Assessment of Bioactivity of *Cassia fistula* Using *Bombyx mori* Lethality Assay

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Summary: In recent times, focus on plant research has increased all over the world and a large body of evidence is being collected to evaluate immense potential of medicinal plants used in various traditional systems. In this regard, the present study was conducted to evaluate the bioactivity of a commonly used medicinal plant *Cassia fistula* against newly selected *Bombyx mori* (silkworm) larvae. *C. fistula* pods were extracted using water, methanol, ethanol, hydro-methanol (1:1) and hydro-ethanol (1:1) and were assayed for their activity against *Bombyx mori*. Methanol extract of *C. fistula* at concentration of 100 mg/L killed half (LC_{50}) of *Bomyx mori* larvae under study. *Bomyx mori* LC_{50} for other *C. fistula* extracts were 400 mg/L for ethanol and hydro-methanol, 800 mg/L for hydro-ethanol and 1600 mg/L for aqueous extract. From the results of the present study it can be concluded that *Bombyx mori* lethality bioassay can be considered a useful preliminary tool for plant extract toxicity evaluation. The main objectives of the present study was to develop a new and simple assay to evaluate claims from traditional, tribal and advanced medicinal lore to suggest directions for future clinical research and commercial importance that could be carried out by local investigators in developing regions.

Introduction

Trees are important to humankind not only environmentally, economically, and industrially but also historically, spiritually and aesthetically, for they sustain human life through direct and indirect gains by providing a wide range of products for survival and prosperity. *Cassia fistula* commonly known as Amaltas, Golden shower, Canafistula Indian Laburnum, Lluvia De Ore or Pudding-Pipe-Tree belongs to the Caesalpiniaceae, a sub group of the Leguminoseae family. *C. fistula* is widely distributed across Pakistan, India, Malaysia and Indochina. Currently *C. fistula* is widely cultivated all over the world due to its ornamental, medicinal and water purifying attributes [1-8]. From spring to mid summer *C. fistula* is covered in pendent racemes of beautiful yellow flowers followed by long cylindrical pods with the seeds imbedded in a stick brown pulp, the specific cepihet refers to these cylindrical shaped pods. The fruits ripen (linear-cylindrical, 30 to 50 cm long, 1.5 to 1.7 cm in diameter), transversely septate, dark brown to black, and indehiscent when ripe in the month of April and May. In folk medicine, *C. fistula* is well known for its antitumor, antifungal, antifertility, anti-inflammatory, antioxidant, antitussive, hypocholesterolaemic, nematicidial, hepatoprotective, and wound healing potentials [3, 6, 9-17]. The medicinal properties of *C. fistula* have been mainly attributed to the presence of alkaloids, a triterpene derivative, anthraquinone derivatives, and polyphenolics comprising flavonoids, catechins, and proanthocyanidins. *C. fistula* seeds galactomannan as a potential binding agent for pharmaceutical formulation found that *C. fistula* seed germ exhibit overall superiority in binding properties when compared to conventional binders like gum Arabic, gum tragacanth, sodium CMC and gelatin.

Traditional medicine is an integral part of culture of many developing countries like Pakistan, India and many others. In these countries, majority of the people are relying for their primary health care on traditional medicine [2]. Due to its large size and ease of culture, *Bombyx mori* has long been a model organism in the study of Lepidopteron and arthropod biology. Fundamental findings on pheromones, hormones, brain structures and physiology were made with the silkworm. Currently, research is focusing on genetics of *Bombyx mori* and genetic engineering. The genome has been sequenced and many projects have worked on genetic engineering of *Bombyx mori* to produce desirable proteins in the place of silk. Such proteins include human drugs. The *Bombyx mori* has been exploited as a silk producer in the silk industry for thousands of years [19]. Recent

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success of *Bombyx mori* lethality bioassay has opened new prospects for this insect species.

In this study, we have used a new bio-lethality assay to verify the efficacy of *C. fistula* fractions extracted using different solvents. The toxicity of *C. fistula* extract was evaluated using *Bombyx mori* lethality bioassay based on its ability to kill laboratory cultured larvae. The reason of *Bombyx mori* for lethality bioassay is presumably due to its weakened immune system which has made the insect highly vulnerable to bacterial and viral infections. Due to these characteristics this worm can respond to minute doses of plant extracts.

Results and Discussion

Throughout the world, the medicinal properties of plants are evaluated for their pharmacologic potency, toxicity level, and economic viability [20-22]. The results of the present study explored that *Bombyx mori* lethality assay is a simple, rapid, effective and inexpensive bioassay for testing medicinal plants extracts bioactivities. The bioassays of medicinal plants in most cases correlate reasonably well with cytotoxic and anti-tumor properties [23-24]. Bioactivity of pods extracts of *Cassia fistula* was evaluated by mortality rate of *Bombyx mori*. Lethal concentration to half of population (LC_{50}) was determined by varying the concentration of each plant extract from 10-5000 mg/L (Figs. 1-5). Methanol extract of *C. fistula* at concentration of 100 mg/L killed half (LC_{50}) of *Bomyx mori* larvae under study. *Bomyx mori* LC_{50} for other *C. fistula* extracts were 400 mg/L for ethanol and hydro-methanol, 800 mg/L for hydro-ethanol and 1600 mg/L for aqueous extract. The LC_{50} value of positive control, podophyllotoxin was 3.1 mg/L. Traditional healers use primarily water as the solvent [25-27] but in our studies we had also used organic solvents (methanol and ethanol) and hydro-alcohol (hydro-methanol and hydro-ethanol for the production of extract from *C. fistula* pods. This was done to rationalize the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay.

Methanol fraction of *C. fistula* pods showed most prominent bioactivity against *Bombyx mori*. The degree of lethality was found to be directly proportional to the concentration of the extract. The observed lethality of *C. fistula* extracts to *Bombyx mori* is an indicative of the presence of potent cytotoxic components which warrants further investigation. The results of the present study support the folkloric usage of the *C. fistula* aqueous, organic and hydro-alcoholic fraction should be further evaluated to isolate active ingredients.

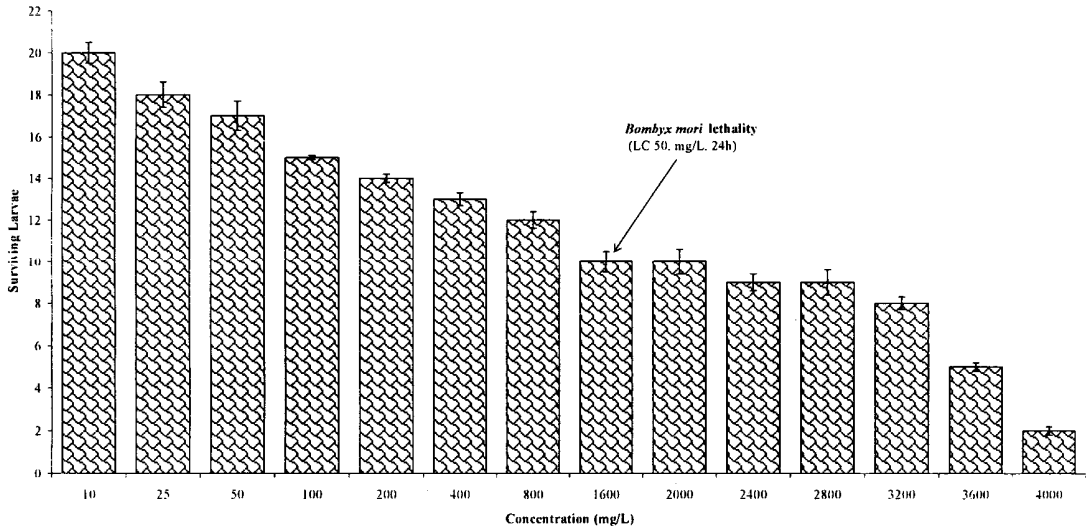

Fig. 1: Assessment of bioactivity of *C. fistula* pods water extract using *Bombyx mori* lethality assay.

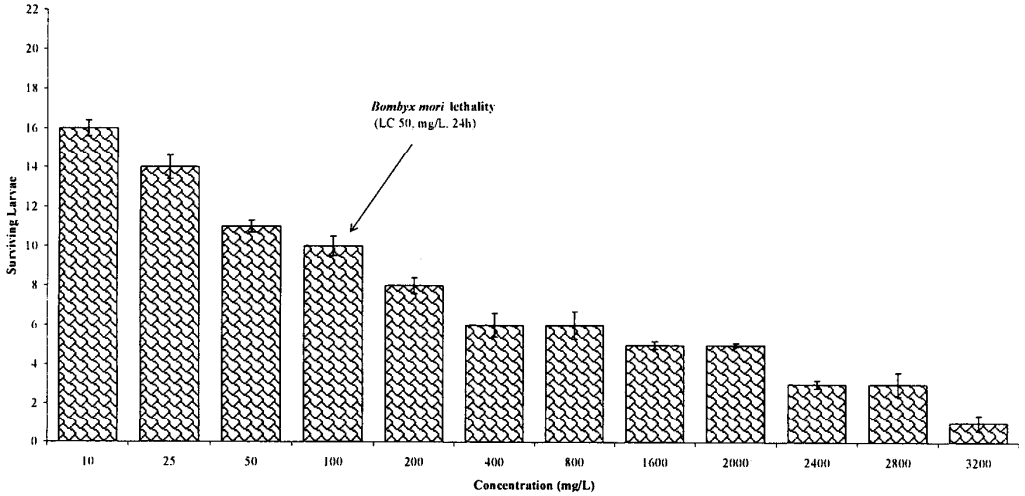

Fig. 2: Assessment of bioactivity of *C. fistula* pods methanol extract using *Bombyx mori* lethality assay.

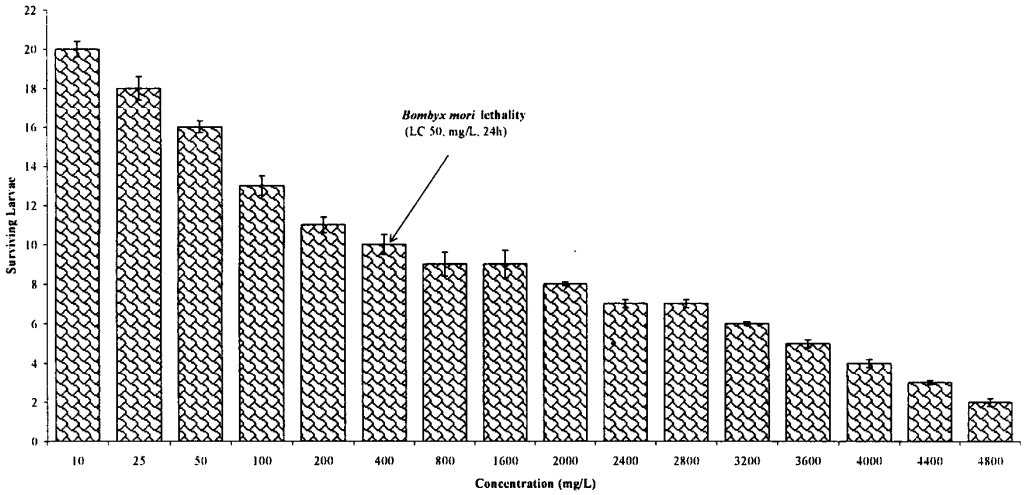

Fig. 3: Assessment of bioactivity of *C. fistula* pods ethanol extract using *Bombyx mori* lethality assay.

Experimental

Collection of Plant Material and its Extraction

Cassia fistula pods used in the present study were harvested by manually removing the matured pods of the plants from University of Agriculture, Faisalabad, Pakistan. Pods were washed thoroughly with deionized distilled water (DDW) to remove any debris and particulate matter. The air dried pods were cut into 5 cm and carefully placed in a built in purring edge peeler of multi purpose wonder mill to remove the *Cassia fistula* pods bark. The dried internal pods mass grounded into fine powder using food processor (Moulinex, France). Powdered pods (each 30 g) were extracted individually with 250 ml of each of water, methanol, ethanol, hydro-methanol (1:1) and hydro-ethanol (1:1) and then filtered. Filtrates were concentrated, dried and subjected for activity studies.

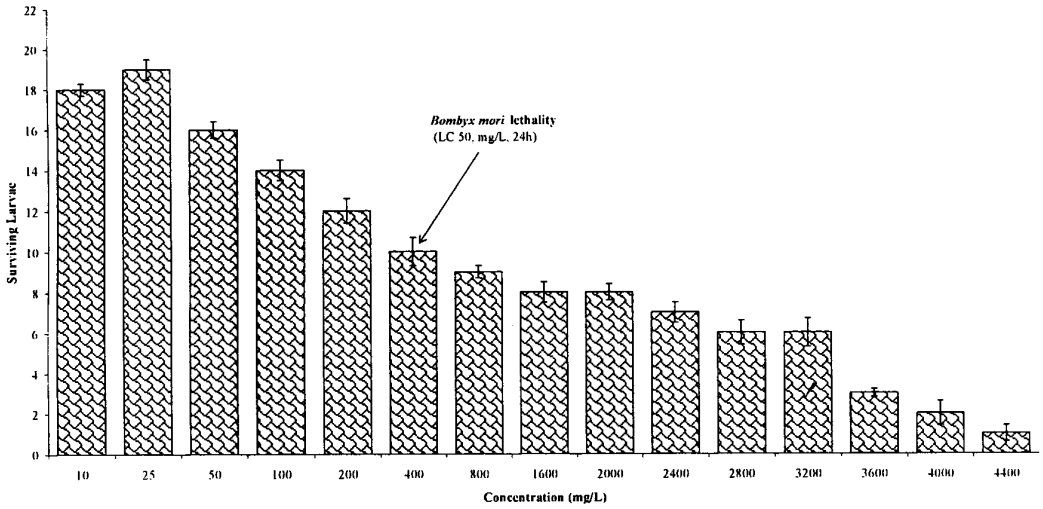

Fig. 4: Assessment of bioactivity of *C. fistula* pods hydro-methanol (1:1) extract using *Bombyx mori* lethality assay.

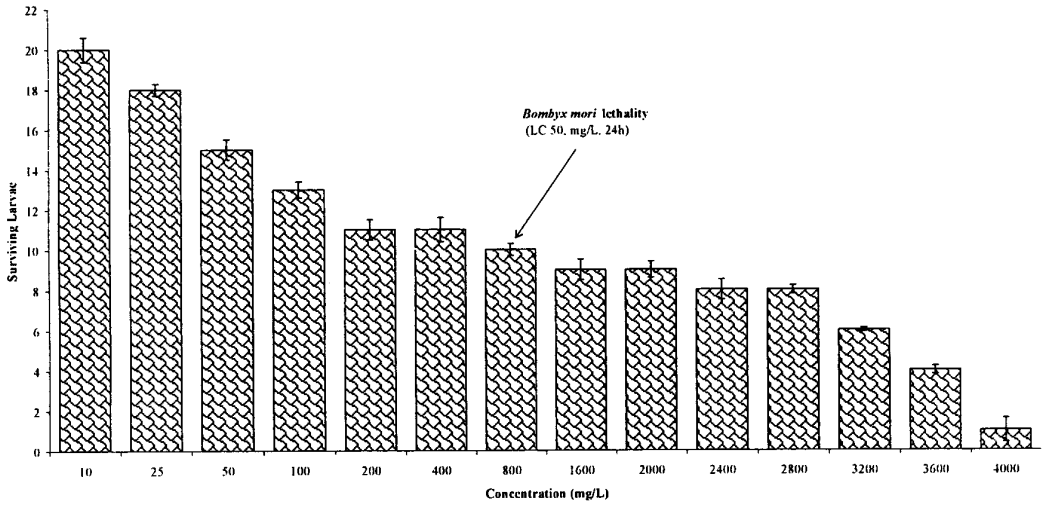

Fig. 5: Assessment of bioactivity of *C. fistula* pods hydro-ethanol (1:1) extract using *Bombyx mori* lethality assay.

Cytotoxicity Bioassay

Lethality bioassay of *Bombyx mori* was performed to check the cytotoxicity of extracts of pods of *Cassia fistula.* Fresh leaves of *Morus indica* (mulberry) were sprayed with solution containing 0.5 mL of plant extract and 4.5 mL of brine solution (prepared by dissolving sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH kept under aeration for 48 hours). The concentration of *C. fistula* pods extract was varied from 1-5000 mg/L. Twenty *Bombyx mori* larvae (5^{th} stage) were fed on freshly sprayed *Morus indica* leaves placed in each plastic tray maintained at room temperature for 24 hours under light and surviving worms were counted. A control assay was also run under similar conditions with the samples. All experiments were conducted in triplicates to ensure reproducibility of results.

Lethality Concentration Determination

The percent lethality was determined by comparing the mean surviving larvae of the test and control assays. Podophyllotoxin was used as a positive control in the bioassay.

Statistical Analysis

The error bars shown in figures represents mean experimental value ± SD.

Conclusions

From the results of the present study it can be concluded that *Bombyx mori* lethality bioassay can be considered a useful preliminary tool for plant extract toxicity. This method is attractive because of simplicity and inexpensiveness. This assay could be further developed as a useful tool for the isolation of bioactive compounds from plant extracts.

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