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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF AEGLE MARMELOS ON HEPATOTOXICITY INDUCED RATS

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ABSTRACT

Herbal drugs are traditionally used in various parts of the world to cure different diseases. The Ayurveda and Siddha medical systems are very famous medical practices in Indian traditional medicines. In the present research studies, Bael leaves (*Aegle marmelos*, family of Rutaceae) which are also called as Bilva in ancient Sanskrit was used as herbal drug and its hepatoprotective effect in INH induced liver injury in albino rat was evaluated using essential biochemical parameters. The experiments were performed with four groups of animals. The experimental animals were administered with EEAM was fed to animals for next 21 days. The results were compared with the standard herbal drug silymarin (133.04 g/g tissue). The experimental results indicate that, the Bael leaves have excellent hepatoprotective effect. A similar experimental result was also observed in other biochemical parameters.

KEYWORDS: Isoniazid, *Aegle marmelos*, Hepatoprotective activity, Marker enzymes.

INTRODUCTION

Liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem for years. However, there are a number of herbal formulations available on liver disorders in ayurvedic medicine. Various formulations like Liv-52, Kamilari, APCL-A Amalkadi Ghrita, Panchagvya Ghrita, Himoliv, and Livex are well known for their hepatoprotective effects. Aegle marmelos, commonly known as Bael, is a spiny tree belonging to the family Rutaceae.[1] It is an indigenous tree found in India, Myanmar, Pakistan and Bangladesh. The leaves, roots, bark, seeds and fruits are edible and medicinal values. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C) no drug has been longer or better known or appreciated by the inhabitants of India than the Bael. The leaves of Bael are astringent, a laxative, and an expectorant and are useful in treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation, and asthmatic complications (Kirtikar and Basu, 1993).[2] It has been claimed the leaf of Aegle marmelos posses contraceptive efficacy (Bhattacharyay, 1982).[3] Fresh aqueous and alcoholic leaf extracts of Aegle marmelos were reported to have a cardio tonic effects in mammals (Haravey, 1968 and Nadkarni, 2000).[4] Aegle marmelos leaf extract has been reported to regenerate damaged pancreatic beta cells in diabetic rats (Das et al., 1996) and increased the activities of peroxidase in the liver tissues of Isoproterenol treated rats (Rajadurai et al., 2005).[5] An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect (Karunanayeke et al., 1984).[6] *Aegle marmelos* leaf extract was found to be a potential antioxidant drug, which reduces the blood sugar level in alloxan induced diabetic rats (Sabu and Ramadasan, 2004). It was found to be as effective as insulin in the restoration of blood glucose and body weight to normal levels on hyperglycemic state (Seema et al., 1996).[7,8]

The ethanolic extract of *Aegle marmelos* leaf possesses anti spermatogenic activity. Considering the diverse medicinal properties of *Aegle marmelos*, the present study was under taken to evaluate the hepatoprotective effect of Aegle marmelos in INH induced liver injury in experimental animal models.[9]

Figure 1: Plant of *Aegle marmelos (Bael/Bilva)* with leaves.

Pharmacognosy of *Aegle marmelos* /Taxonomy of *Aegle marmelos*

Kingdom : Plantae

Subkingdom : Tracheobionta

Super division : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Sapindales

Family : Rutaceae

Genus : Aegle

Species : *Aegle marmelos* L.

Vernacular names

English : Quince, Indian quince, holy fruit or Golden apple

Hindi : Bael, Sirphal

Bengali : Wood apple, Stone apple

Gujarati : Bilvaphala

Tamil : Vilvam

Telugu : Bilva, Bilva chettu

Urdu : Belk, Belk ham

Sanskrit : Bilva or Sriphal or Shivadruma (tree of shiva)

Malayalam : Marredy

MATERIALS AND METHODS

Leaves of *Aegle marmelos* were collected from Vangapally village around Swami Vivekananda Institute of Pharmaceutical Sciences College in the month of January-February 2023.

Successive extraction by using soxhlet apparatus

Principle: To prepare various extracts of *Aegle marmelos*. a successive solvent extraction procedure was adapted. The plant materials were subjected to successive extraction with different solvents, starting from solvent of low polarity to high polarity.[10]

Materials: Dried leaves powder of *Aegle marmelos.*

Solvents: Ethanol.

Procedure: An about 35 gm of powdered drug of the selected medicinal plant was subjected to extraction by using ethanol as solvent in a soxhlet apparatus. The extraction was continued until the solvent in the timble becomes clear or colourless. Then the heating was stopped and the mixture from distillation flask was collected and cooled. Then this mixture was filtered and concentrated by using evaporator at room temperature. The extract was dried at room temperature and stored in amber coloured glass jar in a freezer or desiccator and was used for further experiments. The marc obtained after extraction was removed, dried and recharged, extracted with ethanol (95%) solvent of successively higher polarity to collect ethanolic extract.[11]

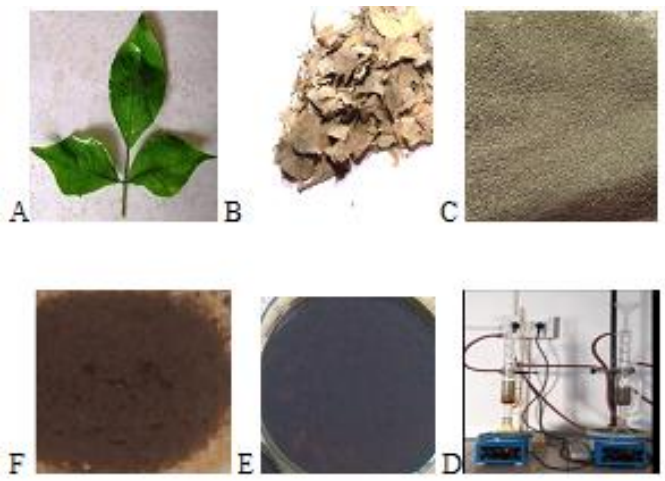

Figure 2: Extraction of *Aegle marmelos* A. Leaves b. Dried Leaves C. leaves powder D. Ethanol Soxhlet apparatus Extraction E. Leaf Crude extract F. extract powder.

Table 1: Extraction values.

S. No	Solvent	Petroleum ether	Chloroform	Ethanol
1	Yield	5.5gm	6.5gm	7.2gm
2	Percentage yield	0.15	0.18	0.2
3	Nature	Semi solid	Semi solid	Semi solid
4	Colour	Green brownish	Green brownish	Green brownish

Thin Layer Chromatography

The dried powdered plant material (Leaves) was extracted in ethanol, and the data showing presence of phytoconstituents in the leaf of EAM. For the study of thin layer chromatography method silica plates is prepared which is as follows. The number of solvent system was tried but the solvent system which shows good resolution was used. The ethanolic extract showed 6 spots with Rf values of 0.41, 0.52, 0.64, 0.75, 0.82, 0.92 using the solvent system Ethyl acetate: Butanol: Formic acid (2.5: 1.5: 0.5).[12]

Animals: All the experiments on animal were conducted according to protocols that were approved by the Institutional Animal Ethics Committee of Swami Vivekananda institute of pharmaceutical sciences. Wister albino rats (150–200 g) of either sex were used for this study. Animals were maintained under standard environmental conditions and had free access to feed and water ad libitum. Acute toxicity study was carried out using albino mice.

Acute toxicity study

The acute toxicity study was carried out by using Swiss albino mice of either sex, weighing about 25– 30g. This study was performed as per OECD (Organization for economic co-operation and development)-423 guidelines. Animals were kept in a temp controlled environment (23 ± 2ºC) at 12 hours light/dark cycle. All the protocols were performed in accordance with Institutional Animal Ethics Committee of Swami Vivekananda Institute of Pharmaceutical Sciences. It was found that the tolerated dose level is 2000 mg/kg body weight.[13]

They were randomized into 5 groups of 6 animals each as follows

Group I: Normal received the vehicle viz. normal saline (2 ml/kg).

Group II: Controls Received INH (100 +50 mg/kg p.o.) at every 72 h for 21 days.

Group III: Received silymarin 50 mg/kg p.o. for 21 days and simultaneously administered INH (100 +50 mg/kg p.o.) every 72 h.

Group IV: Received EAM 200 mg/kg p.o for 21 days and simultaneously administered a

INH (100 +50 mg/kg p.o.) every 72 h.

Group V: Received EAM 400 mg/kg p.o. for 21 days and simultaneously administered INH (100 +50 mg/kg p.o.) every 72 h.

At the end of all experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. The separated serum was used for the estimation of total bilirubin, direct bilirubin, SGOT, SGPT, ALP and total proteins (TP). The animals were sacrificed by administering an excess of ether and their livers were removed.

Assessment of liver function: Biochemical parameters i.e., aspartate amino transferase (AST) alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin and total protein were analyzed according to the reported methods. The liver was removed, weighed and morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase.[14]

Histopathological studies: Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro techniques. 5µm sections of liver grained with alum haematoxylin and eosin were observed microscopically for histopathological changes.

Isolation of liver Liver was carefully removed and washed with ice cold saline solution and pressed between pads of filter paper and weighed. A portion of the liver was preserved in 10% v/v neutral formalin for histopathological studies.

Histopathological Examination A portion of liver tissue was preserved in 10% formaldehyde solution for histopatholigical studies. Haematoxyline and eosin were used for the staining agent and later microscopic slides of the liver photographed at the 100x magnification. Statistical.

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RESULTS

Table 2: Organoleptic Behavior of *Aegle marmelloes* Leaf Powder Treated with Different Reagents.

Chemical treatment	**Reagent**	**Observations**	
For organoleptic behavior	HCI	Powder settles down slowly	Color: Greenish –Black
	Conc. HNO_3	Powder settles down slowly	Color: Brown
	Conc. H_2SO_4	Not observed	Immediately give black color
	5% aq. NaOH	Powder settles down slowly	Color: Reddish Brown
	Iodine solution	Powder settles down Immediately	Color: Brownish

	5% aqueous KOH	Powder float on the surface	Color: Light Green
	Glacial Acetic Acid	Powder settles down slowly	Color: Green
	5% aqueous $FeCI_3$	Powder float on the surface	Color: Reddish Brown

Table 3: Fluorescence Behavior of *Aegle marmelloes* Leaf Powder Treated with Different Reagents.

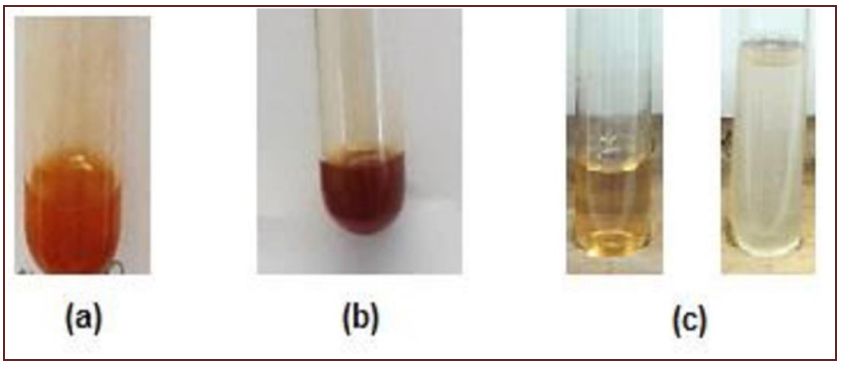
Chemical treatment	Reagent	Under ordinary light or visible light	Under UV light 254 nm	Under UV light 366 nm
For fluorescence behavior	1N NaOH in Methanol	Fluorescent Green	Yellowish brown	Pale green
	50% HNO_3	Yellowish Red	Reddish brown	Pale Green
	50% H_2SO_4	Blackish Green	Reddish Brown	Blackish Green

Preliminary Phytochemical

The early phytochemical analysis of the ethanolic extracts suggests that alkaloids, carbohydrates, glycosides, phytosterol, saponins, tannins, proteins, flavonoids, and diterpenes are among the phytoconstituents present.

Table 4: Preliminary Phytochemical screening of *Aegle marmelloes.*

S. No	Phytocontituents	Report
1	Alkaloids	Present
2	Carbohydrates	Present
3	Glycosides	Present
4	Phytosterol	Present
5	Saponins	Present
6	Tannins	Present
7	Proteins and free amino acids	Present
8	Flavonoids	Present
9	Diterpenes	Present



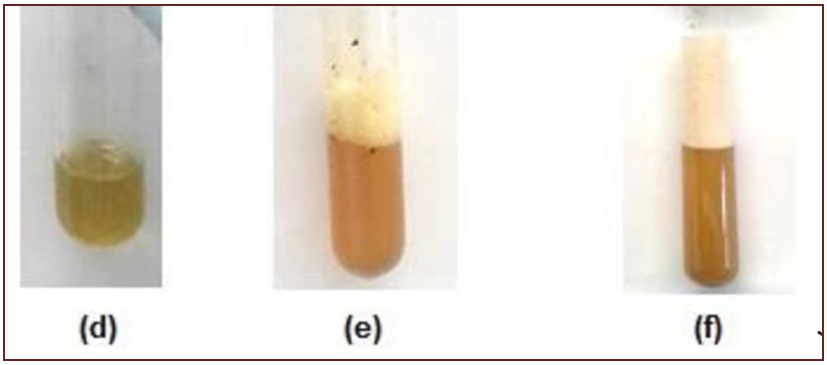


Figure 3: Preliminary Phytochemical screening of *Aegle marmelloes.*

Table 5: Effect of EEAM formulation and silymarin on serum parameters in INH-inducedhepatic damage in rats.

S.No	Treatment	SGPT (I.U/L)	SGOT (I.U/L)	ALP (I.U/L)	LDH (I.U/L)	TB (% mg)	DB (% mg)
Group I	Normal control (no treatment)	43 ± 4.6	51.3 ± 9.15	210 ± 21	135.7 ± 10.14	0.133 ± 0.01	0.144 ± 0.04
Group II	Toxicant control INH (3gm/kg)	120 ± 5.9	130 ± 28	320 ± 25	293.4 ± 10.80	0.266 ± 0.01	0.365 ± 0.11
Group III	Silymarin (50 mg/kg) + INH (3gm/kg)	49 ± 5.2**	54 ± 10**	170 ± 13**	151.2 ±26.65**	0.140 ± 0.01**	0.137 ± 0.02*
Group IV	EEAM (100 mg/kg) + INH (3gm/kg)	78 ± 3.0**	78 ± 18**	230 ± 6.3**	190.5 ± 6.24*	0.170 ± 0.01**	0.162 ± 0.02
Group V	EEAM (200 mg/kg) + INH (gm/kg)	63 ± 7.6**	60 ± 12**	190 ± 10**	157.8 ± 37.6**	0.157 ± 0.02**	0.134 ± 0.03*

(n=6) values are expressed as mean ± S.E.M.; ***P*<0.01 and **P*<0.05, when compared with the toxicant control groups (one-way ANOVA followed by Dunnetts test); The levels of SGPT, SGOT, ALP, and LDH in serum are expressed as IU/L. The levels of TB, DB in serum are expressed as % mg, SGPT = Serum glutamate pyruvate transaminase, SGOT = Serum glutamate oxaloacetate transaminase, ALP = Alkaline phosphate, LDH = Lactate dehydrogenase, TB = Total bilirubin.

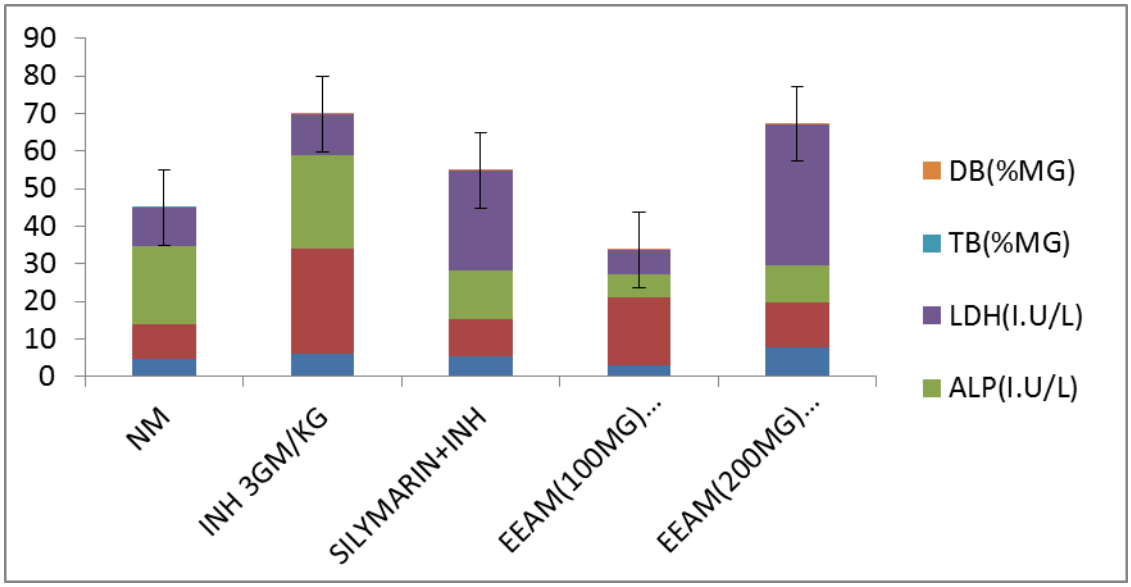


Figure 4: Effect of EEAM formulation and silymarin on serum parameters in INH-inducedhepatic damage in rats.

DISCUSSION

Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used anti-tubercular chemo therapeutic regimens containing INH. The conversion of mono acetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity The plasma half-life of AcHz (metabolite of INH) is shortened by AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH combination. In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by anti-tubercular drugs Earlier it has been well documented that both ALT and AST are considered among the most sensitive markers to assess hepatocellular damage leading to liver cell necrosis. ALP, which is secreted from the lysosomes, is also a marker enzyme for assessing liver damage. Slight to moderate increases in ALP (1-2 times normal) occurred in liver disorders148. Estimating the activities of serum marker enzymes, like AST, ALT, ALP, total bilirubin, can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The enhanced activities of these serum marker enzymes observed in INH treated rats in our study correspond to the extensive liver damage induced by INH. Results indicate that EEAM administration could blunt INH -induced increase in activities of different marker enzymes of heptocellular injury,

viz. AST, ALT, ALP, total bilirubin and total protein, (TABLE suggesting that EEAM possibly has a protective influence against INH induced hepatocellular injury and degenerative changes. Anti tubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane . In previous report suggested that, there did not seem to be clear evidence that INH proves much more injuries t, in this connection, they consider that it is the combination of these two drugs that confer the additive, or even synergistic, potential of liver toxicity than either agent alone, as conjectured. In our study, INH treatment produced the elevation in the levels of LPO Treatment of the rats with EAM significantly reduced the elevated levels of LPO Alterations of various cellular defense mechanisms consisting of enzymatic antioxidant components [superoxide dismutase (SOD), catalase, GSH have been reported in INH -induced hepatoxicity. The INH administered animals exhibited significantly ($p<0.001$) low levels of hepatic enzymatic antioxidant components, and significantly increased with co-administration of EAM (200 and 400 mg/kg) at the all the doses and in silymarin treatment group after 21 days. On the day of 21st enzymatic antioxidant components levels significantly increased with co-administration of EAM at the lower and higher doses (200 and 400 mg/kg) and in silymarin treated group. Histopathological studies, treatment with different doses of EAM produced mild portal triaditis and absence of necrosis when compared with control. Treatment with silymarin also shows mild portal triaditis and absence of necrosis and vacuoles. All these results indicate a hepatoprotective potential of the extract. The results of the present study suggested that EAM possess hepatoprotective activity against the hepatotoxicity induced by the combination of two antitubercular drugs.

CONCLUSION

In conclusion, the results of this experiment demonstrate a potent hepatoprotective action of ethanolic extract of *Aegle marmelloes* in INH-induced oxidative stress and liver toxicity in rats. Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant defence status. Thus ethanolic extract of *Aegle marmelloes* may be used as a safe and effective alternative chemo preventive and protective agent in the management of liver diseases.

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