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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF POLYHERBAL EXTRACT CONTAINING *PUNICA GRANATUM* AND *CLERODENDRUM INERME* LEAVES

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ABSTRACT

Objectives: Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. *Punica granatum* and *Clerodendrum inerme* Leaves have been usually used in Heart diseases, Stomach disorders, Dental care, cancer, osteoarthritis, diabetes, Anemia, liver ailments and kidney disorders. The current investigation was intended to explore the Hepatoprotective activity of a Polyherbal extract containing *Punica granatum* and *Clerodendrum inerme* leaves employing different experimental models.

Methods: A comparative analysis was carried out to investigate the synrgestic hepatoprotective effect of both the plant extracts through a herbal formulation in CCl_4, Paracetamol and Thioacetamide intoxicated rats. Animals were isolated into five sets, each group comprising of six rats. Methanolic extract of *Punica granatum* leaves (MEPGL), aqueous extract of *Clerodendrum inerme* leaves (AECIL) and Polyherbal extract (MEPGL+AECIL) were prepared and Hepatoprotective activity was explored against the selected models.

Results: Results suggests that the serum enzymes such as SGOT, SGPT and ALP were significantly decreased with PHE (400 mg/kg p.o) when compared to toxic controls. However

individual extracts of *Punica granatum* and *Clerodendrum inerme* leaves have exhibited less effect of SGOT, SGPT and ALP when compared to PHE. Total protein levels were significantly increased with PHE (400 mg/kg p.o) whereas total bilirubin and triglyceride levels were significantly decreased with PHE in comparison to individual extracts. Liver weight was significantly decreased by PHE. A histopathological examination of liver fractions has further augmented the biochemical evidences of hepatoprotective function of the *Punica granatum* and *Clerodendrum inerme* Leaf extract by preserving the hepatic architecture of the liver tissue to near normal.

Conclusion: To sum up, the hepatocellular injury was prevented better with PHE of *Punica granatum* and *Clerodendrum inerme* Leaves when compared with the independent extracts indicating synergestic effect of Polyherbal extract. However further research is essential to find out the exact mechanism responsible for the synergistic hepatoprotective effect of the Polyherbal extract.

Keywords: *Punica granatum* leaves, *Clerodendrum inerme* leaves, Polyherbal extract, synergistic effect, hepatoprotective activity, hepatic architecture, protein levels

INTRODUCTION

Nature has reliably been valuable to us in giving flavors of remedial significance[1]. Phytoconstituents of the flavors, for instance, flavonoids, alkaloids, glycosides and lignans have been found to have remedial benefits in the expectation of liver issues and malignant growth[2]. The utilization of regular medications and local treatment by number of patients is growing dramatically[3]. Human usage of local prescriptions for the treatment of diseases has a long history since from Neanderthal ages[4]. Various harms handled from the GIT can impact the metabolic exercises of the liver achieving various kinds of hepatic contaminations. In this manner liver contaminations are considered as most hazardous wellbeing inconveniences[5]. Free radicals are incorporated essentially in various obsessive conditions like cardiovascular issues, harm, hepatic issues, neurological issues and diabetes[6]. Free extremists use oxygen and are consolidated in the mitochondria of mammalian cells and abilities to begin the biochemical responses[7]. Ordinary cell assimilation yields nitrogen species and responsive oxygen (RNS and ROS) which cytotoxically affect microorganisms and other unsafe animals. In any case a couple of proteins are accessible that produce ROS/RNS, for instance,

NADPH oxidases, NOS and myeloperoxidases. In this way an equibrium is essential to be kept up between positive for oxidants and malignant growth anticipation specialists in cells for their common working. However, by and large there is high creation of favorable to oxidants in cells prompting oxidative harm. Oxidative harm is the chief figure included numerous illnesses including hepatic sicknesses [8].

Cancer prevention agent's acts by ending free radicals and their intermediates that causes oxidation by starting a succession of responses that at long last causes cell harm [9].

Antioxidants are lipophilic (Retinoids, flavonoids, tocopherol, ubiquinols and carotenoids) and furthermore water solvent (ascorbic corrosive and urate) substances. These demonstration by extinguishing the oxidative interaction and there by decline the centralizations of hurtful free radicals[10].The leaves of *Punica granatum* plant is accounted for to have various helpful properties, for example, mitigating cardiovascular diseases, anti-anemic, antidiabetic, antipyretic and pain relieving, antifungal, antimicrobial, antibacterial and antiparasitic, against malignant growth and Hepatoprotective activity.[11] The leaves of the *Clerodendrum inerme* plant is accounted for to have antidiabetic, antiarthritic, expectorant, emetic, hepatoprotective, temperament raising and abortificiant impacts.[12]

Antioxidants acts by terminating free radicals and their intermediates that causes oxidation by initiating a sequence of reactions that finally causes cellular damage[13].Antioxidants are lipophilic (Retinoids, flavonoids, tocopherol, ubiquinols and carotenoids) and also water soluble (ascorbic acid and urate) substances. These act by quenching the oxidative process and there by decrease the concentrations of harmful free radicals [14].

Research study on herbal drug shows that they have multidimensional mechanism of action against liver diseases and are also safer than the allopathic drug. However there is no existing pharmacological data that indicate the usefulness of Polyherbal extract of *Punica granatum* and *Clerodendrum inerme* Leaves against hepatic damage. Therefore, this investigation was carried out to evaluate the hepatoprotective action of Polyherbal extract of *Punica granatum* and *Clerodendrum inerme* Leaves and comparison of hepatoprotective efficacy of Polyherbal extract with individual extracts.

MATERIALS AND METHODS

Assortment and confirmation of plant material

For the current assessment, the *Punica granatum* and *Clerodendrum inerme* Leaves

were gathered from the region of the Meerpet, Saroornagar, Hyderabad. Sample specimens of *Punica granatum* and *Clerodendrum inerme* Leaves were kept in a polythene sack. The specimens were kept in new condition by adding 2% formalin. The scientific name of the plant has been checked with http://www.theplantlist.org on fifteenth, March, 2020. Plant materials were recognized and verified by Senior Scientist (Eco Botany), Dr. N. Sivaraj, NBPGR, Hyderabad.

Extraction

The *Punica granatum* and *Clerodendrum inerme* Leaves were dried in shade light independently and precisely pulverised to a coarse powder. The coarse powders weights of *Punica granatum* and *Clerodendrum inerme* Leaves were found to be 1365 g and 1438 g. The powders were presented to hot constant progressive extraction in a Soxhlet extraction with solvents in the increasing order of polarity utilizing petroleum ether, ethyl acetic acetate, acetone, methanol and water under controlled temperature (50-60 °C). The extracts thus obtained were concentrated in vacuum rotary evaporator and extracts were kept in dessicator for further utilization.

Preparation of Polyherbal extract (PHE)

Preparation of Polyherbal extract of *Punica granatum* and *Clerodendrum inerme* Leaves was done by mixing the two extracts in equal ratios of 1:1.

Phytochemical investigation

Phytochemical subjective investigation was performed by oppressing the crude extracts for recognizable proof tests to distinguish the presence of flavonoids, glycosides, alkaloids, sugars, fixed oils, tannoids, phytosterols, proteins, aminoacids, lignins, phenolic mixes, saponins, gums and mucilages.[15]

Methanolic extract of *Punica granatum* leaves (MEPGL) & aqueous extract of *Clerodendrum inerme* leaves (AECIL) were found to possess significant number of active constituents and are selected for antioxidant activity. The PHE was prepared by mixing the extracts of *Punica granatum* and *Clerodendrum inerme* leaves in equal ratio (1:1).[16]

Animals

Albino Rats (180-200 g) and Mice (20-25 g) were acquired from Sainath agencies, Musheerabad, Hyderabad (282/99/CPCSEA) and housed in creature facility of the organization. After haphazardly isolating the animals in to various sets, the rodents were acclimated for a time of one month before commencement of test. Rats were confined in polypropylene cages and protected under standard natural conditions, for example,

temperature (26 ± 2ºc), relative stickiness (45-55%) and 12hr dull/light cycle. The rats were taken care of with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The study protocol was approved from the institutional animal ethical committee with no: 1447/PO/Re/S/11/CPCSEA-36/A.

Single dose oral intense toxicity for multi week with net behavioral study

The Acute toxicity assessment of MEPGL and AECIL were performed based on OECD Guidelines No. 423 by utilizing mice and fixed dose studies were chosen where the limit dosage is 2000 mg/kg.

Experimental methods

Hepatoprotective activity

CCl_4 induced hepatotoxicity [17]

Albino rats were divided in to 6 groups each group consisting of 6 animals.

Group I: Untreated control group (Normal control) (1% liq. Paraffin, 1 ml/kg, s.c.)

Group II: Positive control group (hepatotoxin control) (Vehicle once daily for 7 days (s.c) followed by 1 ml/kg b.w CCl_4:liq.paraffin (1:1) (s.c) on 7^{th} day)

Group III: Standard group (100 mg silymarin once daily for 7 days (p.o) followed by 1ml/kg b.w CCl_4: liq.paraffin (1:1) s.c on 7^{th} day).

Group IV: MEPGL (400 mg/kg, oral, daily for 7 days followed by 1 ml/kg b.w CCl_4:liq.paraffin (1:1) s.c on 7^{th} day)

Group V: AECIL (400 mg/kg, oral, daily for 7 days followed by 1 ml/kg b.w CCl_4:liq.paraffin (1:1) s.c on 7^{th} day)

Group VI: Polyherbal extract (MEPGL + AECIL, 400 mg/kg, oral, daily for 7 days followed by 1 ml/kg b.w CCl_4:liq.paraffin (1:1) s.c on 7^{th} day)

Experimental Details:

All groups of rats were treated as shown above. On 8^{th} day, 18 h after the administration of CCl_4, all the animals were anesthesized with anesthetic ether for the collection of blood from retro-orbital plexus by using heparinized capillary tube. The collected blood was centrifuged at 12000 rpm for 10 minutes and the serum was separated. The separated serum was used for analyzing biochemical parameters such as SGPT, SGOT, ALP, total proteins, total bilirubin and triglycerides.

Paracetamol induced hepatotoxicity [18]

Albino rats were divided in to 6 groups containing six animals in each group.

Group I: Normal control group (2% w/v acacia suspension)

Group II: Toxin control group (vehicle for 7 days + Paracetamol 2 g/kg b.w (p.o) on 5^{th} day)

Group III: Standard group (silymarin 100

mg/kg b.w daily for 7 days and Paracetamol 2g/kg b.w on 5 th day)

Group IV: MEPGL (400 mg/kg, oral, daily for 7 days (p.o) and Paracetamol 2g/kg b.w (p.o) on 5th day).

Group V: AECIL (400 mg/kg, oral, daily for 7 days (p.o) and Paracetamol 2g/kg b.w (p.o) on 5th day).

Group VI: Polyherbal extract (MEPGL + AECIL, 400 mg/kg, oral, daily for 7 days and Paracetamol 2g/kg b.w (p.o) on 5th day).

Experimental details

All groups of rats were treated as shown above. On 8th day, all the rats were anesthesized with anesthetic ether and the blood was collected from retro-orbital plexus with the help of heparinized capillary tube. The collected blood was centrifuged at 12000 rpm for 10 minutes and the serum was separated. The separated serum was further used for the estimation of biochemical markers such as SGPT, SGOT, ALP, total proteins, total bilirubin and triglycerides.

Thioacetamide – induced hepatotoxicity [19]

Rats were divided in to 6 groups of each containing 6 rats.

Group I: Normal control group (2% w/v acacia suspension, p.o)

Group II: Toxin control group (Vehicle for 9 days, p.o + thioacetamide 100 mg/kg, s.c prepared in distilled water on 7th day)

Group III: Standard group (Silymarin 100 mg/kg b.w daily for 9 days, p.o and thioacetamide 100 mg/kg s.c on 7th day)

Group IV: MEPGL (400 mg/kg b.w dose daily for 9 days, p.o and thioacetamide 100 mg/kg s.c on 7th day)

Group V: AECIL (400 mg/kg b.w dose daily for 9 days, p.o and thioacetamide 100 mg/kg s.c on 7th day)

Group VI: Polyherbal extract (MEPGL + AECIL, 400 mg/kg, oral, daily for 9 days and thioacetamide 100 mg/kg s.c on 7th day)

Experimental details

All groups of rats were treated as shown above. After the treatment on 9th day, all the rats were anesthesized with anesthetic ether and the blood was collected from retro-orbital plexus with the help of heparinized capillary tube. The collected blood was centrifuged at 12000 rpm for 10 minutes and the serum was separated. The separated serum was further used for the estimation of biochemical markers such as SGPT, SGOT, ALP, total proteins, total bilirubin and triglycerides.

Isolation of Liver

After sacrificing the rats, liver was dissected out and washed with ice cold saline solution. The weight of the liver was noted after pressing between filter paper pads. A small portion of liver was suspended in 10%

formaldehyde for histopathological studies.

Statistical investigation

Entire values were declared as Mean± SEM for all the trial models.

Findings were deciphered by following assay:

i. Analysis of variance test (one way) continued by Dunnett's test.

ii. The findings were considered to be statistically remarkable when $p<0.05$

RESULTS AND DISCUSSION

Phytochemical investigations

Preliminary phytochemical screening of MEPGL and AECIL indicates the presence of lignins, flavonoids, alkaloids, tannins, glycosides, fixed oils, fats, carbohydrates and saponins. Methanolic extract of *Punica granatum* leaves and aqueous extract of *Clerodendrum inerme* leaves were selected for *in vivo* Hepatoprotective activity. Flavonoids are detected more in methanolic extract of *Punica granatum* leaves and flavonoids are reported to have hepatoprotective properties. Saponins are detected with greater clarity in aqueous extract of *Clerodendrum inerme* leaves and saponins are accounted for to be answerable for antioxidant and hepatoprotective activity.

Preparation of Polyherbal extract of *Punica granatum* and *Clerodendrum inerme* Leaves

Both the leaf extracts were combined in equal ratios (1:1) i.e 10 gms of *Punica granatum* leaf extract with 10 gms of *Clerodendrum inerme* leaf extract.

Acute toxicity studies of MEPNL and AESMF

The methanolic extract of *Punica granatum* leaves (MEPGL) and aqueous extract of *Clerodendrum inerme* leaves (AECIL) were administered to mice at dosages 5, 50, 300 and 2000 mg/kg with oral needle didn't show any side effects of poisonousness. The rodents were analyzed for about fourteen days, twice in a day has not shown harmful signs. Thus oral LD50 of MEPGL and AECIL was finished to outperform 2000 mg/kg. Therefore 2000 mg/kg was viewed as most secure higher portion for methanolic extract of *Punica granatum* leaves and aqueous extract of *Clerodendrum inerme* leaves and one fifth of 2000 mg/kg i.e 400 mg/kg (higher portion) of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) were preferred for the further Hepatoprotective action.

Effects of Methanolic extract of *Punica granatum* leaves (MEPGL), aqueous extract of *Clerodendrum inerme* leaves (AECIL) and Polyherbal extract (MEPGL+AECIL) against CCl_4,

Paracetamol and Thioacetamide induced hepatotoxicity in rats

The hepatoprotective potential of methanolic extract of *Punica granatum* leaves (MEPGL), aqueous extract of *Clerodendrum inerme* leaves (AECIL) and Polyherbal extract (MEPGL+AECIL) was assessed against CCl_4, paracetamol and thioacetamide induced hepatotoxicity. The serum levels of SGPT, SGOT, ALP, total protein, total bilirubin, triglycerides and liver weight were analyzed and compared with CCl_4, paracetamol and thioacetamide treated toxicant groups. The weight of the liver and the levels of SGPT, SGOT, ALP, bilirubin and triglycerides were significantly increased while the protein levels were significantly decreased in the positive control group. Polyherbal extract (MEPGL+AECIL) significantly altered serum parameters thus exhibiting synergistic hepatoprotective activity when compared to individual extracts. SGPT levels were significantly increased to five fold in the toxicant group. However, it was significantly decreased with polyherbal extract (MEPGL+AECIL, 400 mg/kg) treated rats. Individual MEPGL and AECIL extracts exhibited less reduction of SGPT levels when compared to PHE.. Similarly SGOT levels were increased to an extent of 3 fold in hepatotoxic control rats. This was significantly decreased in combined extract (MEPGL+AECIL, 400 mg/kg) treated rats. Alkaline phosphatase levels were nearly doubled in the hepatotoxic group and were significantly reduced with Polyherbal extract (MEPGL+AECIL, 400 mg/kg) treated rats when compared to MEPGL & AECIL independent extracts. Total protein levels in the serum were decreased in the positive control group. Polyherbal extract (MEPGL+AECIL, 400 mg/kg) significantly increased the levels of total proteins. Total bilirubin levels were elevated to 5 folds in the positive control group and significantly decreased with Polyherbal extract (MEPGL+AECIL, 400 mg/kg). There was a 3 fold increase in serum triglyceride levels in the hepatotoxin control group. Whereas with MEPGL 400 mg/kg & AECIL 400 mg/kg the triglyceride levels was not significantly reduced, but with Polyherbal extract (MEPGL+AECIL, 400 mg/kg) the elevated triglycerides were significantly reduced ($p<0.001$). There was a significant increase in the liver weight with toxicant group. However Polyherbal extract (MEPGL+AECIL) at 400 mg/kg significantly decreased liver weight. The standard hepatoprotective silymarin reduced the levels of serum enzymes, total bilirubin, triglycerides and increased the total protein

levels significantly ($p<0.001$). However silymarin exhibited moderate effect on liver weight ($p<0.05$). The results are shown in the **Table No.1, 2 & 3 and Figure No. 1-6**.

Histopathological studies

Normal control group liver revealed normal architecture. The hepatocytes, sinusoids, central veins and portal tracts appear within normal limits. Positive control (CCl_4, paracetamol & thioacetamide treated) groups revealed loss of normal liver architecture with fibrosis, congested sinusoids, ballooning degeneration, lymphocytic infiltration, hemorrhage and centrilobular necrosis (fatty liver). In standard silymarin treated rats, liver architecture was preserved with minimal fibrosis, portal tract and lesser extent of hepatic focal degeneration. In case of MEPGL (400 mg/kg) and AECIL (400 mg/kg) treated rats, the liver architecture was normal with moderate lymphocytic infiltration, light regeneration of hepatocytes and light ballooning degeneration associated with haemorrhage. Polyherbal extract (MEPGL+AECIL, 400 mg/kg) treated rats showed mild lymphocytic infiltration, more regeneration of hepatocytes when compared to individual extracts and negligable ballooning degeneration. The hepatocellular damage was improved with Polyherbal extract (MEPGL+AECIL, 400 mg/kg) when compared to individual extracts. The liver photomicrographs are shown in the **Figure no. 6**.

Table No.1: Effect of MEPGL, AECIL and combined extract (MEPGL+AECIL) on serum parameters and liver weight in CCl_4 induced hepatotoxicity

Groups	Liver weight (g/100g b.w)	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total Proteins (g/dl)	Total bilirubin (mg/dl)	Triglycerides (mg/dl)
Normal control	3.542± 0.17	51.74 ±5.04	98.86 ±7.14	154.63 ±5.04	9.317 ±0.615	0.415 ±0.153	84.36 ±5.65
Positive control	4.271 ±0.32	286.76 ±8.28	328.5 ±27.3	345.82 ±15.4	6.318 ±0.715	2.5 ±0.168	196.82 ±15.6
Standard silymarin	3.65± 0.75	125.24 ±8.13***	128.24 ±11.7***	145.6 ±11.3***	8.914 ±0.16***	0.564 ±0.18***	95.116 ±1.6***
MEPGL (400 mg/kg)	4.364± 0.69	218.91 ±13.4**	213.16 ±8.16***	205.6 ±6.7**	7.651 ±0.11**	1.356 ±0.16**	148.1 ±1.34
AECIL (400 mg/kg)	3.95± 0.08*	165.43 ±12.5***	173.3 ±15.6***	158.14 ±8.21***	8.416 ±0.92***	0.71 ±0.05***	106.26 ±6.94*
Polyherbal extract (MEPGL+ AECIL 400 mg/kg)	3.93± 0.33**	149.32±4.55***	155.4 ±17.47***	152.81 ±7.57***	8.672 ±0.72***	0.683 ±0.124***	102.34 ±4.82**

Findings are represented as mean ± SEM (n=6), ANOVA (one way) continued by Dunnett test, *P<0.05, **P<0.01, ***P<0.001 as equated to control.

Table No. 2: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on serum parameters and liver weight in Paracetamol induced Hepatotoxicity

Groups	Liver weight (g/100g b.w)	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total Proteins (g/dl)	Total bilirubin (mg/dl)	Triglycerides (mg/dl)
Normal control	3.675± 0.164	51.63± 4.17	99.35± 5.12	151.34± 5.07	9.786± 0.952	0.472± 6.278	84.65± 4.216
Positive control	5.718± 0.127	194.36± 5.88	291.17± 7.16	315.6± 7.5	4.932± 0.271	2.568± 0.215	183.19± 3.15
Standard silymarin	4.376± 0.6***	102.94± 4.93***	135.1± 4.21***	111.19± 9.1***	7.318± 0.16***	0.9815± 0.011***	95.12± 3.2***
MEPGL (400 mg/kg)	4.96± 0.017	154.54± 4.08***	215.17± 5.4***	224.15± 6.1***	5.651± 0.715*	1.483± 0.215***	146.13± 3.1***
AECIL (400 mg/kg)	4.566± 0.13**	122.18± 4.95***	152.16± 6.1***	156.72± 4.5***	6.937± 0.15***	1.079± 0.026***	105.81± 3.2***
Polyherbal extract (MEPGL+ AECIL 400 mg/kg)	4.434± 0.67**	112.36± 3.94***	144.12± 5.21***	138.43± 3.76***	7.154± 0.96***	1.026± 0.721***	99.62± 4.6***

Findings are represented as mean ± SEM (n=6), ANOVA (one way) continued by Dunnett test, *P<0.05, **P<0.01, ***P<0.001 as equated to control

Table No. 3: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on serum parameters and liver weight in thioacetamide induced hepatotoxicity

Groups	Liver weight (g/100g b.w)	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total Proteins (g/dl)	Total bilirubin (mg/dl)	Triglycerides (mg/dl)
Normal control	2.972± 0.015	37.6± 4.12	83.19± 7.62	148.16± 7.33	8.415± 0.415	0.491± 0.121	80.7± 4.75
Positive control	4.761± 0.314	195.72± 8.125	240.18± 15.4	285.14± 9.35	5.316± 0.514	2.257± 0.071	165.12± 3.14
Standard silymarin	3.512± 0.73***	81.14± 7.3***	113.68± 7.15***	169.4± 6.5***	7.861± 0.15***	0.751± 0.11***	86.65± 4.12***
MEPGL (400 mg/kg)	4.081± 0.03***	142.35± 3.1***	188.63± 7.05***	223.5± 7.6***	7.152± 0.37***	1.33± 0.53***	128.85± 2.15
AECIL (400 mg/kg)	3.561± 0.05***	106.9± 3.6***	144.26± 7.15***	188.11± 3.7***	7.145± 0.17***	0.885± 0.02***	97.46± 5.17***
Polyherbal extract (MEPGL+ AECIL 400 mg/kg)	3.535± 0.62***	94.31± 2.7***	131.53± 5.82***	174.5± 6.2***	7.432± 0.86***	0.814± 0.08***	92.57± 6.92***

Findings are represented as mean ± SEM (n=6), ANOVA (one way) continued by Dunnett test, *P<0.05, **P<0.01, ***P<0.001 as equated to control.

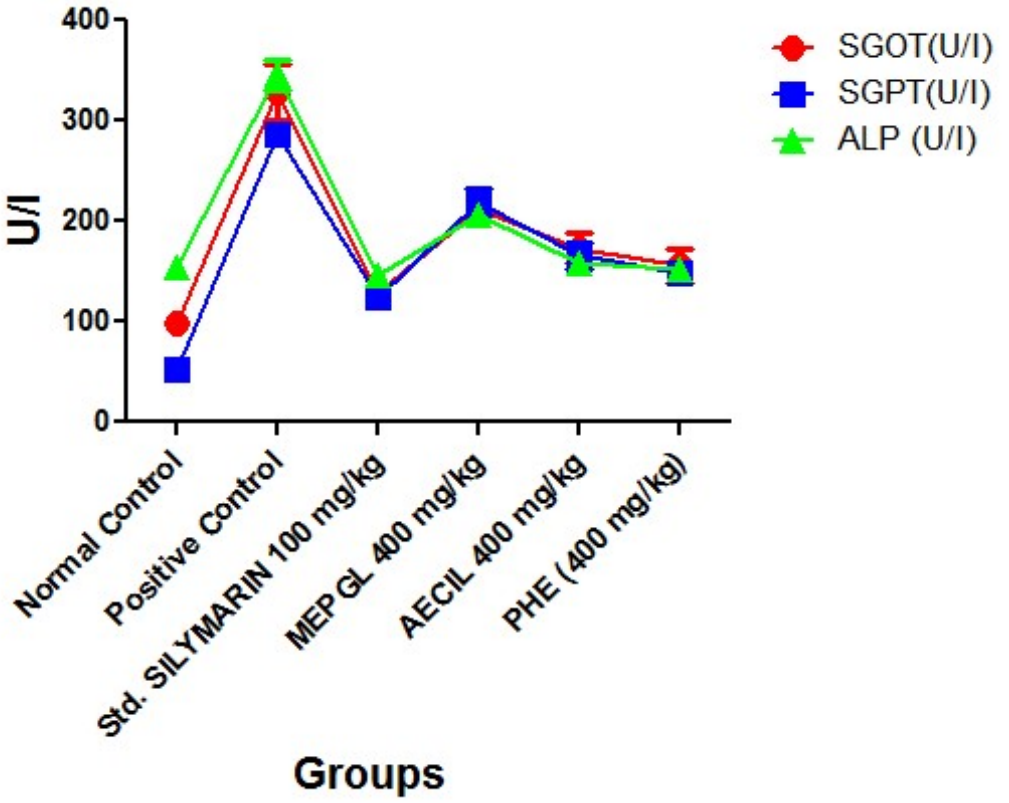

Figure No. 1: MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on SGPT, SGOT and ALP in CCl4 induced Hepatotoxicity

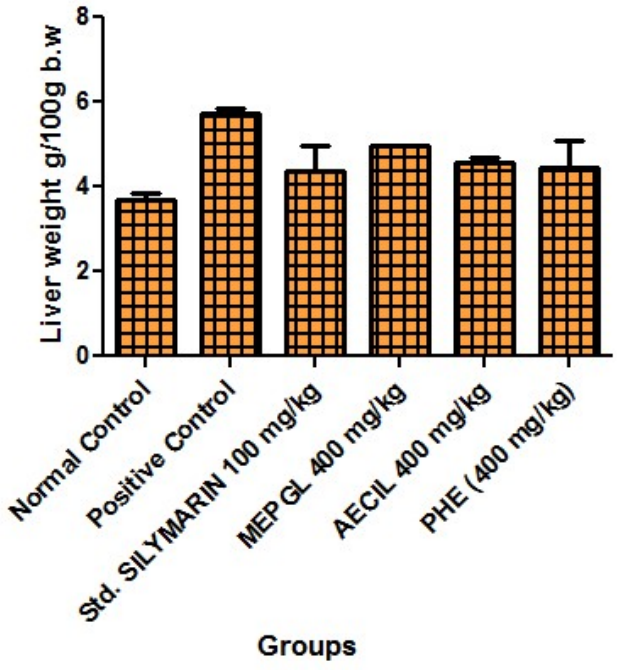

Figure No.2: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on liver weight (g/100g b.w) in Paracetamol induced hepatotoxicity

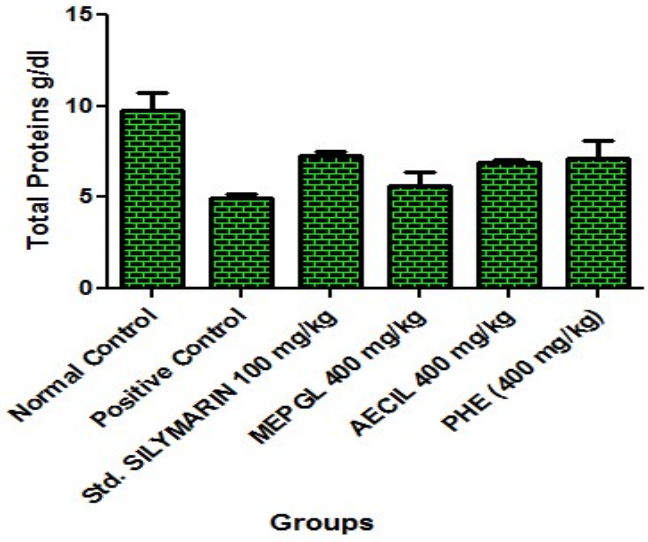

Figure No.3: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on total proteins (g/dl) in Paracetamol induced hepatotoxicity

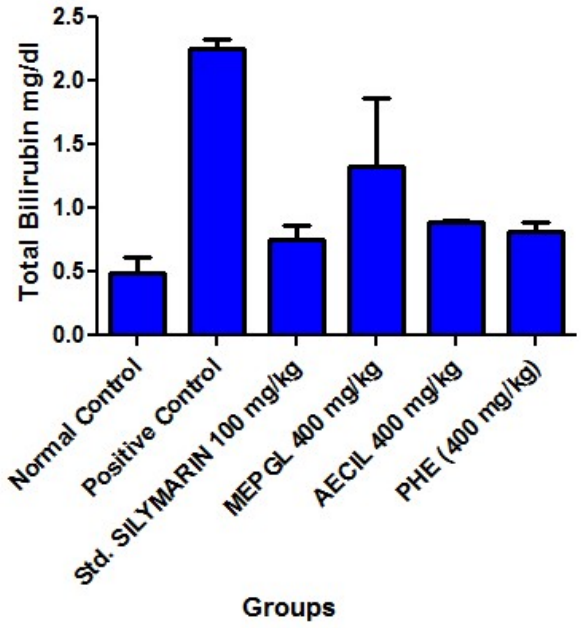

Figure No. 4: Effect of MEPGL, AECIL and Polyherbal extract extract (MEPGL+AECIL) on total bilirubin in thioacetamide induced induced hepatotoxicity

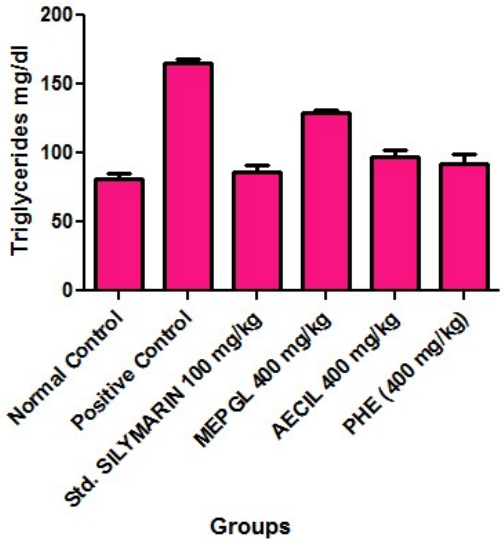

Figure No. 5: Effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on triglycerides in thioacetamide induced induced hepatotoxicity

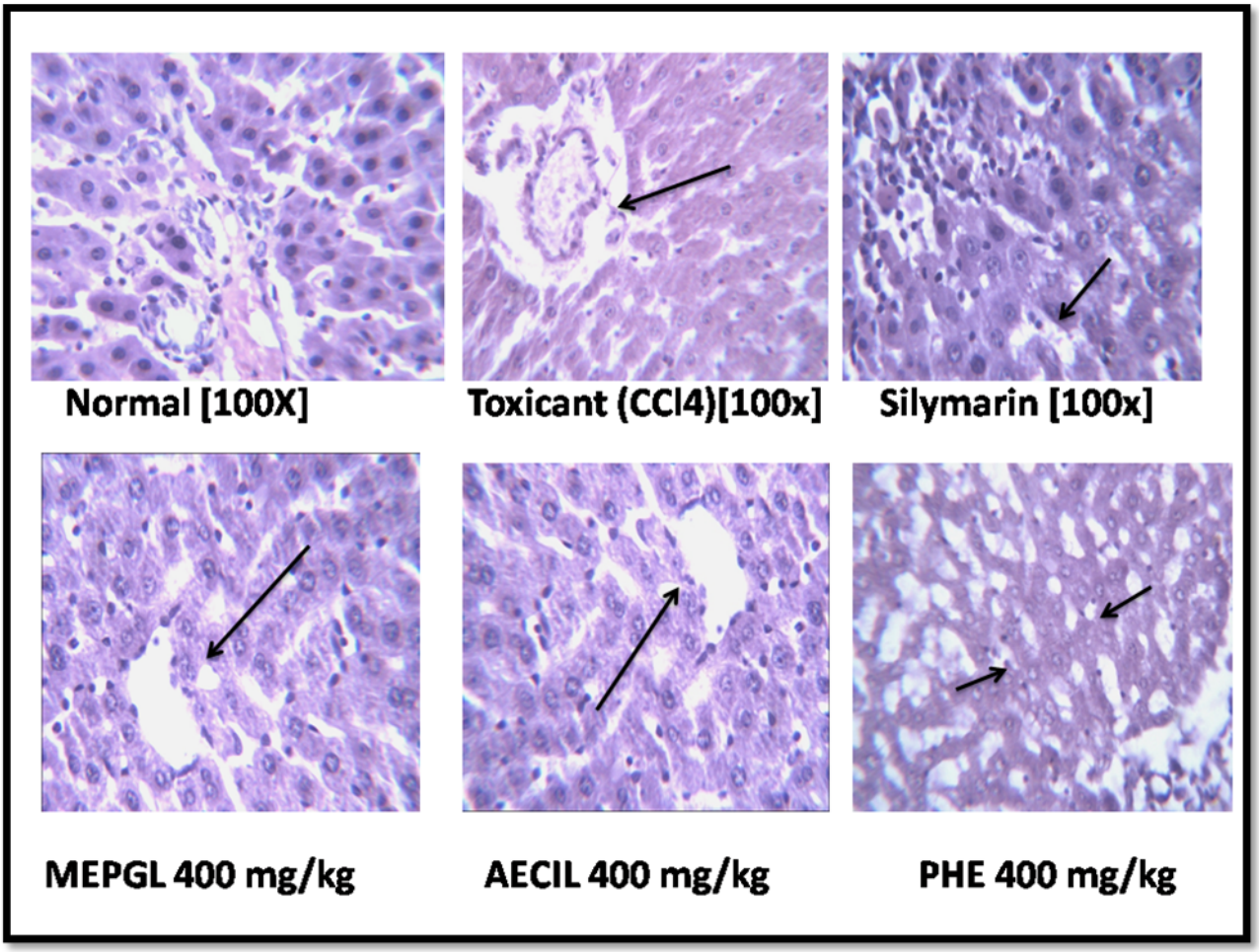

Figure No. 6: Histopathological photographs showing the effect of MEPGL, AECIL and Polyherbal extract (MEPGL+AECIL) on liver architecture in CCl_4 induced hepatotoxicity

CONCLUSION

Phytochemical screening of methanolic extract of *Punica granatum* leaves and aqueous extract of *Clerodendrum inerme* leaves has confirmed the presence of flavonoids, Saponins, tannins, alkaloids, glycosides and lignans. With acute toxicity studies of MEPGL & AECIL on rats, therapeutically safest dose is 400 mg/kg. Further hepatoprotective activity reveals that the Polyherbal extract of *Punica granatum* leaves and aqueous *Clerodendrum inerme* leaves was found to be more effective in reversing the hepatotoxicity induced by CCl_4, Paracetamol and thioacetamide when compared to individual extracts. Hence it can be hypothesized that the above mentioned active principles in the leaves and fruits of the plants may have synergistic hepatoprotective potentials. However further studies are essential to discover the exact mode of hepatoprotective action of the polyherbal extract.

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