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Original Article

THE PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ORAL ACUTE TOXICITY STUDY OF STEM BARK OF *SYZYGIUM CUMINI*

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ABSTRACT

Objective: The current subject field was carried out to evaluate phytochemical analysis and acute toxicity study of the stem bark of *Syzygium cumini*.

Methods: The ethanol extraction was made by the Soxhlet extraction method. Phytochemical screening was performed by applying standard methods. The oral administration of the drug in treating a group is done by using a curved ball-tipped intubation needle affixed to a 3 ml syringe and animal's observed from cage side observations. All the animals were anesthetized with ether anesthesia; blood was collected from orbital puncture and analysis was made by standard methods. Several organs were collected by dissecting abdominal, thoracic and cranial cavities and each weighed by using a sensitive electronic balance. Fixation was made with 10% buffered formalin and processed for histological embedding by Carleton's histological techniques. Statistical data analyzed by statistical software Sigma Plot 10.

Results: The phytochemical screening revealed the presence of flavonoids, tannins, carbohydrates, sterols and amino acids. The oral acute toxicity study showed no detectable clinical signs of toxicity and mortality during the study period of 14 d. There were no statistically significant alterations in body weights, organ weights, and hematological parameters compared to control group. The microscopic anatomy of the reproductive organs and liver showed normal architecture.

Conclusion: The outcome of the study showed that the LD50 of the drug was greater than 5000 mg/kg body weight. The phytochemicals present in stem bark of *Syzygium cumini* can be employed in the fabrication of innovative medication for several diseases.

Keywords: Toxicity study, *Syzygium cumini*, Stem bark, Phytochemicals.

INTRODUCTION

The role of medicinal plants in folklore medicine is a well-known fact from ancient time in India. Herbal medicines involving the use of fresh or dried plant parts are overtaken modern synthetic drugs by having minimal or no side effects [1]. The World Health Organization survey estimated that close to 80% of the world's populations of developing nations rely on traditional medication in primary healthcare sectors [2].

The phytochemicals present in plants are known for their anticancer, antioxidant, immunity-potentiating, neuropharmacological and detoxifying functions. The phytochemicals are classified into flavonoids, alkaloids, tannins, glycosides, steroids, saponins, phenolics, terpenes, anthraquinones and essential oils [3]. Previous reports support that flavonoids are used as free radical scavengers, antioxidants, antimicrobial activity, anti-inflammatory activity, cytotoxic activity and estrogen activity agents [3, 4].

In a number of diseases, the tannin-rich medicinal plants are used as curing agents in Ayurveda. Further, they are employed for the treatment of diseases like diarrhea, leucorrhoea, and rhinorrhoea. The antiseptic activity of tannins is due to the phenolic group present in it [5]. Plant sterols exist in both the sterol and stanol forms, the most abundant plant sterols are β-sitosterol, campesterol, stigmasterol. The well-known effects of plant sterols is to bring down the blood cholesterol levels, the cholesterol modulating actions of plant sterols may overlap with their anti-cancer actions [6]. Likewise, the phytochemicals present in plants have various health beneficiary effects.

The acute toxicity is the toxicity produced by a substance when administered in one or more doses during a period not exceeding 24 h, acute toxicity studies in animals are usually necessary for any medicinal substance intended for human use [7]. Acute toxicity studies are routinely applied to influence symptoms of toxicity and effects on hematological, biochemical, and histological parameters [8]. Toxicity studies before clinical trials demonstrate the efficacy and safety of natural and herbal drugs [9]. Herbal preparation in a suitable animal model helps to estimate toxicity and get guidelines for choosing a safe dose in human [10].

The species *Syzygium cumini (L)* Skeels are a big evergreen tree belonging to the genera of the myrtle family Myrtaceae found throughout India up to an elevation of 1,800 m [11]. The native place of *Syzygium cumini* is India or East Indies. It is likewise shown in many other regions of the world [12]. The former studies on stem bark of the *Syzygium cumini* has reported several medicinal properties like carminative, astringent, refrigerant, digestive, stomachic, anthelmintic, antidiabetic, and used for gastric disorder, intrinsic hemorrhage, sore throat, asthma, bronchitis, thirst, diuretic, antibacterial, Febrifuge, skin diseases, strangury, wounds, leucorrhoea, fever, dysentery, chronic diarrhea and menorrhagia [11-15].

The previous phytochemicals investigation carried out on the ethanolic extract of the stem bark of *Syzygium cumini* showed the presence of flavonoids, tannins, carbohydrates, sucrose, saponins, proteins, phenols, euginin and a fatty acid ester, resin, phytosterol, myricyl alcohol, bergenin, friedelin, friedelinol, lignan derivatives, pentacyclic triterpenoid-betulinic acid [12-18]. Even though the reports regarding the phytochemicals and toxicity studies exist, the correlative cellular architecture of various organs lacks in the literature. Therefore, the present work is planned to evaluate the phytochemicals composition in the stem bark of *Syzygium cumini* and the effect of ethanolic extract of it on the toxicity along with the microscopic structural evaluation of various organs.

MATERIALS AND METHODS

Plant material

The plant material was collected in the month of June and July from Hyderabad-17 ° 22' 31" North, 78 ° 28' 28" East, Telangana, India, Asia and authenticated by Head of Botany department, Osmania University, Hyderabad, Telangana, India. The plant specimen is identified as *Syzygium cumini (L)* Skeels it belongs to the family Myrtaceae. The voucher No.0271 specimen sample of the plant was deposited in the department herbarium for future reference.

Extraction

After recognition of the plant, the bark was removed and rinsed thoroughly in tap water and dried in shade for about 20 d under controlled temperature (25±2 °C). And so the crude material was powdered and passed through a 40 mesh sieve and stored in a well-closed container for further use. Coarsely powdered bark was successively soxhlet using petroleum ether, chloroform, and ethanol for 72 h. The extracts were filtered, and the solvents were evaporated to dryness under reduced pressure in an Eyela rotary evaporator at 40 to 45 °C. The preliminary phytochemical investigations of the bark extract of *Syzygium cumini (L.)* were carried out by the standard methods.

Phytochemical analysis

The preliminary phytochemicals analysis tests for flavonoids, tannins, carbohydrates, sterols, amino acids, alkaloids, steroids and terpenoids, gums and mucilage, saponins and fixed oils were performed according to standard methods [19-23].

Animals

Inbred fifteen adult nulliparous and non-pregnant female Wistar rats of age around six months and weighing close to 180-200 g were used for the experimental study. The rats were procured from Teena Biolabs Pvt. Ltd, Hyderabad. The rats were acclimatized to the testing ground conditions for a week before the beginning of the experiments, were maintained as per the Institutional Ethical Committee (IAEC) norms. The rats were maintained on 12 h dark and light cycle with food and water at ad libitum. The study procedures involving the handling and treatment of animals were approved by the Institutional Ethical Committee Teena Biolabs Pvt. Ltd. Reg. No. 177/PO/cb/99/CPCSEA. Project No: TBLSTPRJ0032014.

Acute toxicity study

The aggregate of fifteen rats were randomly separated into three groups, each containing five rats. The control group (C) was given the normal standard diet. The treated groups-SE2 and SE5 were administered intragastric gavage (i. g.) single dose ethanol extract of the stem bark of *Syzygium cumini* 2000 mg/kg and 5000 mg/kg body weight respectively. Immediately after administration of a single dose of extract the animal's behavior, toxic signs and mortality rates were continuously followed for the first thirty minutes and at hourly intervals during the first twenty-four hours, special attention given during the first four hours and thereafter daily for a total of 14 d [24].

Cage side examination

The examinations included changes in behavior pattern, skin, fur, eyes-for dullness, ptosis, pupil diameter, tremors, convulsions, salivation, diarrhea, breathing abnormalities, sleep, and gait.

Body weight

The body weight was recorded on day zero (before dosing), day seven and day fourteen.

Hematology

On the day of sacrifice, all the animals were anesthetized with ether anesthesia, blood was collected. Right away after collection, the blood was analyzed for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT).

Organ weight

The anesthetized animals were carefully dissected for ovaries, uterine horns, liver, heart, lungs, kidneys, suprarenal glands, spleen and brain. Each organ was cleaned for connective tissue and fat, examined macroscopically and immediately weighed by using a sensitive electronic balance.

Microscopic anatomy

The collected ovaries, the uterine horns, and liver were fixed at 10% buffered formalin and processed for histological embedding [25]. The 5 µm thick sections of the ovary, uterus (uterine horns) and liver were cut using a rotary microtome. The sections were stained with Haematoxylin and Eosin [H&E] stains [25]. The stained sections of ovarian, uterine horns and liver were examined for any alteration in cellular morphology.

Statistical data analysis

All the information was compiled and tabulated by using Microsoft excel worksheet. The mean, standard deviation (SD), standard error (SE) and t-test were performed by using statistical software Sigma Plot 10 to find out the significance level. The P value<0.05 is considered as statistically significant. All the values are shown as mean±SE.

Table 1: Phytochemical constituents of ethanolic extract of stem bark *Syzygium cumini*

Compounds	Phytochemicals test	Inference	Results
Flavonoids	Alkaline reagent test	Yellow color precipitate	+
	Lead acetate test	Yellow color precipitate	+
Tannins	Gelatin test	White precipitate	+
	Lead acetate	Yellow or red precipitate	+
	Ferric chloride reagent	Dark green or blue color precipitate	+
Carbohydrates	Molisch's test	Violet or Purple Color Ring	+
Sterols	Salkowaski reaction	Greenish yellow fluorescence	+
	Liebermann Burchard's test	Brown ring	+
Amino acids	Ninhydrin test	Purple or blue color	+
Alkaloids	Mayer's test	Yellow colored or white, brown precipitate	-
	Dragendroff's test	Orange brown or red precipitate	-
	Wagner's test	Reddish brown precipitate	-
	Hager's test	Yellow colored precipitate	-
Steroids and terpenoids	Steroids test	Brown ring	-
	Terpenoids		
Gums and mucilage	Gums and Mucilage test	Precipitation	-
Saponins	Froth test	Foam	-
Fixed oils and fat	Filter paper test	Oil Stain	-
	Saponification test	Soap	-

Note: "**+**" means presence and "**-**" means the absence of compounds.

RESULTS

Preliminary phytochemicals

The phytochemical screening of the ethanolic extract of the *Syzygium cumini* plant bark revealed the presence of flavonoids, tannins, sugars, proteins, amino acids, sterols and absence of steroids, alkaloids, saponins, terpenoids (table 1).

Acute toxicity study

The results of acute toxicity study of stem bark of *Syzygium cumini* showed no noticeable signs of acute toxicity and mortality was not noticed at any dose up to 5000 mg/kg body weight.

Cage side observation

The study of each animal on a day-to-day basis from the 0 d of study to the 14th day of the study did not show any significant change between control and treated groups (table 2).

Body weight

Body weight was recorded on Day 0 (before treatment), Day 7 and Day 14. The body weight of all treated group rate was slightly decreased, but the decrease was not statistically significant (P>0.05) when compared with the control group (table 3).

Organ weights

The macroscopic examination of each organ showed normal anatomical features without any gross changes. There was no statistically significant change (P>0.05) in organ weight between control and treated groups (table 4).

Hematology

Results of blood parameters in table 5 showed a slight increase of HB, WBC, RBC, HCT, MCV, MCH, MCHC & PLT in the treated groups, but these changes were not statistically significant compared to the control group.

Table 2: Cage side observation control and treated animals

Parameter	Cage side observation	
	Control	Treated
Behavior pattern	Normal	Normal
Condition of the fur	Normal	Normal
Skin	Normal	Normal
Convulsions	Nil	Nil
Tremors	Nil	Nil
Eyes-Dullness	Nil	Nil
Eyes-Opacities	Nil	Nil
Pupil diameter	Normal	Normal
Ptosis	Nil	Nil
Color & consistency of the feces	Normal	Normal
Salivation	Normal	Normal
Diarrhea	Nil	Nil
Breathing abnormalities	Nil	Nil
Gait	Normal	Normal

Table 3: Body weight control and treated animal in grams (g)

Body weight	Control	Treated	
	C	SE2	SE5
0 D	200.7±1.90	201.2±1.10	199.5±0.71
7th Day	214.4±1.89	210.2±0.87	210.6±1.43
14th Day	226.6±2.44	221.9±1.71	222.4±0.61

The data were expressed as mean±SE, n=5, C-control, SE2-Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight and, SE5-Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight.

Table 4: Organ weight control and treated groups in grams (g)

Organs	Control	Treated	
	C	SE2	SE5
Ovary *	47.4±0.40	45.6±1.01	48.9±0.66
Uterus	0.302±0.01	0.309±0.01	0.320±0.01
Spleen	0.412±0.01	0.425±0.01	0.426±0.01
Kidneys	1.51±0.02	1.57±0.06	1.66±0.04
Suprarenal Glands *	43.4±1.91	48.0±1.92	49.0±2.95
Liver	7.04±0.21	7.12±0.28	7.28±0.16
Heart	0.720±0.01	0.742±0.03	0.750±0.02
Lungs	1.40±0.09	1.46±0.01	1.52±0.03
Brain	1.47±0.02	1.49±0.01	1.53±0.02

All the data were expressed in g except * in mg as mean±SE, n=5, C-control, SE2-Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight and, SE5-Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight.

Microscopic anatomy

The results of the microscopic anatomy of ovaries showed the normal histo architecture by having the epithelium and with the presence of different stages of the follicle, further the medulla also showed the normal stromal tissue (fig. 1).

The histological sections of uterine horns revealed the normal cytoarchitecture with respect to the endometrium, myometrium, and perimetrium (fig. 2). The liver histology was normal with central vein and radiating hepatocytes arranged in lamellae, the portal triad was also clearly visible without any alterations (fig. 3).

Table 5: Hematological parameter control and treated groups

Parameter (units)	Control	Treated	
	C	SE2	SE5
Hb (g/dl)	12.92±0.29	13.44±0.27	13.25±0.25
WBC (10^3/µl)	11.68±0.24	12.14±0.40	12.28±0.30
RBC (10^6/µl)	8.23±0.18	8.60±0.16	8.69±0.27
HCT (%)	40.54±0.63	41.21±0.71	41.45±0.51
MCV (fL)	54.62±0.54	55.29±0.22	54.27±0.40
MCH (pg)	17.18±0.31	17.76±0.20	17.84±0.31
MCHC (g/dl)	31.82±0.32	32.09±0.21	32.46±0.26
PLT (10^3/µl)	765±5.32	767±4.11	776±5.12

The values were expressed as mean±SE, n=5, C-control, SE2-Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight and, SE5-Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight.

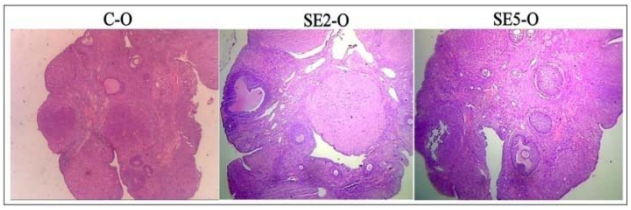

Fig. 1: Photomicrograph of ovary-H&E stain (4x magnification)

C-O (control ovary)

SE2-O (ovary of Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight)

SE5-O (ovary of Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight)

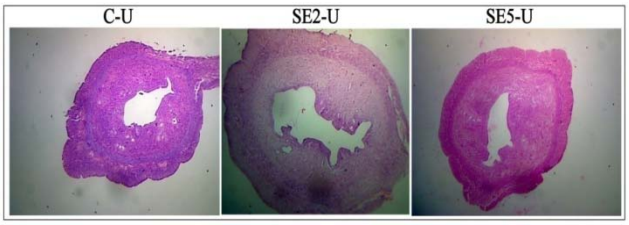

Fig. 2: Photomicrograph of uterine horns-H&E stain (4X magnification)

C-U (control uterine horn)

SE2-U (uterine horn of Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight)

SE5-U (uterine horn of Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight)

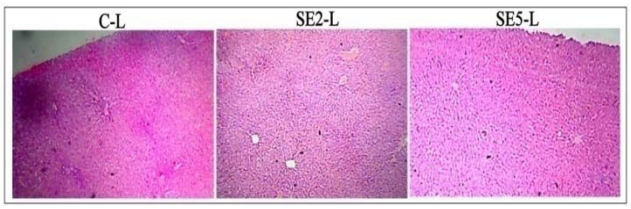

Fig. 3: Photomicrograph of the liver-H&E stain (4X magnification)

C-L (control Liver)

SE2-L (Liver of Ethanolic extract of the stem bark of *Syzygium cumini* 2000 mg/kg body weight)

SE5-L (Liver of Ethanolic extract of the stem bark of *Syzygium cumini* 5000 mg/kg body weight)

DISCUSSION

The initial phytochemical screening investigations may be helpful in the designation of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate separation of pharmacologically active chemical compounds and their qualitative and quantitative estimation [21].

Herbal extracts contain different phytochemicals with biological activity that can be of the valuable therapeutic index. The present work of preliminary phytochemical screening of the ethanolic extract of the *Syzygium cumini* plant stem bark revealed the presence of flavonoids, tannins, sugars, proteins, amino acids, sterols and absence of steroids, alkaloids, saponins, terpenoids. These results concur with the previous study of methanolic extract except for the presence of saponins, terpenoids, and absence of amino acids [11, 17]. This study demonstrates that the bark contains many secondary metabolites; these metabolites are of importance in the phytomedicine.

For instance, the flavonoids present in the stem bark of *Syzygium cumini* may have the free radical scavenging capacity, antioxidative activity, coronary heart disease prevention, anti-inflammatory, antiviral, anticancer and hepatoprotective activities [26]. The tannins present in the stem bark of *Syzygium cumini* may responsible for anticarcinogenic, antimutagenic, antioxidative, antimicrobial activities, as well as reduce blood pressure, accelerate blood clotting, decrease the serum lipid level, modulate immune responses [27]. The tannins also have been reported to be responsible for decreased intake of food, net metabolizable energy, growth rate, and protein digestibility in experimental animals this may be responsible for the decreased body weight noticed in the present study but not statistically significant compared to control group [27]. The presence of sterols has a pharmacological activity like lowering blood cholesterol and anti-inflammatory [16].

The oral acute toxicity study results of the present study indicated no significant alterations in final body weight, organ weights, blood parameters and microscopic anatomy of ovaries, the uterine horns and liver of rats treated with ethanolic extract of the stem bark of *Syzygium cumini* in comparison with the control group. Further, during the study period of 14 d there were no detectable signs of acute toxicity. Lack of death at all doses showed that the LD50 of ethanolic extract of the stem bark of *Syzygium cumini* is greater than 5000 mg/kg body weight these results are similar to the previous reports [24, 28].

CONCLUSION

We resolve that the oral administration of ethanolic extract of the stem bark of *Syzygium cumini* was found to be nontoxic up to 5000 mg/kg body weight, so the LD50 of ethanolic extract of the stem bark of *Syzygium cumini* is greater than 5000 mg/kg body weight. Hence, the present work provides a satisfactory preclinical proof of safety of stem bark of *Syzygium cumini* in toto including maintenance of cellular architecture. Phytochemicals present in the stem bark may have remarkable pharmacological activities so it is mandatory to do further analysis to isolate such pharmacological active compounds which can be employed in the fabrication of innovative medication for several diseases.

CONFLICT OF INTERESTS

There is no conflict of interest to declare.

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