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PHYTOCHEMICAL INVESTIGATIONS AND HEPATOPROTECTIVE EFFECTS OF AQUEOUS FRUIT EXTRACT OF *AEGLE MARMELOS* CORR.

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ABSTRACT

Aegle marmelos Correa (Rutaceae), commonly known as Bael has been used in ethno-medicine to exploit it's medicinal properties including astringent, antidiarrheal, antidysentric, dermulcent, antidiabetic and anti-inflammatory activities. *Aegle marmelos* Corr. is widely used in the treatment of hepatitis in folk medicine. Owing to its overwhelming ethnomedicinal significance, it is proposed to investigate it chemically and to verify the hepatoprotective activity of the crude aqueous extract of the fruit of *Aegle marmelos*. A novel compound was isolated from the aqueous fruit extract. The aqueous crude extract showed significant hepatoprotective activity in Wistar albino rats treated with paracetamol.

Key words: Paracetamol, Aegle marmelos, SGPT, SGOT, ALP, Bilirubin, Hepatoprotective, Silymarin.

INTRODUCTION

Liver is the vital organ in the body that is concerned with the detoxification of toxic substances. It is exposed to a wide variety of xenobiotics, hepato toxins and chemotherapeutic agents that leads to damage and subsequent impairment of its function[1]. Therefore herbal and other indigenous sources have been adequately explored for the safe and effective hepatoprotective action.

Aegle marmelos corr, (Rutaceae)[2] known as Bael is common through out India. Bael is generally grown in temples dedicated to lord shiva, whose worship cannot be completed without the leaves of this tree[3]. In recent times it is frequently eluded as an emblem of fertility[4]. The parts of this plant at all stages of maturity are used as ethano medicine against

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various human ailments. The Bael fruits are used in Ayurveda as well as Unani medicines for the treatment of many diseases such as chronic diarrhoea, dysentery and typhoid. Ripe fruits show antiviral activity.

Aqueous fruit extract lower blood sugar and it shows antidiabetic activity in streptozotocin induced wistar diabetic rats[5]. The fruit extract of *Aegle marmelos* protects the mice against radiation induced lethality, cure for dyspepsia, constipation and body healing problems[6]. Earlier several chemical constituents have been isolated and characterised from different parts of the tree. These include coumarins[7], anthraquinones[8], sterols[9], lignan glycosides, protoliminoids and alkaloid amides[10].

According to literature fruits of *A. marmelos* are prescribed in the treatment of tuberculosis and hepatitis but scientific study reported regarding its hepatoprotective activity is very sparse[11]. Therefore the present study was conducted to investigate its hepatoprotective activity.

EXPERIMENTAL

Materials and methods

Plant extract

The finely powdered (25 g) aqueous extract of the plant was procured from Laila Impex, Vijayawada, Andhra Pradesh.

Animals

Albino rats (Wistar strain) and albino mice (Swiss strain) used in the present studies were procured from the animal house of Ghosh enterprises, Kolkata. All animals were kept under standard laboratory conditions and fed on standard diet supplied by Reyans Biotechnologies Pvt Ltd., Hyderabad. The study was approved by Institutional animal ethical committee. (516/01/A/CPCSEA).

The finely powdered (20 g) aqueous fruit extract was collected with methanol by using soxhlet apparatus. After evaporation of the solvent under reduced pressure 9 g of crude extract was obtained. The resulting residue was dissolved in methanol and adsorbed on to 20 g silica gel (100-200 mesh). It was then subjected gradient column chromatography by hexane, ethyl acetate, methanol in increasing polarity. The fractions were collected and monitered on TLC. Upon purification of the collected fractions a novel alkaloid amide derivative was obtained along with β-amyrin. The new alkaloid amide derivative was

obtained at 5% ethyl acetate in hexane fractions. The structure was established by interpretation of the spectral data (^{1}H NMR, ^{13}C NMR, IR and Mass).

Acute toxicity studies

Albino mice (Swiss strain) were divided into five groups of 6 each. One group was set as control group and remaining four groups received (800, 1000, 2000 and 3000 mg/kg, b.w) of the extract orally. The mice were observed continuously for 1 hr for any gross behavioural changes and death, if any intermittently for the next 6 hrs and then again at 24 hrs after dosing. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed.

Paracetamol induced hepatotoxicity

The rats were divided into six groups I-VI, each group consisting of six rats. The rats in group-I served as vehicle control. Liver toxicity was induced in rats by administering paracetamol orally in a 1% CMC at a dose of 2 g/kg body weight for three days in all the groups except control (group-I).

Group-II served as paracetamol control.

Group-III received silymarin (25 mg/kg, b.w) for seven days.

Group-IV-VI received aqueous extract (100, 200 and 400 mg/kg, b.w) for seven days. All samples were administered orally.

Assessment of hepatoprotective activity

Blood samples of the rats were withdrawn on 1st, 4th and 10th day from the retro-orbitol plexus. Serum was coagulated after coagulating at 37^{o} for 30 min and centrifuging at 2000 rpm for 15 min, and estimated for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate trans aminase (SGPT)[8], alkaline phosphate (ALP)[9] and serum bilirubin (SBLN)[10] using kits supplied by Bayer diagnostics India Limited.

The hepatoprotective activity was confirmed through histopathological studies on liver of rats. After collection of blood for biochemical estimation, the rats were sacrificed and the liver was carefully dissected, cleaned of extraneous tissue and fixed in 10% formalin and kept for 24 h. Then the paraffin sections were prepared (automatic tissue processor, Autotechnique) and cut into 5 µm thick sections, using a rotary microtome. The sections were stained with Haematoxylin-Eosin dye and studied for histopathological changes[11].

Statistical analysis

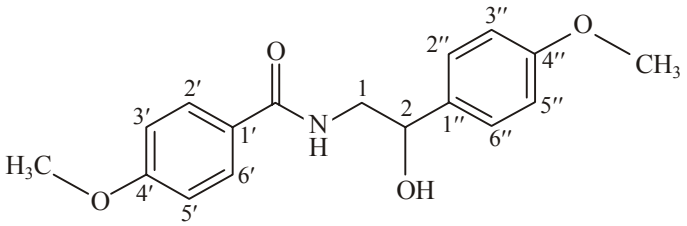
All the results were expressed as mean ± SEM. The siginificance of difference between mean values for the various groups was tested by using one way analysis of varience (ANOVA). The level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

Chemistry

The new compound which was obtained as white needles, m.p 286-288^{o}C. It was analysed for $C_7H_{19}NO_4$ which was supported by mass peak at *m/z* 301.5. The IR spectrum showed a peak at 1680.59 for an aromatic carbonyl and 3310.16 for a hydroxyl. Its ^{1}H NMR spectrum exhibited a signal at 3.88 and ^{13}C NMR values 66.37 (6H, s) accounting for two aromatic methoxyls. It showed two sets of four aromatic protons as AB quartets each corresponding to the presence of two para substituted aromatic rings in the molecule. This is supported by ^{13}C NMR at 115.21 and 122.74.

The two aromatic rings and the two aromatic methoxyls accounted for 14 carbons, out of the 17 carbons in the molecule. The ^{1}H NMR spectrum showed a methylene doublet (1) and a proton α to the hydroxyl as triplet (2). This suggest that the carbonyl carbon as an aromatic amide which appeared at (δ 166). The carbon connected to the hydroxyl was shown at δ 51.37. The methylene carbon connected to nitrogen on one side and hydroxyl methylene on the other side appeared at δ 29.66. Based on the foregoing data the structure of the new aromatic compound was deduced as N-(2-hydroxy-2-4'' methoxy phenyl)-4'-methoxy benzamide.



N-(2-Hydroxy-2-(4" -methoxy phenyl) ethyl)-4' -methoxy benzamide

Pharmacology

Results presented in Table 1 indicates that the elevated levels of SGOT, SGPT, ALP and BLN (total) due to paracetamol intoxication were reduced significantly ($P < 0.001$) in

rats after treatment with aqueous extract at 100 mg, 200 mg and 400 mg/kg b.w, silymarin used as standard.

Table 1: Effect of crude aqueous extract of fruit of *A. marmelos* on serum parameters in paracetamol induced (2 g/kg) hepatic damage in rats

Parameters	Control Group-I	Paracetamol Group-II 2g/kg	Silymarin Group-III 25 mg/kg	Extract 100 mg/kg Group-IV	Extract 200 mg/kg Group-IV	Extract 400 mg/kg Group-IV
T. Bilirubin (mg/dl)	0.61 ± 1.08*	2.39 ± 1.16*	0.94 ± 1.21*	1.8 ± 0.12*	1.5 ± 0.66*	1.22 ± 1.11*
SGOT/AST (IU/L)	130 ± 6.54*	222.5 ± 11.12***	144 ± 17.34*	218.3 ± 10.49***	192 ± 11.05***	176 ± 4.63*
SGPT/ALT (IU/L)	31 ± 5.29*	55.75 ± 7.85**	36.751 ± 5.34*	54.33 ± 11.02*	44 ± 5.03***	43 ± 2.64***
ALP (IU/L)	82 ± 7.82*	133 ± 12.83***	106.5 ± 10.43*	168 ± 10.33***	129 ± 6.928***	123 ± 11.32***

The data were represented as as mean ± S.E.M of six animals in each groups.

Student t test was used for statistical analysis of blood serum parameters.

* the value $P < 0.05$ consider to be significant, ** the value were $P < 0.01$ consider to be significant., *** the value $P < 0.001$ consider to be significant.

Comparison

Group-IV, V, VI (Aqueous Extract only) is compared with group-I (Control Group);

Group-II (Paracetamol only) was compared with group-I (Control Group)

Group-III (Standard Group) is compared with group-II (Paracetamol).

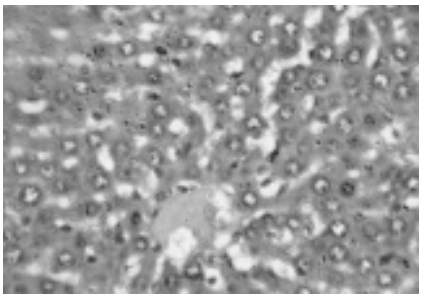
Histopathological studies in liver

Histopathological studies of section of liver of control and 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. 1).

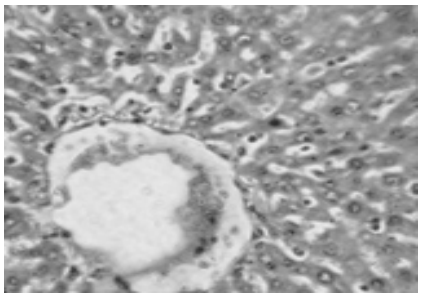
Group-II (Paracetamol treated) shows loss of the normal liver architecture. There are extensive areas of patchy and confluent hepatocyte necrosis and lobular inflammation is intense, (Fig. 2).

In the liver section of the animals treated with aqueous extract (100 mg/kg b.w) + paracetamol, the nuclei's are not very clear as in normal hepatocytes, however when compared to the CCl_4 damage group, the number of hepatocytes with normal nucleus was much more.



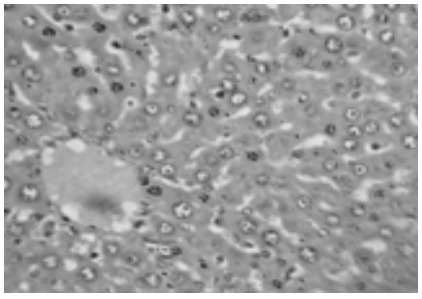
Section of control group liver

Fig. 1: The parenchymatous tissue showing the regenerative changes by emptying of cytoplasm of Hepatocytes and nucleus was centrally located



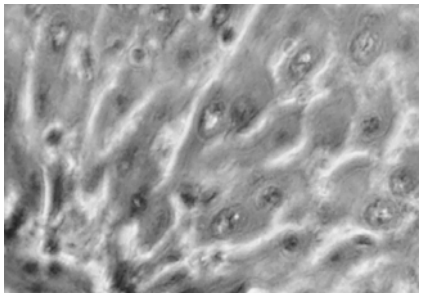
Section of paracetamol treated group liver

Fig. 2: Liver showing mild congestion, increased space of canaliculi moderate vacuolation and foci of necrosis



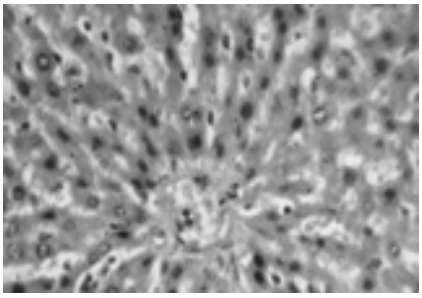
Section of plant extract (100 mg/kg) + paracetamol treated group liver

Fig. 3: Hepatic parenchyma revealing a large area of necrosis and serve degenerative changes normal space of canaliculi was observed



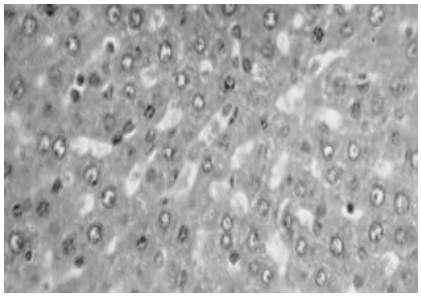
Section of plant extract (200 mg/kg) + paracetamol treated group liver

Fig. 4: Hepatic parenchyma revealing a large area of necrosis and serve degenerating changes. Normal space of canaliculi was observed



Section of plant extract (400 mg/kg) + acetaminophen treated group liver

Fig. 5: Hepatocytes were regenerative and showed a milder degree of vacuolation but prominent nuclei, indicating returning to normalcy



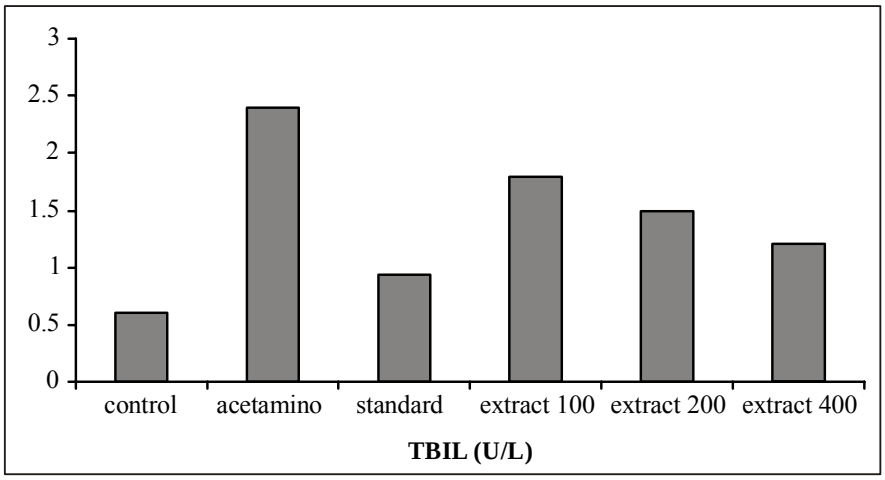
Section of paracetamol + silymarin treated group liver

Fig. 6: The sheets of hepatocytes were positioned in typical radiation pattern hepatocytes were individually demarked with canalicular space

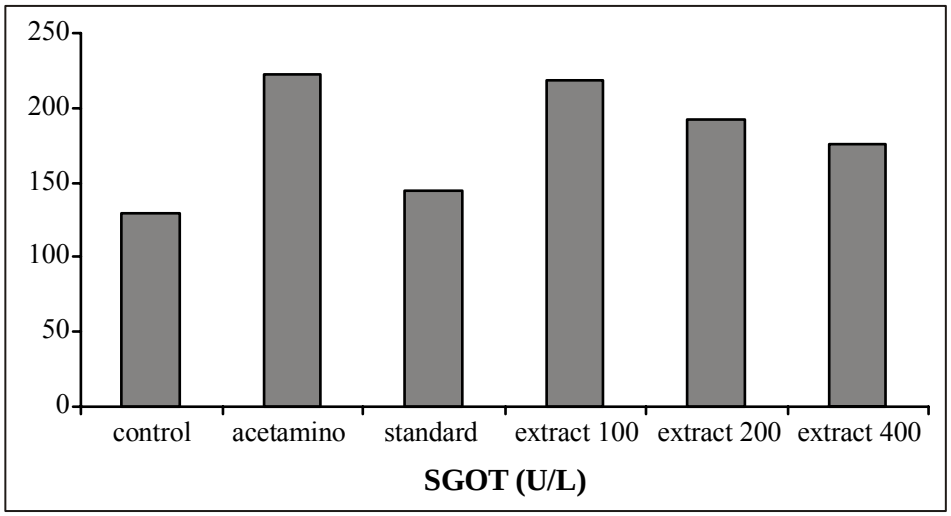
The hepatic cells of the rats treated with aqueous extract (200 mg/kg, b.w and 400 mg/kg, b.w) and intoxicated with paracetamol were radially arranged. The vacuolation was present. The recovery was comparable to that of silymarin a standard hepatoprotective agent.

Acute toxicity studies

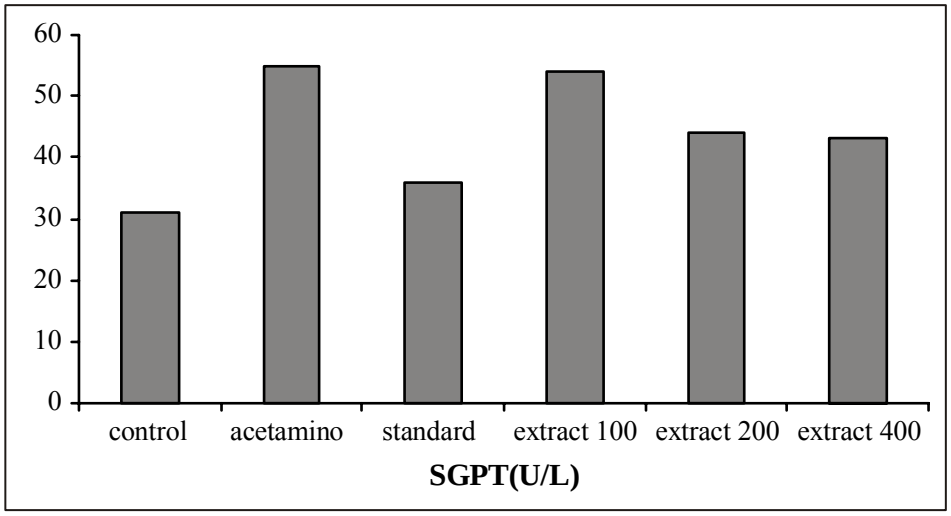
The extract was found to be safe for further biological studies as no lethal effect was observed even at 3000 mg/kg b.w in mice.



Graph 1: The effect of various groups on bio-chemical parameter, bilirubin in rat serum



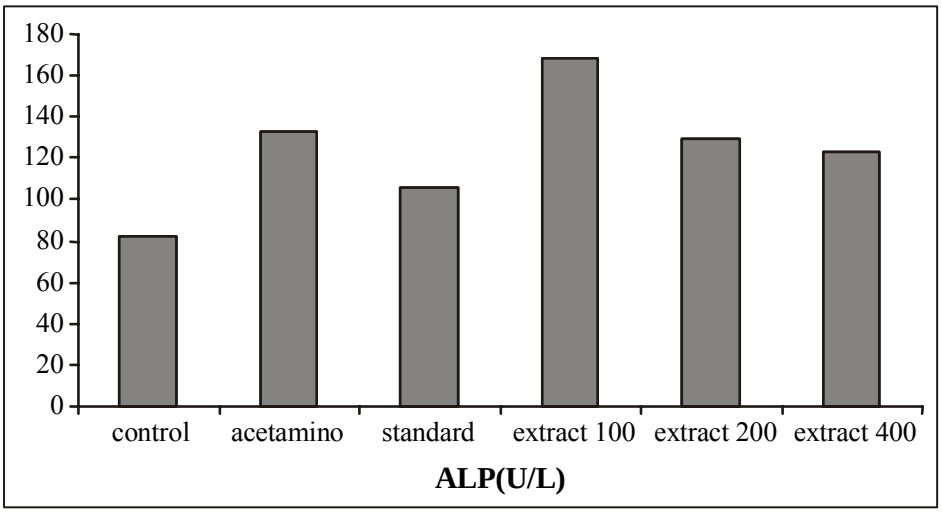
Graph 2: The effect of various groups on bio-chemical parameter, SGOT (U/L) in rat serum



Graph 3: The effect of various groups on bio-chemical parameter, SGPT (U/L) in rat serum

Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by paracetamol in higher doses in experimental animals and humans[12] for screening of hepatoprotective agents, paracetamol induced hepatotoxicity has been used as reliable method. Paracetamol is

metabolised primarily in liver and eliminated by conjugation with sulphate and glucuronide, and then excreted by the kidney. More over paracetamol hepatotoxicity has been attributed to the formation of toxic metabolities, a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N-acetyl-P-benzo quinone imine (NAPQI)[13]. Toxic metabolites (NAPQI) can alkylate and oxidise intracellular glutathione, which results in liver GSH depletion subsequently leads to increased lipid peroxidation[14,15].



Graph 4: The effect of various groups on Bio-chemical parameter, ALP (U/L) in Rat serum

Administration of paracetamol caused a significant elevation of enzyme level such as SGOT, SGPT, ALP and total bilirubin, 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[16,17]. The administration of aqueous extract of *A.marmelos* exhibits excellent hepatoprotective activities by reducing significantly increased serum biochemical parameters on paracetamol induced toxicity.

CONCLUSION

The study clearly indicates aqueous fruit extract of *Aegle marmelos* is effective in the treatment and prevention of paracetamol induced hepatic cytotoxicity. The hepatoprotective activity of the above extract was also found to be dose dependent. Further the new alkaloid amide derivative which was isolated was of very low yield, we were unable to conduct the activity for it.

More over it is very important to study the specific phytochemical compounds responsible for this hepatoprotective effect.

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