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STUDY ON THE EFFECT OF *Aganosma cymosa* AND *Plumeria rubra* METHANOL EXTRACT ON DIFFERENT MODELS OF INDUCED LIVER TOXICITY IN EXPERIMENTAL RATS

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Abstract- The Hepatoprotective effect of *Aganosma cymosa* and *Plumeria rubra* methanol extracts investigated on Carbon tetra chloride (CCl4), Paracetamol (PCM) and Antitubercular drugs induced liver damage in rats. The variations in the level of hepatic biomarkers viz. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Bilirubin levels were measured in a normal and treated group of rats. The potency of the plant extract in two different doses (200 mg/kg and 400 mg/kg b.w.) were compared with a popular hepatoprotective drug silymarin. From the results, the plant extract were found to be effective in reducing the elevated enzyme levels that were caused due to CCl_4, PCM and Antitubercular drug intoxication. Among the two administered doses of *Aganosma cymosa* methanol extract (ACE) and *Plumeria rubra* methanol extract (PRE), 400 mg/kg b.w. of ACE and PRE gave significant (p<0.001) results. The Histopathological studies also support the results with reversal of damaged liver cell architecture in the ACE and PRE treated groups. Hence, both the plants selected showed good Hepatoprotective effect.
Keywords- Hepatoprotective effect, *Aganosma cymosa*, *Plumeria rubra*, Carbon tetra chloride, Paracetamol, Antitubercular drug

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Introduction

The traditional systems of medicine use the herbal drugs for various disorders for centuries. In recent times, the focus on herbal drug usage as protective measures are found extensively. The reason may be due to the minimal side effects and less known usage of allopathy medicines in the treatment of liver diseases. The Naturopaths discovered that the treatment for various disorders is more often indicated with liver toxicity [1]. The liver is a vital organ that plays an important role in metabolizing the drugs and other substances [2]. This makes the liver exposed to toxic substances. The imbalance caused during liver damage is not detectable in the early stages [3]. The damaging effect of toxins towards liver cells gave the root to discover Hepatoprotective substances. As there is, no standard synthetic medicine invented until now as a Hepatoprotective agent. Since the herbal medicines were found more effective in protecting the liver, the extensive research on several herbal medicines is necessary to establish its activity.

Aganosma cymosa also known as *Echites cymosa* (Apocynaceae) is a climbing shrub found commonly growing in hill slopes. The distribution is restricted to peninsular region in India and Bengal [3, 4]. The traditionally plant is used as an emetic and anthelmintic. It is also used in the treatment of bronchitis, leprosy and skin diseases. The flower is said to be effective in diseases of eye [5]. The plant has been reported to contain highest oil content of about 10.3% [6].

Plumeria rubra [7,8] belongs to family Apocynaceae found to possess several medicinal uses. The extensive research on the plant constituents revealed the presence of various phytoconstituents [9] such as saponins, glycosides, tannins, flavonoids, alkaloids etc. In folklore the plant in used as purgative, counter irritant, in the treatment of arthritis and rheumatism [10]. The detailed literature study revealed the plant is a potent anticancer agent [11], anti hyperglycemic agent [12]. Therefore, in this current investigation the methanol extract of *Aganosma cymosa* and *Plumeria rubra* were evaluated for Hepatoprotective effect on CCl_4, PCM, and Antitubercular drug induced liver toxicity in experimental rats.

Materials and Methods

Plant Collection

The whole plant of Aganosma cymosa and the leaves of Plumeria rubra collected from the Eastern Ghats of South India and surrounding places of Hyderabad. A sample was authenticated by Dr. B. Prathibha Davy, HOD, Department of Botany, Osmania University, Hyderabad and a voucher specimen were preserved in the institutional herbarium.

Extract Preparation

The plant material of *Aganosma cymosa* and *Plumeria rubra* washed cleaned, dried and grinded to obtain coarse powder. The powder subjected to maceration for 72 hrs. in 75% methanol. The

resulting extract filtered to obtain clear solution and evaporated under vacuum in Rota evaporator. The percentage yield of the dried extract was found to be 5.5%w/w and 3.8%w/w respectively. The resultant dried extracts were obtained used for experimental work.

Preliminary Phytochemical Screening

The dried extracts obtained were screened for phytoconstituents such as alkaloids, glycosides, saponins, tannins, terpenoids, flavonoids etc. by standard procedures [13,14].

Animals

Wistar Albino rats weighing (150-200gm) and Wistar mice weighing (25-30gms) of either sex were procured and housed in an Institutional animal house. The rats provided with proper cross ventilation, feed and water. All the experiments conducted in accordance with approval obtained from the Institutional Ethical Committee CPCSEA, India (Reg. No. 1217/2008).

Acute Toxicity Study

The acute toxicity conducted according to OECD guidelines No.423 [15]. Wistar albino mice of either sex were divided into five groups with six mice in each. The plant extract from the dose of 250, 500, 1000, 1500 and 2000 mg/kg b.w. administered per oral as a single dose and the animals observed for any behavioural symptoms and death within 24 hrs. and for 14 days.

Experimental Protocol

Paracetamol Induced Toxicity

The animals were divided into seven groups with six in each. Group 1 received 2.5% gum acacia once daily for 3 days and served as normal control. Group 2 received paracetamol [16] (3gm/kg, b.w., p.o) as a single dose on day 3 and served as negative control. Group 3 served as a reference, which received silymarin [17] (25 mg/kg, B.w., p.o) once daily for 3 days. Groups 4, 5 and 6,7 received ACE and PRE at a dose 200 mg/kg and 400 mg/kg each resp. once daily for 3 days. On day 3 after 30mins of test material administration Group 3-7 received paracetamol (3gm/kg) as a single dose to induce hepatotoxicity. After 48 hrs. of PCM administration, the blood collected by retro-orbital puncture under light ether anaesthesia and taken for determining biochemical parameters.

Carbontetrachloride Induced Hepatotoxicity

The rats divided into seven groups of six rats in each. Group 1 and 2 served as normal control and intoxicated control resp. receives 2.5% gum acacia 2ml/kg. Group 3 served as reference that receives silymarin (100 mg/kg, b.w.,p.o). Group 4, 5 and 6,7 received ACE and PRE extracts resp. at a dose of 200 mg/kg and 400 mg/kg b.w p.o. The treatment continued for seven days. On day 7, 30min post dosing the Groups 2-7 received CCL_4 [18] (1.5ml/kg, 1:1 of CCL_4 in olive oil, p.o.). After 36 hrs. the blood collected by retro orbital puncture under mild ether anaesthesia for biochemical parameter estimation.

Anti-tubercular Drugs Induced Hepatotoxicity

The rats were divided into seven groups of six rats in each. Group 1 received 2.5% gum acacia (2ml/kg b.w.) and served as normal control. Group 2 received a combination of anti-tubercular drugs (Isoniazid - 7.5 mg/kg, Rifampicin - 10 mg/kg and PyraZinamide - 35 mg/kg) [19] for 35 days per oral route in 2.5% gum acacia served as negative control. Group 3 received silymarin, (Sigma chemicals company, USA), at a dose of 100 mg/kg, p.o., daily for 35 days, 45 min prior to anti-tubercular drugs dosing and served as reference. Group 4, 5, 6 and 7 were treated with ACE and PRE each with 200 mg/kg and 400 mg/kg, b.w. p.o., daily for 35 days, 45 min prior to administration of antitubercular drug. After the experimental period blood was, withdrawn from retro orbital puncture under mild ether anaesthesia and the animals were sacrificed for Histopathological studies

Estimation of Biochemical Parameters

The blood samples collected were allowed to clot and centrifuged at 3000 RPM for 15min to obtain serum. The estimation of biochemical markers like serum enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total Bilirubin (TBL) and Direct Bilirubin (DBL) were determined spectrophotometrically by standard method using enzyme assay kits (Span diagnostic Ltd., India).

Histopathological Studies

For the histological studies, the liver tissues were isolated from rats and stored in 10% phosphate buffered neutral formalin. Further, thin section were cut and stained with hematoxylin and eosin stain for microscopic examination.

Statistical Analysis

The results were expressed as mean±SEM for six rats and all comparison was made by one way ANOVA followed by Dunnett's test using Graph Pad Prism computer package Version 6 for determining the level of significance. The values $p<0.05$ were considered statistically significant.

Results

The whole plant extract and the leaf extract of *Aganosma cymosa* and *Plumeria rubra* produced no lethality upto 2000 mg/kg. The preliminary phytochemical screening of the extracts was done, ACE showed the presence of saponins, terpenoids, glycosides, tannins, phenolic compounds and carbohydrates. PRE on analysis showed the presence of alkaloids, glycosides, coumarins, phenolic compounds etc.

The comparative effect of *Aganosma cymosa* and *Plumeria rubra* methanol extract of various doses on serum biochemical markers was studied in CCL_4, PCM and antitubercular drug induced liver toxicity, the results were summarized from [Table-1]. The intoxicated rats showed significant ($p<0.001$) liver cell damage with increased level of serum hepatic enzymes AST, ALT, ALP and Bilirubin in comparison with normal control. The elevated enzyme levels were significantly reduced ($p<0.001$) at higher dose (400 mg/kg, b.w.) of ACE and PRE when compared with silymarin while the low dose (200 mg/kg, b.w.) of plant extract ACE and PRE showed moderate hepatoprotection viz. partial decrease in the elevated enzyme levels.

The Histopathological study [Fig-1] of normal liver tissues (a) was found with typical architecture with central vein from which chords of hepatocyte radiates. The portal triad consisting of hepatic artery bile duct and portal vein was observed. The intoxicated animal liver section showed hemorrhagic necrosis, lymphocytic infiltrate with inflammatory cells, sinusoidal dilation and balloon degenration (b-d). Complete reversal of liver architecture with regeneration of hepatocytes with minimal cell necrosis, mild dilation of sinusoids and mild lymphocytic infiltrate supported the significant reduction of serum enzymes at 400 mg/kg of ACE and PRE (e-g).

Table 1- Effect of Aganosma cymosa and Plumeria rubra methanol extracts on serum enzymes and bilirubin levels against Paracetamol, CCl4 and Antitubercular drug induced hepatotoxicity in rats.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TBL (mg/dL)	DBL (mg/dL)
Effects against Paracetamol					
Control	22±8.7303	13±2.73	68±.1.461	0.33±1.489	0.33±1.489
PCM toxic	212.2±13.83***	83.51±10.497***	88.33±2.789***	59.98±2.987***	60±2.981***
Silymarin	67.8±6.036[a]	42.22±6.127[b]	66.67±1.667[c]	33.33±2.996[c]	33.33±2.983[c]
ACE 200	52.69±7.563[a]	35.66±4.058[a]	76.5±6.658[c]	33.33±1.872[c]	33.33±1.653[c]
ACE 400	30.3±3.233[a]	25.81±9.943[a]	52.66±3.57[a]	2.22±1.404[a]	1.11±1.11[a]
PRE 200	147.6±3.381[b]	35.43±7.407[a]	57.83±2.587[a]	31.07±2.825[c]	31.11±2.812[c]
PRE 400	94.65±2.796[a]	27.65±8.589[a]	42.85±1.465[a]	26.66±1.679[c]	31.33±4.135[c]
Effects against CCl4					
Control	22±8.7303	13±2.73	68±.1.461	0.33±1.489	0.33±1.489
CCL4 toxic	505.9±41.310***	494.7±22.32***	124±7.323***	49.00±4.025***	52.00±3.795***
Silymarin	283.70±13.700[b]	294±8.190[a]	62.83±2.283[a]	23.11±1.394[b]	24.44±1.404[c]
ACE 200	380.05±12.164[c]	397.72±17.41[c]	71.28±5.432[c]	21.33±0.394[b]	15.33±0.143[b]
ACE 400	189.62±15.687[a]	203.78±24.853[a]	51.33±3.612[a]	4.23±1.35[a]	3.33±1.489[a]
PRE 200	374.80±1.783[c]	370.70±6.1[c]	89±1.932[c]	30.00±0.894[c]	30.67±0.843[c]
PRE 400	175.8±11.00[a]	172.3±18.510[a]	46.5±0.836[a]	15.33±0.894[b]	16.00±0.843[b]
Effects against Antitubercular drug					
Control	22±8.7303	13±2.73	68±1.461	0.33±1.489	0.33±1.489
I+R +P Toxic	84.97±3.233***	34.72±2.584***	120.166±3.487***	4.32±0.745***	2.33±0.638***
Silymarin	39.99±2.022[a]	12.88±0.5818[a]	78.176±1.72[a]	0.66±0.242[b]	0.88±0.506[a]
ACE 200	51.25±2.983[b]	35.79±0.846[n]	112.53±3.483[c]	3.33±0.658[c]	3.33±0.782[n]
ACE 400	29.84±1.556[a]	16.83±0.601[a]	82.5±2.045[a]	0.66±0.420[b]	0.33±0.147[a]
PRE 200	48.51±8.570[b]	28.57±10.574[b]	95.62±16.41[b]	0.66±0.784[b]	0.33±0.275[a]
PRE 400	23.75±15.805[a]	16.48±5.483[a]	74.113±6.427[a]	0.33±0.785[a]	0.108±0.147[a]

*[n]: not significant; [a]: p<0.001; [b]: p<0.01; [c]: p<0.05; ***: p<0.001 (compared with control group); PCM: paracetamol; CCl_4: carbontetra chloride; I: isoniazid; R: rifampicin; P: pyrazinamide; ACE: Aganosma cymosa extract; PRE: Plumeria rubra extract.*

Discussion

In the present study *Aganosma cymosa* and *Plumeria rubra* was evaluated for Hepatoprotective activity using CCl4 induced and drug induced hepatotoxicity in rats. As we are aware, that the highest percentage of the population suffers from liver damage due to one or the other reason. The cause mainly due to chronic treatment in several diseases. The liver damage can be easily studied by serum enzymes and bilirubin levels such as AST, ALT, ALP, Total Bilirubin and Direct Bilirubin levels. In the process these enzymes are directly or indirectly responsible for the conversion of amino acids to ketoacids. In CCl_4 induced hepatotoxicity, it is believed that CCl_4 undergoes biotransformation to highly reactive trichloromethyl free radicals by the cytochrome P 450 system [20, 21]. The free radicals produced are responsible for lipid peroxidation wherein it binds covalently to cell membranes and organelles lead to hepatic cell damage.

In PCM model, a small amount of drug is metabolized via the cytochrome P 450 system to the alkylating metabolite N-acetyl-p-benzoquinone imine (NAPQI) [21] which is responsible for the toxic side effects of PCM. However, the larger portion eliminated mainly as sulfate and glucuronide. The toxic doses of PCM saturate the sulfation and glucuronidation [22, 23] routes, thus allowing the major portion of the drug oxidized into highly reactive NAPQI. The high level of NAPQI alkylates and oxidizes intracellular glutathione (GSH) and protein thiol groups, which in turn deplete the liver GSH concentration and lead to lipid peroxidation.

Antitubercular drugs not only produces hepatic damage individually but also when given in combination enhances the liver toxicity in a synergistic manner [24]. Isoniazid is converted into its metabolite monoacetyl hydrazine a toxin via the cytochrome P 450 system leads to hepatotoxicity. Rifampicin induces Cytochrome P450 enzyme to convert acetyl hydrazine (AcHz) [25] to toxic metabolites, it also converts isoniazid to isonicotinic acid and hydrogen, both are hepatotoxins. Pyrazinamide also produces hepatotoxicity in combination with isoniazid and rifampicin. Antitubercular drugs not only produce hepatotoxins but also exert oxidative stress induced hepatotoxicity.

The results of the present study showed that *Aganosma cymosa* and *Plumeria rubra* are potent Hepatoprotective herbal drug against hepatotoxicity induced by CCl_4, PCM and the combination of three antitubercular drugs. Administration of ACE and PRE of two different doses (200 mg and 400 mg) normalized the increased levels serum biomarkers AST, ALT, ALP and Bilirubin produced by a toxicity inducing substance. This was further accomplished by the Histopathological examinations. The hepatoprotection was found more effective in 400 mg/kg treated animals than 200 mg/kg dose level. The research on the various phytoconstituents have shown in its effect on the ability to protect the liver from damage due to lipid peroxidation [26]. Phytoconstituents [27] like flavonoids, triterpenes, saponins and alkaloids were known to possess Hepatoprotective activities and free radical scavenging activities. The *Aganosma cymosa* and *Plumeria rubra* revealed the presence of flavonoids, saponins, triterpenes, glycosides. Hence, it is possible that the Hepatoprotective nature of the plant may due to its antioxidant property produced by these phytoconstituents.

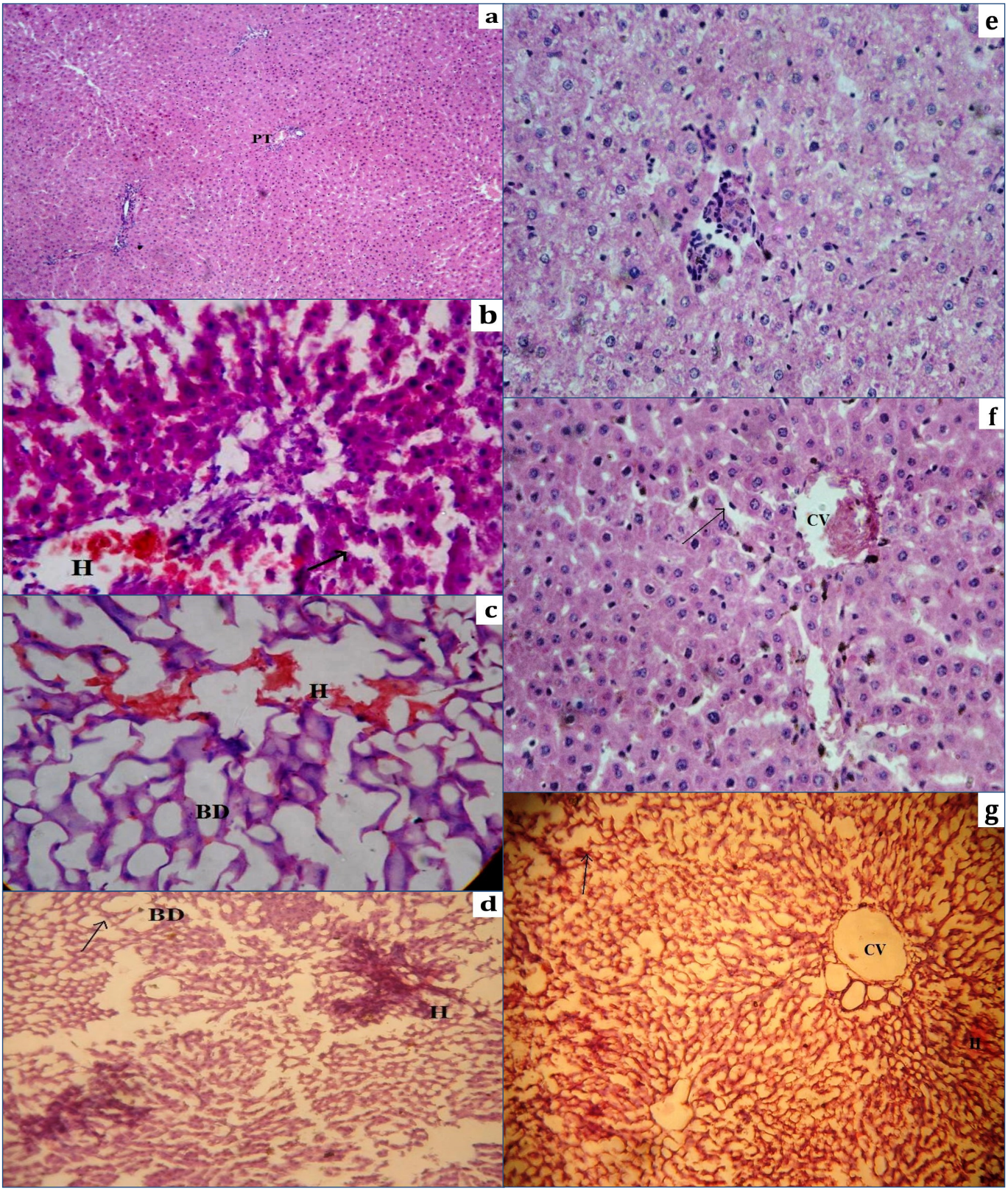

Fig. 1- Histopathology of liver tissues. **1a.** Normal control showing portal tract (PT, normal architecture) with mild dilation of sinusoids; 1**b, 1c & 1d.** Liver sections showing necrosis, massive fatty changes, haemorrhage (H), balloon degeneration (BD) and dilation of sinusoids (indicated with arrow) in intoxicated rats with paracetamol, carbontetra chloride and antitubercular drugs; **1e.** Liver section of carbontetra chloride intoxicated rats treated with ACE (400 mg/kg) showing less inflammatory cells, mild dilation of sinusoids and partial reversal of lymphatic infiltration; **1f.** Liver section of paracetamol intoxicated rats treated with PRE (400 mg/kg) showing almost normal architecture with slight congestion in the central vein (CV); **1g.** Liver section of antitubercular drugs intoxicated rats treated with ACE (400 mg/kg) showing partial reversal of liver architecture, mild balloon degeneration with normal central vein; ACE. *Aganosma cymosa* extract; PRE. *Plumeria rubra* extract

From the results it is evident that the *Aganosma cymosa* and *Plumeria rubra* is potent Hepatoprotective agent and the higher dose showed comparatively greater activity to that of standard group which is evidenced by its liver Histopathological studies. Further, the investigations are in process to isolate the constituents responsible for Hepatoprotective activity and antioxidant activity.

Conclusion

The methanol extracts of *Aganosma cymosa* and *Plumeria rubra* were prepared. The test samples exhibited dose dependent Hepatoprotective activity in various hepatotoxic models in comparison with silymarin when administered orally

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Conflict of Interest : None Declared.

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