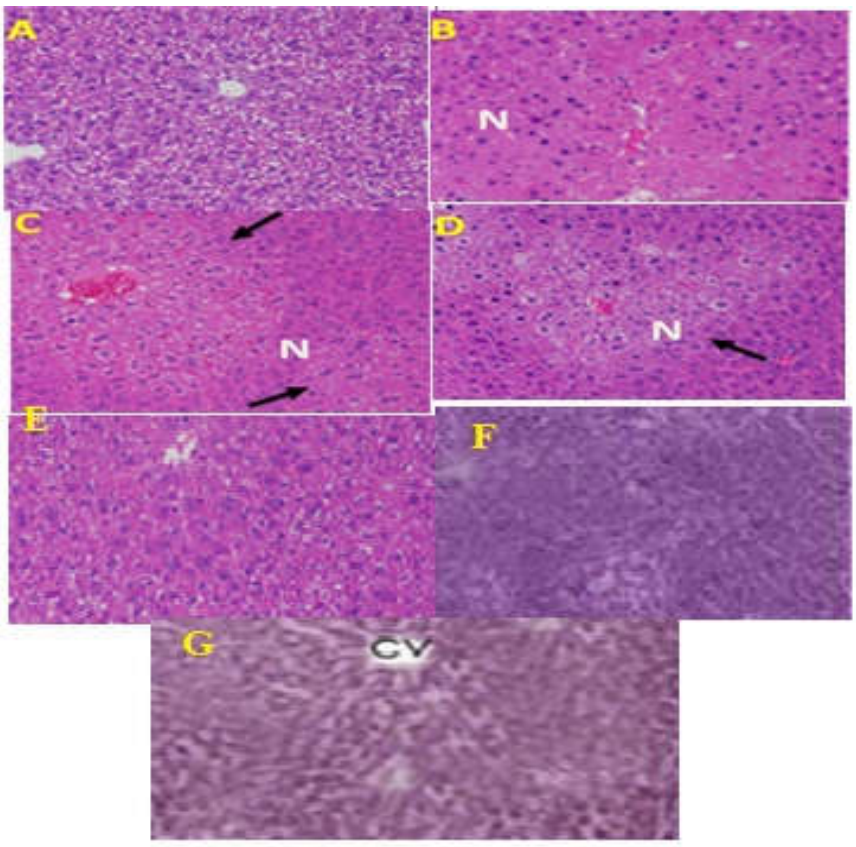

Figure 1 Hematoxylin and eosin stained paraffin portions of the liver for histopathological changes. (A) Liver portion of the test group demonstrating standard liver histology layout. (B) PCM liver (2gm / Kg) (C) Liver segment PCM + MEAC (200 mg / kg) (D) Liver segment PCM and MEAC (400 mg / kg) (E) Liver section PCM and MECB(200 (F) liver section PCM and MECB(400 mg / kg) (G) Liver section PCM and Silymarin (25 mg / kg)

and liver specific Variables	**(Normal Control: 0.5% Tween 80 1ml/kg b.wt)**	**(Hepatotoxic Control: 0.5% Tween80 1ml/kg b.wt+PCM (2g/Kg b.wt)**	**(MEAC 200mg/kg b.wt + PCM 2g/kg. b.wt)**	**(MEAC 400mg/kg b.wt +PCM 2g/kg. b.wt)**	**(MECB 200 mg/kg b.wt +PCM 2g/kg. b.wt)**	**MECB 400 mg/kg b.wt +PCM 2g/kg. b.wt)**	**(Silymarin 25 mg/kg b. wt. + PCM 2 g/kg b. wt.)**
AST (U/L)	48±4.12	97±9.63*	64±2.85*	53±5.44*	78±2.85*	63±5.44*	46.23±2.25*
ALT (U/L)	42±3.23	104±5.36*	68±5.58*	49±3.85*	86±5.58*	54±3.85*	36.52±2.58*
ALP (U/L)	108±8.36	276±11.85*	143±11.23*	118±8.58*	155±11.23*	132±8.58*	102±6.36*
Total Bilrubin (mg/dl)	0.37±0.05	1.26±0.08*	0.69±0.03*	0.46±0.03*	0.86±0.03*	0.52±0.03*	0.34±0.02*

Table 1 Effects of pre-treatment with MEAC (200 and 400 mg/kg), MECB (200 and 400 mg/kg) and Silymarin on the serum levels of AST, ALT, ALP and Bilirubin in PCM induced hepatotoxicity in rats

Results and Discussion

Plant medicines contribute greatly to the treatment of different diseases by their multiple products. All of them are already researched and scientifically validated. We designed experiments to examine the MEAC MECB hepatoprotective activity to grow into safe and natural drug candidates.

Paracetamol is widely used as a therapeutically effective antipyretic drug. Nevertheless, it could cause fatal hepatic damage to humans and animals at higher toxic doses. PCM organic activation by hepatic cytochrome P-450 leads to the formation of a N - acetyl - p-benzoquinone imine (NAPQI) that is very volatile and harmful. NAPQI is usually detoxified by mixture of reduced glutathione (GSH) in order to produce mercapturic acid eradicated by urine. The toxic overdosis correlated with PCM allows it weaker for hepatic GSH content, and thus for free, NAPQI attaches covalently to cellular mitochondrial proteins, avoiding mitochondrial protein, which induces mitochondrial fatty acid oxidation and leads to hepatocyte necrosis and apoptosis. ALT is more liver responsive and a key factor for hepatic injury research. Higher AST levels mean cell exudation together with the decreased cell membrane functional capacity in the liver. Serum ALP often entails damage to the liver cell. High ALP concentration leads to severe hepatic damage in PCM controlled rats (Bhattacharyya et al.,22013) The liver is a critical source of most serum proteins. Bilirubin is an inhome part of the reticuloendothelial system; it may be induced by overproduction, increased hemolysis, reduced conjugation or defective bilirubin activity in the bloodstream Bilirubin is used for measurement of the normal function of the liver, not the extent of hepatocellular injury. Phytoconstituents including flavonoids and phytosterols are well known for their hepatoprotective antioxidants activity.

Recent findings have demonstrated a strong decrease in serum liver enzyme activity (AST, ALT, ALP) and bilirubin concentrations in MEAC and MECB significantly inhibited the acute liver toxicity in rats by increased doses of PCM. Furthermore, the liver morphology as well as the findings of histopathology support the protective action of this extract against hepatic damage caused by PCM as is apparent because the centri-lob necrosis of hepatic parenchyma is reversed by administration of above three extracts.

The extract then restored the hepatocytes to normal architecture was observed as illustrated in Figure 4.6. Although the SGPT, SGOT, ALP and total bilirubin levels of MEAC and MECB have significantly reduced, these biochemical parameters are completely returned to the normal values.

The results of this research indicate, in fact, that MEAC and MECB have hepatoprotective strategies against PCM-induced rat liver damage. This property was allocated to the nature of Flavonoids, i.e. rutin, quercetin which could prove to normalize damaged antioxidants, likely through the protection of glutathione levels by inhibiting the production of malondialdehyde or by inhibiting the activation of toxicants and by raising the body's defense function. The hepatoprotection of the sterols i.e. β-sitosterol and stigmasterol was linked to an increase in mitochondrial glutathione redox status, possibly with the raise in mitochondrial glutathione redox cycling by glutathione reductase. This flavonoids and phytosterols therefore act as a potential mitohormetic to rising oxidative stress in the liver (Lei Cao et al., 2017)

Conclusion

In the hepatoprotective action of the paracetamol is utilized as concoction initiating specialist in heaptic harm. Paracetamol (PCM) organization, actuated devastation to hepatocytes affirmed by the raised degree of liver chemicals (ALT, AST, and ALP), absolute bilirubin and cholesterol when contrasted with control. Raised degrees of these chemicals are characteristic of cell harm and loss of practical honesty of hepatocytes. A solitary portion of PCM (2 g/kg) essentially higher ($P < 0.001$), raised the ALT, AST, ALP and bilirubin levels (104, 97, 276, 1.26 units/ml) when contrasted with the ordinary creatures (42, 48, 108, 0.37 units/ml) separately showing height in catalyst levels. Treatment of the rodents with the MEAC (200mg/kg, 400mg/kg), and MECB (200mg/kg, 400mg/kg) have diminished the chemical levels in the scope of 49-68 units/ml for ALT, 52-64 units/ml for AST, 108-143 units/ml for ALP and 0.39-0.69 units/ml for bilirubin which were seen as similar to the protein levels (AST, ALT, ALP and TP) raised by PCM initiated rodents. Standard medication Silymarin likewise diminished the protein levels in the scope of 36.52, 46.23, 102 and 0.34 units/ml, for ALT, AST, ALP, bilirubin and cholesterol levels individually. Results for histopathological assessment were gives data that the harm of the hepatic cells decreased after the admistartion of above methanolic extricates.

Conflict of interest statement

We declare that we have no conflict of interest.

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