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Evaluation of Hepatoprotective Effect of Polyherbal Extracts in Rats

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***Abstract*: The hydro alcoholic herbal extracts of *Aegle marmelos, Eclipta alba* and *Lippia multiflora* leaves combinations was screened for hepatoprotective activity in Paracetamol induced hepatotoxicity in albino rats. The degree of protection was measured by estimating biochemical parameters like Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase, Serum alkaline phosphatase, triglycerides and level of total Serum bilirubin. Hepatoprotective activity of Hydro alcoholic leaves extract mixture of *Aegle marmelos, Eclipta alba* and *Lippia multiflora* at different formulations were compared with Liv.52 (500mg/kg, p.o.) treated animals.**

***Keywords*: Paracetamol, Hepatoprotective activity, *Aegle marmelos, Eclipta alba,* Liv.52 and *Lippia multiflora*.**

1. Introduction

Liver is the largest internal organ weighing about 1400grams in adults. It constitutes 2.5% of total body weight. The bile secreted by the liver plays an important role in digestion. Over that, liver is integral part of drug metabolism and removal of xenobiotics from the body thus protecting against foreign substances by detoxifying and eliminating them. Various chemotherapeutic agents like paracetamol (high dose), carbon tetrachloride, thioacetamide etc. during their metabolism inside the liver cause severe damage to hepatocytes. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation and other oxidative damages in liver. A drug having beneficial effect on the liver is known as Hepatoprotective.

Acetaminophen (paracetamol, N-acetyl-*p*-aminophenol; APAP) is a widely used analgesic and antipyretic drug. At therapeutic doses, it is believed to be safe, having analgesic and antipyretic effects similar to those of aspirin and ibuprofen. Although considered safe at therapeutic doses, at higher doses, acetaminophen produces a centrilobular hepatic necrosis that can be fatal. The metabolism, acetaminophen was converted by drug metabolizing enzymes to a reactive metabolite that covalently bound to proteins. At nontoxic doses,

the metabolite was efficiently detoxified by glutathione forming an acetaminophen-glutathione conjugate. However, at toxic doses, the metabolite depleted hepatic glutathione by as much as 80–90% and subsequently covalently bound to protein. Subsequently, the reactive metabolite of acetaminophen was identified to be N-acetyl-*p*-benzoquinone imine (NAPQI). It was found to be formed by cytochrome P-450 (CYP) by a direct two electron oxidation of acetaminophen, a previously unrecognized mechanism of CYP. A major problem is that hepatic CYP enzymes or glutathione levels important in metabolic activation of acetaminophen and detoxification may be altered by treatments or genetic modification of the animal. These results decrease in formation of NAPQI, less glutathione depletion, less covalent binding, and less toxicity. The only hepatocytes that developed necrosis had acetaminophen-protein adducts [1].

The hepatotoxicity of acetaminophen appears to occur by a complex mechanistic sequence. These events include: (1) CYP metabolism to the reactive metabolite NAPQI which depletes glutathione by a conjugation reaction and covalently binds to proteins; (2) loss of glutathione causing an increased oxidative stress response (decreased detoxification of reactive oxygen and nitrogen species); (3) increased oxidative stress, possibly associated with alterations in calcium metabolism, initiation of signal transduction responses and mitochondrial permeability transition; (4) mitochondrial permeability transition occurring with an even larger increase in oxidative stress, loss of mitochondrial membrane potential, and loss of the ability of the mitochondria to synthesize ATP; and (5) loss of ATP which causes necrosis. Associated with these essential events there appears to be a number of modulators of inflammatory responses that can alter the severity of liver injury following the initiation of toxicity [2].

Liv.52, a multi-ingredient formulation, offers a new dimension in the management of hepatic cell damage. It markedly improves liver function by acting as a stimulant. Experimental studies have reported that Liv.52 offers significant protection against carbon tetrachloride; alcohol and beryllium induced hepatic damage. The present study was undertaken to investigate the Liv.52 protection against paracetamol induced hepatotoxicity. The potential cytoprotective effect of Liv.52 was studied in vitro studies, it improves copper and tetra-butyl hydroperoxide (t-BHP) toxicity in HepG2 cells by inhibition of lipid peroxidation and

increase of GSH content and antioxidant enzyme activity. Another recent study found that Liv.52 abrogated the ethanol-induced PPARγ suppression and ethanol-induced TNFα gene expression, it also up regulated PPARγ mRNA.

The Liver is very important organ of our human system having various multifunctional activities like metabolism and excretion. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction.

2. Plant Profile

A. Bael leaves [3]

- Botanical name : *Aegle marmelos*
- Kingdom : Plantae
- Orde r : Sapindales
- Family : Rutaceae
- Genus : Aegle
- Species : *Aegle marmelos*

Uses: Anti-inflammatory, Anti-Ulcer, Antidiarrheal activity, and laxative, expectorant and also to treat cold, respiratory infections, backache, abdominal disorder, vomiting, cut and wounds, dropsy, beriberi, cholera, cardiac tonic, nervous disorders and veterinary medicine.

B. Bhrngarajah leaves [4]

- Botanical name : *Eclipta alba*
- Kingdom : Plantae
- Division : Tracheophyta
- Orde r : Asterales
- Family : Asteraceae
- Genus : EcliptaL.
- Species : Ecliptaalba(L.) Hassk

Uses: Treatment of jaundice, antihelmentic, scorpion sting, anti-venom, prevents abortion and miscarriage.

C. Lippia tea leaves [5]

- Botanical name : *Lippia multiflora*
- Order : Lamiales
- Family : Verbenaceae
- Genus : Lippia
- Species : L. abyssinica

Uses: The infusion of the leaves is traditionally used to treat fevers, coughs, influenza. Some rural dwellers cook the herbs and use it to relieve stress and enhance sleep.

3. Methodology

A. Preparation of plant leaf extract

The collected *Aegle marmelos*, *Eclipta alba* and *Lippia multiflora* were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 100g of powdered materials were extracted with 70% ethanol (60˚-80˚C) using soxhlet apparatus. The extraction was carried out until the powder become colourless. The extract is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in carboxy methyl cellulose and used for the experiment.

B. Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on the aqueous extract of the leaves of Murraya Koenigii for qualitative identification.

C. Identification of Hepatoprotective activity

Animals used: Male wistar albino rats (175-200g) were maintained in a 12h light/dark cycle at a constant temperature 25˚C with free access to feed and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover, the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals.

D. Acute toxicity study

The procedure was followed according to the OECD guidelines 423. The acute toxic class method is a step wise procedure with 3 male sex animals per group. Depending on the mortality and or morbidity status of the animals, on an average 2-4 steps may be necessary to allow judgment on the acute toxicity of the testing substance. According to this procedure minimum number of animals were to be used for acceptable data band scientific conclusion. The method uses defined doses of *Aegle marmelos*(AM), Eclipta *alba*(EA) and *Lippia multiflora*(LM) leaves extract (50:50:100 mg/kg, 100:200:300 mg/kg, 200:300:500 mg/kg, 400:400:900 mg/kg and 500:800:1200 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system for the classification of chemical which causes acute toxicity.

Adult male Wistar rats were used for this study. The starting dose of AM, EA and LM leaves extract was 50:50:100 mg/kg body weight. The dose was administered to overnight fasted rats and food was withheld for further 3-4 hours after administration of the drug and observed for signs of toxicity.

Body weight of the rats before and after treatment were noted and any changes in skin, eye and mucous membranes, salivation, nasal discharge, urination and behavioural (sedation, depression), neuromuscular, cardiovascular, lethargy, sleep and coma were noted. The onset of toxicity was also noted. The animals were kept under observation for 14 days.

The acute toxicity of Ethanolic leaf extract of AM, EA and LM were determined as per the OECD guidelines no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at high dose. Hence, AM, EA and LM leaves extracts 50:50:100 mg/kg bw, 100:200:300 mg/kg bw and 200:300:500 mg/kg bw of this dose were selected for further study.

E. Paracetamol induced hepatotoxicity in rats

- The liver protective effect was evaluated using the paracetamol (PCM) model.
- Wistar albino rats (180-220g) were divided into six groups and were subjected to the following treatments:
- Group-I served as normal control; received vehicle only i.e., Carboxy methyl cellulose.
- Group-II served as toxicant group; received only PCM for three days, to assist assessing the severity of toxicity produced by PCM administration.
- Groups III-VI-V served as treated groups; received AM, EA and LM at the dose of 50:50:100 mg/kg, 100:200:300 mg/kg, 200:300:500 mg/kg p.o. and standard drug Liv.52 at a dose of 500 mg/kg p.o. were administered orally to rats of the respective groups for 15 days.
- Group VI is given only Liv 52 (5 mg/kg).
- PCM diluted with Carboxy methyl cellulose (CMC) (1:1) was administered in dose of 1ml/kg, p.o. for 3 days to all animal groups except for normal control. After 72h of PCM treatment and 15 days of post treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Serum was separated by centrifugation at 2500 rpm at 37˚C for 15min and analyzed for various biochemical parameters.

Biochemical estimation: The Separated serum was subjected to estimate SGOT, SGPT, alkaline phosphatise (ALP), total serum bilirubin and triglycerides(tg).

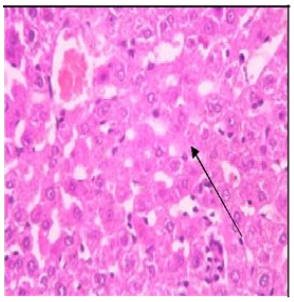
Fig. 1. Normal control

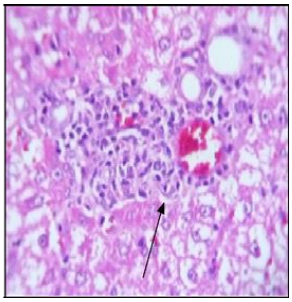
Fig. 2. PCM treated groups

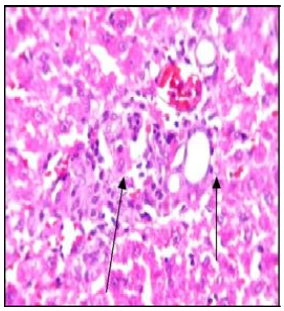
Fig. 3. Test dose 1

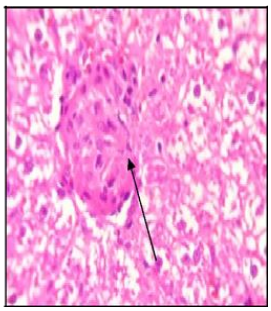
Fig. 4. Test dose 2

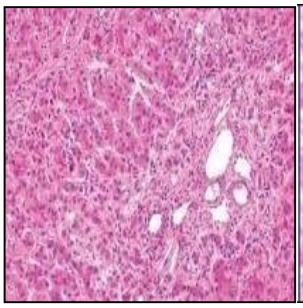
Fig. 5. Test dose 3

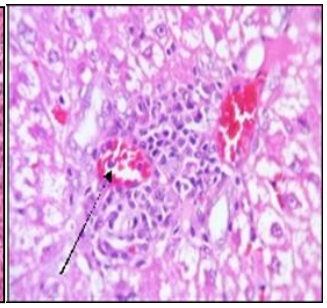
Fig. 6. Liv.52+ PCM

4. Results

C. Histopathological studies in liver

Histopathological studies of section of liver of control and

A. Acute Toxicity Studies

Table 1
Acute Toxicity Studies

Group	Dose(mg)/kg AM:EA:LM	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration
		Before Test	After Test			
I	50:50:100	206 g	200 g	No signs	Nil	14 Days
II	100:200:300	192 g	182 g	No signs	Nil	14 Days
III	200:300:500	191 g	178 g	No signs	Nil	14 Days
IV	400:400:900	202 g	174 g	No signs	Nil	14 Days
V	500:800:1200	212 g	210 g	No signs	Nil	14 Days

B. Effect of plant extract on paracetamol induced hepatotoxicity

Table 2
Effect of plant extract on hepatotoxicity

Group (n=6)	Biochemical Parameters				
	SGOT (IU/L)	SGPT (IU/L)	ALP (KAunits/Dl)	Total serum Bilirubin (mg/dL)	TGs (mg/dL)
I	66±12.6	73±30.23	101±21.1	0.8±0.04	128±16.6
II	221±16.5	246±16.8	171±25.55	0.9±0.02	139±12.32
III	42.5±7.63	49±10.2	83±26.8	0.7±0.06	91±12.89
IV	46±5.2	47.5±5.65	86±35.7	1.0±0.04	123±44.5
V	62.5±6.52	72±8.88	86±8.58	0.8±0.15	112.5±60.2
VI	41±2.64	67±5.10	94±9.88	0.75±0.11	111±52.3

experimental rats were compared in the following figures. In Group-I (normal control) rats, liver showed normal architecture. The central liver, portal tracts, hepatocytes and sinusoids appear normal. The lobular unit is well identified (Fig 3). Group-II (PCM treated) shows loss of the normal liver architecture. There are extensive areas of patchy and confluent hepatocyte necrosis and lobular inflammation is intense, (Fig 4). The hepatic cells of the rats treated with test groups and intoxicated with PCM were radially arranged. The vacuolation was present. The recovery was comparable to that of silymarin a standard hepatoprotective agent.

Histopathological examination of the liver section of the rats treated with toxicant showed mild congestion, increased space of canaliculi moderate vacuolation and foci of necrosis, Apoptotic hepatocytes and Congested central veins. The rats treated with Liv.52 and extract along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of Effaced architecture, Apoptotic hepatocytes and Congested central veins.

5. Discussion

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. The lowering of enzymes level is definite indication of hepatoprotective action of the drug. In the present study the hepatoprotective activity of polyherbal mixture was evaluated in drug (Paracetamol) induced liver toxicity. Chronic administration of PCM for 3 days produced elevation of the serum levels of these markers in treated animals (Group II) compared to that of the control group (Group I). The present investigation also revealed that the given dose of PCM produced significant elevation in SGOT, SGPT, ALP, triglycerides and total bilirubin levels and also histopathology study indicating all impaired liver function, which leads to damage of structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages, indicating development of hepatotoxicity.

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Interest in a large number of traditional natural products has increased. In the present study, we used an animal model to reveal the protective role of *Aegle marmelos*, *Eclipta alba* and *Lippia multiflora* extract formulations against Paracetamol-induced hepatic toxicity. Further, in the present investigation, phytochemical analysis of leaf extract revealed the presence of flavonoids, steroids, saponins, tannins, flavonoids, Carbohydrates, Xanthoproteins, Coumarin and phenolic compounds. flavanoids (Yoshikawa et al., 2003) and saponins (Baek et al., 1996) are well known for their antioxidant and hepatoprotective activities.

Liv.52 at 500 mg/kg dose significantly prevented such rise in prophylactic study. The protective effect of Liv.52 was well established in several models of hepatotoxicity and was reported to be due to its antioxidant and membrane stabilizing activities. The reduction of PCM-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver, Which was markedly reduced by the dose of Liv.52 and Test dose formulations (AM:EA:LM=100:200:300 mg/kg, b.w. and 200:300:500 mg/kg, b.w.). Moreover, in the present study, the effects of Liv.52 and leaves of *Aegle marmelos*, *Eclipta alba* and *Lippia multiflora* extract mixture on blood parameters were demonstrated by the significant reduction in Granulocytes, PLT, MCV, MCH, MCHC and PCT compared to induced group while WBC, Lymphocytes, Monocytes, RBC, Hb, HCT, RDW-CV, RDW-SD, MPV and PDW counts are improved.

Rita Bouagnon et al, Arun K et al has suggested that *Lippia multiflora*, *Aegle marmelos* and *Eclipta alba* extracts are most widely consumed beverages in the world and more attention is paid to its health benefits particularly those generated by ethanol or drug consumption.

The histopathological study also supported the biochemical evidence for the hepatoprotection shown by Polyherbal mixture. The normal hepatic cell is a polygonal cell and binucleated with nucleolus and abundant eosinophillic cytoplasm. The above features were found in normal control group (Fig. No: 1). In PCM treated group i.e group II all the above mentioned structures were modified and there was macrovesicular steotosis, necrosis and degeneration indicating hepatic damage (Fig. 2). Where, the polyherbal mixture/Liv.52 treated groups the normal structures were protected in prophylactic study (Fig. 3 to 6). Dose dependent protection/regeneration were observed in extract/standard treated groups.

The total serum bilirubin did not vary significantly during the study. However, a significant increase of biochemical parameters was noticed the inflammatory process initiated by Paracetamol. The results also show that the polyherbal mixture at the dose of about 100:200:300 mg/kg bd. wt. was found to be more effective than 200:300:500 mg/kg bd. wt. and standard Liv.52-500mg/kg bd. wt. doses.

6. Conclusion

The herbal extract mixture is proved to be effective against paracetamol induced hepatic toxicity in Rats. The results also show that the polyherbal mixture at the dose of about 100:200:300 mg/kg bd. wt. was found to be more effective than 200:300:500 mg/kg bd. wt. and standard Liv.52-500mg/kg bd. wt. doses. Thus the presence of flavonoids, terpenoids, saponins, tannins and polyphenolic compounds in the herbal extracts might be responsible for the hepatoprotective activity. The study clearly indicates Ethanolic leaf extract of *Aegle marmelos*, *Eclipta alba* and *Lippia multiflora* are effective in the treatment and prevention of paracetamol induced hepatotoxicity.

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