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Research Article

PHYTOCHEMICAL AND TOXICITY STUDY OF *EMBLICA OFFICINALIS* (AMLA)

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ABSTRACT
In the present study, phytochemistry and toxicities (acute and chronic) of *Emblica officinalis* fruit (Amla) have been determined. The hydroalcoholic extract (HAE) of amla was prepared and its extractability was found to be 46.9%. Different chemical tests showed the presence of various active principles or phytoconstituents, viz., alkaloids, glycosides, reducing sugars, tannins, resins, saponins, sterols and fixed oils. For acute toxicity, including median lethal dose (LD_{50}) of amla, its HAE was administered @ 250, 500 and 1000 mg/kg body weight to female albino rats of groups 2 to 4, respectively. Rats of group 1 were given normal saline to serve as control. There was no mortality up to 48 hr, hence this drug showed the LD_{50} above 1000 mg/kg. For chronic toxicity of *E. officinalis* HAE, similar drug dosage schedule was applied in groups 1 to 4 of rats as used for acute toxicity study; however, the drug was given for 3 weeks. During this period, *E. officinalis* did not cause any untoward effect.
KEYWORDS: Active principles, *Emblica officinalis* (Amla), Phytochemistry, Toxicity, Rat.

INTRODUCTION

The knowledge of extractability of a plant provides an idea regarding the amount of extract present in a definite quantity of drug. The extractability also serves as a tool for quality control of plant-drug. The acute and chronic toxicity studies drug-extract provide a preliminary information regarding the useful properties likely to possessed by the extract, and at the same time provide the LD_{50}. Different signs and symptoms during gross observational studies of a drug give an idea regarding the type of drug action and the dosage to be employed. Therefore, on the basis of acute and chronic toxicities, the therapeutic dose and route of administration of drug can also be known[1-2]. It is well known that the medicinal activities of herbal drugs are due to the presence of various active principles or phytoconstituents such as alkaloids, glycosides, reducing sugars, tannins, saponins, resins, phytosterols, flavonoids, organic acids, essential oils, fixed oils, etc. The active principles can be extracted with different solvents, e.g., petroleum ether, alcohol, benzene, chloroform and distilled water. By doing so, the per cent extractability of herbal drugs can be determined. In view of these facts, the present study was undertaken to determine the phytochemical and toxicity (acute and chronic) studies of *E. officinalis* fruit (Amla) extract.

Emblica officinalis Gaertn. *(Phyllanthus emblica* Linn., Amla, Indian Gooseberry) belongs to the plant family *Euphorbiaceae.* Amla fruits are acrid, cooling, refrigerant, astringent, diuretic and laxative[3-4]. The raw fruits are aperient, while dried fruits are useful in haemorrhage, diarrhoea and dysentery, and in combination with iron, used for anaemia, jaundice and dyspepsia. The fermented liquor prepared from the fruits is used in jaundice, dyspepsia and cough. *Sherbet* of amla with lemon juice is taken for arresting the acute bacillary dysentery. Exudation from incision on the fruit is used as external application for the inflammation of the eye. Flowers of *E. officinalis* are also cooling, refrigerant and aperient, while root and bark are astringent. *E. officinalis* seeds are used for asthma, bronchitis and biliousness. Amla fruit is a rich natural source of vitamin C (70-72%) and tannin. Due to rich vitamin C, amla is successfully used in the treatment of human scurvy. Fruit has also been reported to contain phyllemblic acid (6.3%), gallic acid (5%), lipid (6%), emblicol, flavaniod, colloidal complexes and micic acid. Phyllembin, from fruit pulp identified as ethyl gallate potentiate the pharmacologic action of adrenaline *in vitro* and *in vivo.* Bark and leaves are also rich in tannin, while seeds contain fixed oil, essential oil and phophatides. Leucodelphinidin, a phytochemical of bark has also been isolated. *E. officinalis* is common in the mixed deciduous forests of India ascending to 4,500 ft. on the hills, and is often cultivated in gardens and homeyards[3]. Amla fruit

contains 20 times as much as vitamin C as orange juice. The fruits are eaten as raw or cooked. They are also used in hair dyes, and dried ones are detergent and are used for shampooing hair. Seeds yield a fixed oil, and fruits, bark and leaves are rich in tannin containing 28%, 8-21% and 22%, respectively[4].

Further, amla is also hepatoprotective, antioxidant, immune-stimulator and antitumour agent. Vitamin C (ascorbic acid or ascorbate), tannins and flavaniods present in amla have very powerful antioxidant properties. Amla fruit contains 18 compounds that inhibit the growth of gastric, uterine and breast cancers. It enhances natural killer (NK) cell activity in various tumours. Its extract reduced the ascites and solid tumours induced by Dalton's lymphona ascites cells in mice. The extract also increased the life span of tumour bearing animals[5]. The intraperitoneal maximum tolerated dose (MTD) and LD_{50} of *E. officinalis* fruits after administration of its 50% ethanolic extract in rat could not be found up to any dose[6].

MATERIALS AND METHODS

Animals: Healthy inbred female albino rats (100-160 g) were kept in colony cages under standard laboratory conditions in the Small Animal House of Govt. NSCB Medical College, Jabalpur. The rats were quarantined for one week after arrival. They were fed on standard pellet diet and drinking water ad libitum. The experimental designs and protocols received the approval of Institutional Animal Ethics Committee (IAEC).

Drugs and chemicals: *E. officinalis* fruits in powder form were obtained from Indian Herbs Research & Supply Co. Ltd., Saharanpur, UP. The chemicals and reagents required for phytochemical study have been purchased from the authorized chemical shops.

Preparation of extract and determination of extractability: Hydroalcoholic extraction of powdered *E. officinalis* fruits was done as per the method used by Pandey[1]. The HAE was prepared with 50% distilled water and 50% ethyl alcohol (ethanol). The per cent extractability of *E. officinalis* fruit was then calculated.

Determination of active principles: *E. officinalis* HAE was analyzed for the presence of different active principles, viz., alkaloids, glycosides, reducing sugars, tannins, resins, saponins, sterols and fixed oils as per the methods employed by Pandey[1,7].

Determination of acute toxicity and median lethal dose (LD_{50}): To determine the acute toxicity, including LD_{50} of *E. officinalis,* its HAE was administered orally to the rats as per the methods described by earlier workers[1,6-8]. To dissolve the extract completely in distilled water, a pinch of *Gum acacia* powder was mixed and the aqueous suspension of extract was prepared. *E. officinalis* HAE was administered @ 250, 500 and 1000 mg/kg to the rats of groups (each group had 6 animals) 2 to 4, respectively. Rats of group 1 were given normal saline to serve as control. The mortality in rats occurred within 48 hr was noted. The gross observational changes recorded were the effects on central nervous system (stimulation or depression), respiration, spontaneous motor activity (SMA), posture, gait, secretion, piloerection, tremor and response to stimuli, etc.

Determination of chronic toxicity: The chronic toxicity and gross observational effects of *E. officinalis* HAE were determined as per the method followed by Pandey[7] in different groups of rats. Similar drug dosage schedule was applied in groups 1 to 4 of rats as used for acute toxicity study. However, the extract was administered for 3 weeks and the gross effects were observed.

RESULTS AND DISCUSSION

Phytochemical study: The extractability of HAE of *E. officinalis* fruit was 46.9%. The extract was greenish-brown, while its consistency was viscous to semiliquid. The higher extractability of *E. officinalis* in hydroalcohol indicates its sufficient absorption through the gastrointestinal tract. Different chemical tests revealed the presence of active principles or phytoconstituents, viz., alkaloids, glycosides, reducing sugars, tannins, resins, saponins, sterols and fixed oils. The presence of phytoconstituents as seen in *E. officinalis* has also been reported[3-4,6] in many medicinal plants, which confirms the findings of the present study. The reported[3-5] pharmacological activities of *E. officinalis* may be due to the presence of active principles in it.

Toxicity study: During acute toxicity study, *E. officinalis* HAE administered @ 250, 500 and 1000 mg/kg, once orally to different groups of rats caused no mortality up to 48 hr. Hence, the LD_{50} of *E. officinalis* is more than 1000 mg/kg. The chronic toxicity of *E. officinalis* HAE was determined in different groups of rats according to the doses as given for acute toxicity study. During 3 weeks, *E. officinalis* did not cause any untoward effect. No gross observational effects were observed at any of the doses of *E. officinalis* during acute and chronic toxicity studies.

The acute and chronic toxicity studies with extract provide a great information regarding the useful properties likely to possessed by the extract and at the same time provide the LD_{50}. The development of signs and symptoms during gross observational studies gives an idea regarding the type of drug action, and the therapeutic dose and route of administration of drug to be used[1-2]. On the basis of present study, it can be concluded that *E. officinalis* appears to be free from

acute and chronic toxicities. Many workers, including Dhar et al.[6] screened out several medicinal plants for their phytochemical and biological activities, and they have also not found untoward effects (or acute and chronic toxicities) and LD_{50} of *E. officinalis* fruit.

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Table 1: Extractability and active principles (chief phytoconstituents) of hydroalcoholic extract (HAE) of *E. officinalis* fruit

Extractability		**Active principles**	
		Test applied for active principle	**Present / absent**
Part used	Fruit	For Alkaloids- Wagner's reagent	Present
Solvent used	50% distilled water and 50% ethyl alcohol	For Reducing sugars- Benedict's reagent	Present
		For Glycosides- Benedict's reagent	Present
		For Tannins- Ferric chloride	Present
Extractability	46.9%		
		For Resins- Alcohol containing extract in distilled water	Present
Colour of extract	Greenish brown		
		For Saponins- Sodium bicarbonate foam test	Present
Consistency of extract	Viscous to semiliquid	For Sterols- Ferric chloride	Present
		For Fixed oils- Filter paper	Present

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