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ATCC 8668, *Aspergillus fischeri*

## Evaluation of acute and sub-acute toxicity effect of ethanol extract from *Caesalpinia bonducella* seed kernel.

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### Abstract

Seed kernel of *Caesalpinia bonducella* comprises of various influential components. The ethanol extract of these seed kernels have potential antibacterial activity against pathogenic micro-organisms. The antibacterial activity is more prominent against fungi. The acute toxicity studies of extract in albino mice were investigated. Various doses of extract from 5 to 2000 mg/kg bodyweight were administered orally to the test groups of mice, while distilled water was given to the control group to evaluate the toxicity in mice after ingestion of the extract for one day (acute model) and for 14 days (sub-acute model). The histopathological studies of liver, heart, and kidney indicated either no or less alteration in the treated group. However, a slight change in liver cellular architecture was observed with tubular congestion, tubular desquamation, glomerular congestion and interstitial inflammatory cells of test animal. The results indicated that LD50 of the extract is higher than 2000 mg/kg and no changes were observed in any behavioral parameters in mice.

**Keywords:** *Caesalpinia bonducella*, toxicity

### 1. Introduction

Plants are rich source of many natural products which have been extensively used for human health care, seasonings, beverages, cosmetics and dyes. Given their potential to produce significant effect, they can be useful in the treatment of various diseases. Many western drugs have origin from plant extract. They are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. In recent years, there is an increase in interest in the use of medicinal plants for their pharmacological evaluation. Herbal drugs or medicinal plants extracts and their isolated compounds have demonstrated spectrum of biological activities. They are associated with both harmful as well as beneficial effects to the biologic system. *Caesalpinia bonducella* (Family: Caesalpinaceae), commonly known as NataKaranja (Hindi). *Caesalpinia bonducella* belonging to the family: Caesalpinaceae. Found throughout India and tropical countries of the world [1]. The seed of the plant is known as fever nut, bonduc nut and physic nut. Seed coat is hard, glossy, and greenish to ash grey in color [2]. Phytochemical analysis of the seeds revealed the presence of flavonoids, terpenoids, glycosides, saponins, tannins, alkaloids and amino acid were found in the seed kernel with varying concentration [3-5]. The major components were aspartic acid, lysine, glycine, leucine, histidine, isoleucine and serine [6]. The application is found in the treatment of tumour, inflammation and liver disorder [7, 8]. The plant possess therapeutic properties such as antipyretic [9], anthelmintic and antimicrobial [10], anticonvulsant [11], anti-inflammatory [12], antiasthmatic [13], nematocidal [14], abortifacient [15], activities. Recent studies on antidiabetic [16] antifilarial activity [17], antioxidant activity [18], effect on female reproductive system of albino rat [19] and immunomodulatory [20] activities of ethanol extract from *C. bonducella* seed's has been reported. However, the toxicity of ethanol extract of *C. bonducella* seed kernel has not been studied so far and is attempted here.

### 2. Materials and Methods

#### a) Microorganism

The following organisms were procured from National Collection of Industrial Microorganisms (NCIM), Pune: *Escherichia coli* ATCC9661, *Streptococcus pyogenes* NCIM 508, *Staphylococcus aureus* NCIM 2079.

**b) Plant material**

The seeds of *C. bonducella* (Caesalpiniaceae) were procured from the local market of Bidar district, Karnataka, India. Taxonomic identification was conducted by Department of Botany, Karnataka University, Dharwad, India.

**C) Animal Model**

Healthy young adult Swiss albinos were used for studies. The studies were approved in Institutional Animal Committee meeting, resolution BMK/IAEC/Res-11/2011.

**2.1 Preparation of the extract**

The seed coat was separated from the seed kernel and 100 grams of dried seed kernels of *Caesalpinia bonducella* were ground into powder using a pestle mortar and stored in an air tight container. The extraction was carried as described by Shukla *et al.* [21] using Soxhlet apparatus by 95% ethanol for 24 hrs. The crude extract obtained was filtered through Whatman paper and the filtrate. The sample was dried on rotary flash evaporator.

**2.2 Antimicrobial activity by Disc diffusion**

Antimicrobial activity of the cultures was checked by the disc-diffusion assay method [22]. The pathogens were cultured in a nutrient broth for 24 hrs and diluted with sterile saline solution. 100 µl of 24 hrs culture of pathogens was plated on to nutrient agar plates and incubated at respective growth temperatures of organism for 12–14 hrs. 20 µL of extract solution (4%w/v) was applied on sterile filter discs (diameter 6 mm) which were placed on the surface of plates. The plates were incubated at respective temperatures for 14–16 hrs. Inhibition was noted by measuring the clearance zone diameter. The experiment was repeated in triplicate for statistical significance.

**2.3 Selection of animal species and feeding conditions**

Healthy young adult Swiss albinos were used for the proposed study as sanctioned by the ethical clearance committee. Male and female mice were used for lethal dose toxicity and for 14 day study. Females are nulliparous and non-pregnant. Each animal, at the commencement of testing, is between 8 and 12 weeks old. The temperature of the experimental animal room is maintained between 22±3 °C. Artificial lighting is provided, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets is used with an unlimited supply of drinking water. Animals are group-caged by dose, but the number of animals per cage does not interfere with clear observations of each animal. The animals are randomly selected and marked to permit individual identification.

**2.4 Administration of doses by sighting studies**

The test substance is administered in a single dose by gavages using a stomach tube. In the unusual circumstance that a single dose is not possible, the dose is given in smaller fractions over a period not exceeding 24 hours. The animals are weighed and the test substance is administered. After the substance has been administered, food is withheld for a further 1-2 hours.

**a) Sighting study**

The purpose of the sighting study is to allow selection of the appropriate starting concentration for the main study. The test substance is administered to single animals in a sequential manner following 5, 50, 300 and 2000 mg/kg. A period of at least 24 hours will be allowed between the dosing of each animal. All animals were observed for 14 days. The sighting study is completed when a decision on the starting concentration can be made, based on signs of evident toxicity or if a death is seen at the lowest fixed concentration.

**2.5 Acute toxicity**

The bioassay was conducted according to the OECD 420 guidelines (Acute Oral Toxicity). The animals were divided into control and test groups. 10 mice in each group (5 male and 5 female) The control group received distilled water per and remaining groups received 2000 mg/kg body weight for *Caesalpinia bonducella* extract. The animals were observed continuously for 4h, and then observed each hour for 24 h for behavioral and physiological activities, body weight and mortality rate. The LD<sub>50</sub> was determined at the end of 24 hrs.

**2.6 Sub-acute toxicity**

For the sub-acute toxicity studies two groups of 10 mice each (5 male and 5 female) were given daily with the lowest lethal dose of 1/10th (200 mg/kg) of LD<sub>50</sub> for 14 days. The aim of this test was to evaluate the maximum tolerated dose and also to study the nature of toxic reactions and toxic potential of the extract. Control group received distilled water every day. During the study, the animals were weighed, and food and water intake were monitored every day. On 14<sup>th</sup> day, all the surviving animals were left for fasting overnight and then sacrificed by overdosing of anesthesia. The heart, lung, kidney and liver were collected after intense washing and preserved in 10% formalin for further analysis of histological parameters.

**2.7 Histological examination**

The tissues were first examined for gross pathology and then fixed in 10% neutral buffered formalin solution. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The tissue sections were cut and photographed under microscope for histological examination.

**3. Results and Discussion****3.1 Antimicrobial activity**

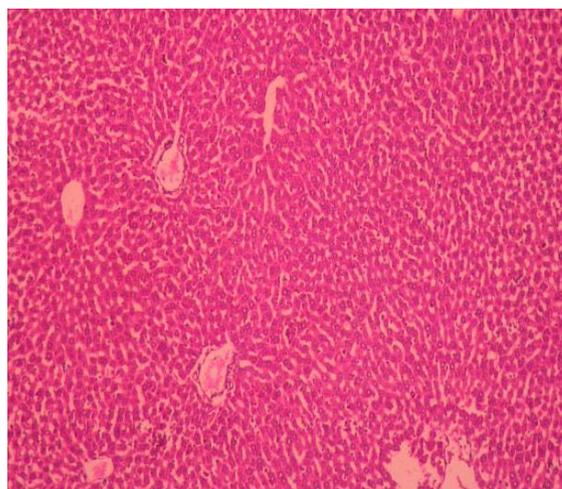
The antimicrobial activities of the ethanol extract of *C. bonducella* seed kernel obtained by the disc diffusion method are presented in Table 1. The zone of inhibition was observed against two gram-positive, one gram-negative bacterium and one fungus. The maximum zone of inhibition was against *Aspergillus fischeri* as shown in Fig 1. The data indicates that the extract comprises of prominent antifungal agent. This property of antifungal can be used for various applications. Antibacterial activity of extract indicates that it comprises of compounds which would inhibit broad spectrum of microorganisms.



**Fig 1:** Zone of inhibition by *C. bonducella* extract against *Aspergillus fischeri*

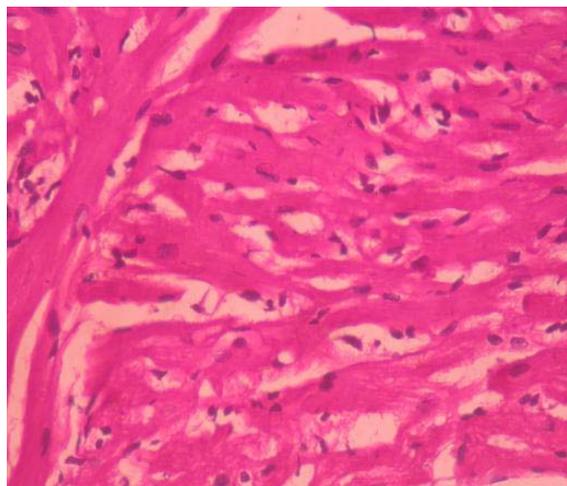
**Table 1:** Zone of Inhibition by disc diffusion method. Values are represented as mean± Standard error of mean.

Micro-organism	diameter (mm)
<i>Escherichia coli</i>	8.5±0.52
<i>Streptococcus pyogenes</i>	9.2±0.15
<i>Staphylococcus aureus</i>	8.2±0.43
<i>Aspergillus fischeri</i>	20.5±0.3

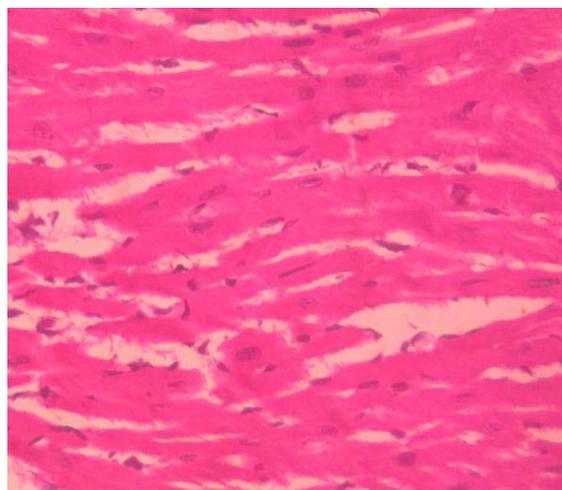


(C)

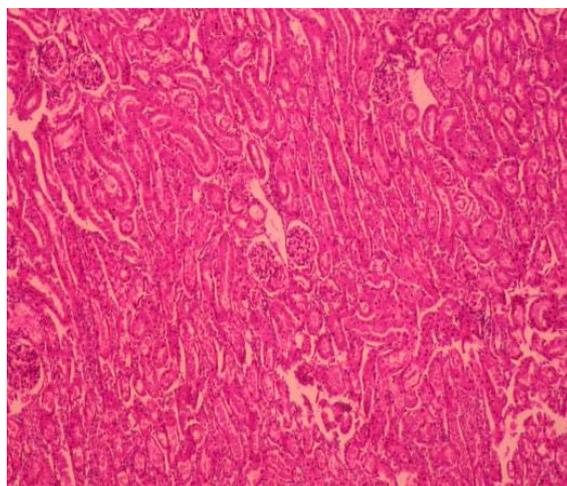
**Fig 2:** **A:** Heart tissue, **B:** Kidney tissue, **C:** Liver tissue dissected from control mice.



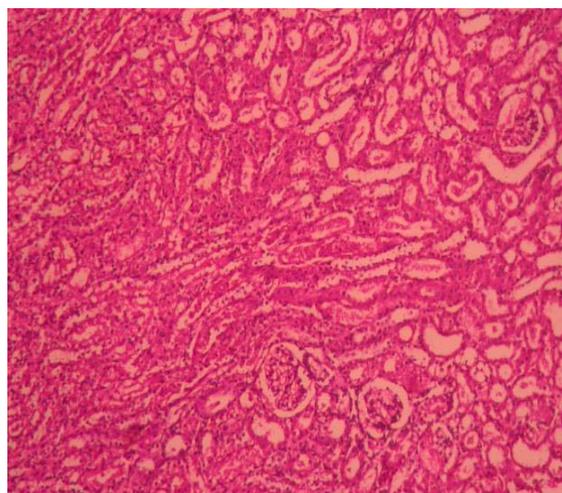
(A)



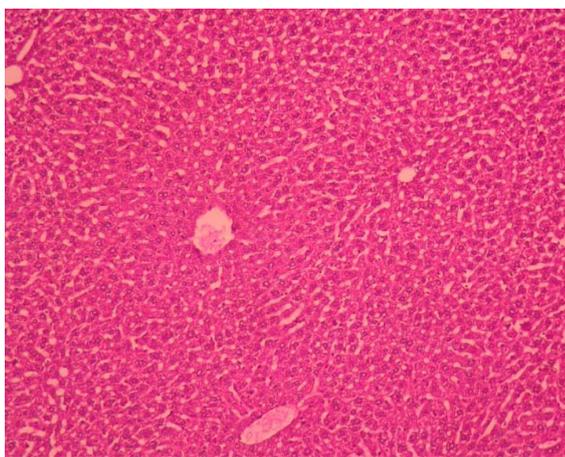
(A)



(B)



(B)



(C)

**Fig 3:** A: Heart tissue, B: Kidney tissue, C: Liver tissue dissected from test mice

### 3.2 Acute toxicity

The acute toxicity of ethanol extract of *C. bonducella* was studied. The groups of mice were treated with doses from 5 to 2000 mg/kg body weight showed no behavioral changes after oral administration after 24 hrs. Thus, the dose did not produce significant change in behavior and sensory nervous system responses in female mice. During 24 h of the experiment, no death occurred in any of the groups. Hence from the above studies it can be concluded that the maximum lethal dose for ethanol extract from seed kernel of *C. bonducella* is higher than 2000 mg/kg body weight.

### 3.3 Sub-acute toxicity

It's appeared that the ethanol extract of *C. bonducella* at 200 mg/kg body weight did not produce any marked change in both male and female mice. No mortality was observed during 14 days of drug.

### 3.4 Histological observations

The light microscopy examinations of vital organs are presented in Fig. 2 and Fig.3. Fig.2 represents vital organs of control and Fig. 3 represents vital organs of test mice. The histological study of *Caesalpinia bonducella* at a dose of 2000 mg/ kg body weight by oral route for 14 days shows mild damages to the liver i.e. spotty necrosis, sinus congestion and central vein congestion observed, whereas there is no toxic effect observed in the Heart. Further the kidney having mild damage shows tubular congestion, tubular desquamation, glomerular congestion and interstitial inflammatory cells.

### 4. Conclusion

The present study demonstrated that ethanol extract from seed kernel of *C. bonducella* was nontoxic at 2000 mg/kg body weight to Swiss albino mice as evidenced by behavioral and histological studies. However, a slight change in the cellular architecture was revealed in histology of liver. Further, study on the mode of mechanism of toxicity of the drug should be undertaken in order to identify the site of action. The extract has prominent antibacterial properties which can be further tested against more pathogenic bacteria and fungi.

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