Acute toxic effects of Medicinal Plant *Jatropha gossypifolia* on non-target Fish and Mice

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The medicinal properties attributed to *Jatropha gossypifolia* L. (Ratanjot) are extensive. The safety of *J. gossypifolia* is important in relation to its medicinal applications. This study was performed to elucidate the possible toxic effects and mortality caused by crude latex and compound apigenin isolated from leaves of *J. gossypifolia* on freshwater snakehead fish, *Channa punctatus* and Swiss albino mice *Mus musculus*. Toxicity of both the tested materials against *Channa punctatus* was time and dose-dependent. But in case of *Mus musculus*, the toxicity was only dose dependent and the LC50 doses was much higher in comparison to fish *Channa punctatus*.

**Key words:** *Jatropha gossypifolia*, Apigenin, *Channa punctatus*, *Mus musculus*, Piscicidal activity, Muricidal activity.

**INTRODUCTION**

The use of plants and their products for curing and managing various ailments has been known to the world since time immemorial, and is gaining momentum these days due to toxic effects by synthetic drugs. Crude extract of local plants are frequently being used by traditional communities like tribal and rural population of India and elsewhere. This, and medicines manufactured on principles of natural compounds even by pharmaceutical companies, may lead to a large-scale exposure of humans to natural products (Awasthy et al., 2000). Natural products have always played a major role in the development of organic chemistry. The various structural types of natural products contribute not only to new finding or pose challenging synthetic problems but also provide hope that they may become the basis for new biologically active substances of commercial significance (Sukari et al., 1992). Though apparently believed non-toxic, a clean chit offered to these plant products requires strict scientific tests, besides clinical ones on different vital systems, moreso, because these natural products may contain a few harmful ingredients in them as secondary metabolites (Nakamura and Yamamoto, 1982) which may have perilous side effects including mutagenic potentials.

It is, therefore, biological testing has played an important role in toxicity studies of crude latex and its compound. There are numerous bioassay studies on plant extract. The first of such work involving some local plants was reported as early as 1965 (Nakanishi et al., 1965). This study was conducted as part of the research to study the biological activity of crude latex and compound apigenin isolated from leaves of local medicinal plant *Jatropha gossypifolia* L. (Ratanjot). The report discusses the piscicidal and muricidal activity of both the tested plant material on fish and mice respectively.

**PLANTS**

*Jatropha gossypifolia* (Family Euphorbiaceae) was collected locally from the Botanical garden of D.D.U. Gorakhpur University, Gorakhpur, (U.P.), India and identified by Prof. S.K. Singh, Plant Taxonomist, Department of Botany, D.D.U. Gorakhpur University,

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Gorakhpur, U.P. (India) where the boucher specimen was deposited.

Uses in Traditional Medicine

*Jatropha gossypifolia* is used in different countries in many ways. The plant is antibiotic, insecticidal and used for toothache and as blood purifier (Balee, 1994). It possesses significant anticancer, hepatoprotective and pesticidal activity (Hartwell, 1969; Chatterjee et al., 1980; Panda et al., 2009; Panda et al., 2009). The roots, stems, leaves, seeds, and fruits of the plant have been widely used in traditional folk medicine in many parts of different countries. The young stem of the plant is used as toothbrush as well as to clean the tongue in the treatment of thrush (Ogundare, 2007). The stem latex has been shown to possess coagulant activity and its mechanism of action as haemostatic agent found to be by precipitation of coagulant factors (Oduola et al., 2005).

The leaves of *J. gossypifolia* are used for intermittent fevers, carbuncles, eczema, itches, and sores on the tongues of babies, swollen mammae, stomachache, and venereal disease and as blood purifier (Balee, 1994). The leaf decoction is used for bathing wounds (wound healing) (Morton, 1968; Morton, 1981). In Southern Nigeria, the fresh leaf extract applied with crushed leaf by herbalists and local people used to stop bleeding from skin and nose (Morton, 1981; Omorogbe et al., 1996). The leaf extract has been used as an anticoagulant for biochemical and haematological analysis (Oduola et al., 2005). From leaves of *Jatropha gossypifolia*, three flavonoids were isolated viz. apigenin, vitexin and isovitexin (Subramanian et al., 1971). The latex of *J. gossypifolia* yields the cyclic heptapeptide, cyclogossine A (Horsten et al., 1996) and cyclic octapeptides Cyclogossine B (Auvin-Guette et al., 1997).

**EXPERIMENTAL MATERIALS**

Experimental material 1

The yellowish milky latex of *Jatropha gossypifolia* drained into glass tubes by cutting the stem apices. The latex was centrifuged at 1000 rpm for 20 minutes to remove the resin. This resin free latex lyophilized at -40°C, and the lyophilized powder stored for further use. The wet weight of one ml latex of *J. gossypifolia* was 1.04 gm and dry weight (lyophilized at -40°C) was 0.140 gm.

Experimental material 2

Extraction of Apigenin was done by washing the leaves of *Jatropha gossypifolia* properly with water and dried in incubator at 37°C. The dried leaves were then powdered. About 50 gm powdered leaf was subjected to extraction through Soxhlet apparatus with about 250-300 ml ethyl alcohol for 72 hours at 20-40°C. After extraction, a little amount of crude yellow powder was obtained. Dilute NaOH solution was added to crude powder and then filtered. Again dilute HCl solution was added to filtrate and again filtered. Obtained precipitate was crystallized with Methanol, and it was Apigenin. The pure compound isolated by this procedure was tested for their UV spectra, as well as their respective shifts (bathochromic and hypochromic) that occur due to addition of specific agents: NaOMe, NaOAc, NaOAc + H2BO3, AlCl3 + HCl. IR spectra were also made. For the determination of Rf value in the TBA system (tertiary butanol acetic-water 6:2.2 v/v/v) Whatman 1 chromatographic band was used.

Apigenin is the main compound found in *J. gossypifolia* leaf and its molluscicidal (Singh and Agrawal, 1988) as well as piscicidal activity (Singh and Singh, 2002) has already been known.

Molecular weight: 270.24 g/mol
Molecular formulae: C15H10O5
Melting Point: 345-350°C

**EXPERIMENTAL ANIMAL USED**

Experimental animal 1

The fish Channa punctatus (Bloch) (19.50 ± 2.50 cm in length, 50.00 ± 4.00 gm in weight) were collected locally and acclimatized in aquarium for 72 hours containing 100 L of de-chlorinated tap water. The water in the aquarium was changed every 24 hour. The bioassays were carried out at specific conditions, as recommended by the American Public Health Association (APHA 1998). The measured values of pH, temperature, dissolved oxygen (DO) and hardness of water were 6.8-7.0, 26°C, 7.2mg/l and 41mg/CaCO3, respectively.

Experimental animal 2: Four to six weeks old laboratory Swiss albino mice Mus musculus of body weight 37 ± 3 gm, male were used for the study. The total five groups of experimental animals, having three replicates, each comprising five mice. All the experimental animals were housed in stainless steel cages in a room maintained at 25 ± 2°C with 12 hr day/night cycle. “Gulmuhar” (Hindustan Lever Limited, Mumbai, India) diet was the basal food for all the experimental animals. Drinking water was made available *ad libitum*. The animals used in the present study were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),
India and approved by the Institute's ethical committee.

TOXICITY EXPERIMENTS

Toxicity Tests on Experimental animal 1

Toxicity experiments were performed according to the method of (Singh and Agrawal, 1988). The fishes were exposed for 24h, 48h, 72h and 96h at four different concentrations of both tested material of J. gossypifolia. Concentrations (w/v) of J. gossypifolia crude latex and apigenin used for toxicity experiments were 10, 13, 16 and 19 mg/l, 50, 55, 65, and 80 mg/L respectively. Three aquaria were set up for each dose and each aquarium contains ten fishes in six litre of de-chlorinated tap water. Control animals were kept in similar condition without any treatment.

Mortality was recorded every 24h throughout the 96h exposure period. Fishes were considered dead if they failed to respond to vigorous poking with glass rod. Dead fishes were removed from aquaria as soon as possible in order to prevent their body decomposition.

Toxicity Tests on Experimental animal 2: An initial test was done to determine the approximate lethal and non-lethal doses of the extract according to method of (Turner, 1965). Five groups of five mice, having three replicates of each, were used in the experiments and treated with the aqueous latex extract and compound apigenin at the plant doses of 120, 150, 170, 200 mg extract /kg body weight and 1100, 1150, 1300, 1450 mg/kg body weight respectively. Single dose was administered to animal intraperitoneally (i.p.), by tuberculin syringe with needle no. 26. The control group was given an equal volume of water. The animals were observed for 24 hr for signs of toxicity including death and the number of dead mice was recorded and used in the calculation of the acute toxicity value (LD50).

RESULTS

The results are reported in Table 1 and 2. Toxicity experiments were performed by the method of (Singh and Agrawal, 1988) in case of fish and by the method of (Turner, 1965) in case of mice. Toxicity data obtained from this study was computed through POLO PLUS computer program version 2.0 of (Robertson et al., 2007). By using this program lethal concentration (LC values); upper and lower confidence limits and slope values were calculated through probit log analysis method. Table 1 and Table 2 shows the effective doses (LC10, 50, 90 Values) of dried latex powder and compound apigenin respectively, isolated from J. gossypifolia for period ranging from 24h to 96h against Channa punctatus and Mus musculus.

From present study, it was clear that toxicity of crude latex and apigenin from J. gossypifolia was time and dose dependent in case of fish. There was a significant negative correlation between LC50 values and exposure periods. Thus LC50 of crude latex and apigenin decreased from 22.334 mg/l (24h), 16.637 mg/l (48h), 12.614 mg/l (72h) and 10.490 mg/l (96h) to 21.344 mg/l (24h), 16.156 mg/l (48h), 12.143 mg/l (72h) and 10.092 mg/l (96h) respectively. On administration of both tested materials, behavioral and physicals changes were noted i.e. the skin color of the fishes became light grey. Black spots on fins were found to lose their intensity and fishes started scratching their nostril at the bottom of aquarium and frequently came at the water surface for gasping air. Within 15-30 minutes, fishes try to escape from test aquaria. After 30 minutes, their movement was slowed down, but they continue to swim near the water surface. Their after, fish shows irregular, erratic and sometimes jerky movement that was increase as exposure period increases.

At higher doses after 10-12 hours, loss of body equilibrium and hemorrhage occurred, which manifested itself as reddish color in head region and finally fishes were died. In control group there were no such changes observed, that means another factors other than the plant moieties were responsible for alterations in behavior and mortality of fish.

In case of mice, the median acute toxicity value (LC50) of crude latex and apigenin determined to be 148.34 mg/kg body weight within 95% confidence limits (148.34-165.2 mg/kg body weight) and 1218.17 mg/kg body weight within 95% confidence limits (1218.17-1294.4 mg/kg body weight) respectively after 24h (Table 1 and 2). No further mortality was observed after 24h exposure periods (Table 1 and 2).

On administration of the extract, no immediate behavioral changes were noted. The mice moved and fed normally in both case of tested materials. After twenty minutes, piloerection was noticed and the animals become restless, some trying to escape through the holes in the cages. The animals did not vomit, neither was there ptosis. The animals that received higher doses went into convulsions and died in hyperextension.

DISCUSSION

The mortality of the fish and mice was chosen as the measurable effect to determine the toxicity of the crude latex and apigenin. It is clear from the results that J. gossypifolia crude latex and compound apigenin is toxic against both freshwater fish Channa punctatus and Swiss albino mice Mus musculus, thus, the plant is piscicidal as well as muricidal respectively.

The plant J. gossypifolia may be used as a potential source of piscicides as a crude preparation of the latex as well as compound apigenin has sufficient time dependent piscicidal activity. As mentioned in result portion, the type of behavior responses indicate that both tested materials are neurotoxic, which might be active at the neuromuscular system of fish C. punctatus. Animal
Table 1. Toxicity (LC_{10,50,90} Values) of the crude latex of *J. gossypifolia* at different time intervals to the fish *Channa punctatus* and Swiss albino mice *Mus musculus*.

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Effective dose (mg/L)</th>
<th>Slope value</th>
<th>Effective dose (mg/kg body weight)</th>
<th>Slope value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC_{10} = 11.28</td>
<td>4.322±1.404</td>
<td>LC_{10} = 101.272</td>
<td>7.731±2.269</td>
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<tr>
<td></td>
<td>(6.46-13.26)</td>
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<td>(55.6-120.9)</td>
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<tr>
<td></td>
<td>LC_{50} = 22.33</td>
<td></td>
<td>LC_{50} = 148.340</td>
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<td></td>
<td>(18.46-46.14)</td>
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<td>(127.2-165.2)</td>
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<td></td>
<td>LC_{90} = 44.20</td>
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<td>LC_{90} = 217.283</td>
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<td></td>
<td>(28.68-95.55)</td>
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<td>(187.4-351.2)</td>
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<tr>
<td></td>
<td>LC_{10} = 8.278</td>
<td>4.228±1.196</td>
<td>LC_{10} = -</td>
<td>-</td>
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<tr>
<td></td>
<td>(4.029-10.364)</td>
<td></td>
<td>(4.36-8.79)</td>
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<tr>
<td></td>
<td>LC_{50} = 16.63</td>
<td></td>
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<td></td>
<td>(14.63-21.43)</td>
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<td>(10.64-14.11)</td>
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<td></td>
<td>LC_{90} = 33.435</td>
<td></td>
<td>LC_{90} = -</td>
<td>-</td>
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<tr>
<td></td>
<td>(24.36-96.68)</td>
<td></td>
<td>(19.12-38.13)</td>
<td>-</td>
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<td></td>
<td>LC_{10} = 6.87</td>
<td>4.861±1.192</td>
<td>LC_{10} = -</td>
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<td></td>
<td>(3.52-8.79)</td>
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<td>(3.52-8.79)</td>
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<td></td>
<td>LC_{50} = 12.61</td>
<td></td>
<td>LC_{50} = -</td>
<td>-</td>
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<td></td>
<td>(10.64-14.11)</td>
<td></td>
<td>(10.64-14.11)</td>
<td>-</td>
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<tr>
<td></td>
<td>LC_{90} = 23.14</td>
<td></td>
<td>LC_{90} = -</td>
<td>-</td>
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<tr>
<td></td>
<td>(19.12-38.13)</td>
<td></td>
<td>(19.12-38.13)</td>
<td>-</td>
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<tr>
<td></td>
<td>LC_{10} = 5.66</td>
<td>4.785±1.249</td>
<td>LC_{10} = -</td>
<td>-</td>
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<tr>
<td></td>
<td>(2.25-7.70)</td>
<td></td>
<td>(2.25-7.70)</td>
<td>-</td>
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<tr>
<td></td>
<td>LC_{50} = 10.490</td>
<td></td>
<td>LC_{50} = -</td>
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<tr>
<td></td>
<td>(7.70-11.98)</td>
<td></td>
<td>(7.70-11.98)</td>
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<tr>
<td></td>
<td>LC_{90} = 19.435</td>
<td></td>
<td>LC_{90} = -</td>
<td>-</td>
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<tr>
<td></td>
<td>(16.55-29.65)</td>
<td></td>
<td>(16.55-29.65)</td>
<td>-</td>
</tr>
</tbody>
</table>

- Values could not be calculated because no further mortality after 24 h.
- There was no mortality in control groups.
- Mortality was determined at every 24h.
- Regress coefficient showed that there was significant (P<0.05) negative regression between exposure time and different LC values.

Values given in parenthesis are LCL (Lower confidence limit) and UCL (Upper confidence limit) at 95% confidence limits.

Behavior is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances (Bullock *et al.*, 1977). The stressful breathing behavior exhibited by fish may be as a result of respiratory impairment due to effect of toxicant on the gills. Mortality caused by both tested materials showed a significant positive correlation between dose and mortality. It may be due to increase of extract concentration in water resulted in more intakes of their active moieties in fish body. Data also shows the significant negative correlation between LC values and exposure periods. The increase in mortality with increased exposure periods could be affected by several factors, which may be acting separately or jointly (Goodmann *et al.*, 1985). Stability (life span) of active moieties in environment and their detoxification rate in animal body also alters the mortality and exposure periods, relationship (Mitra *et al.*, 1978; Matsumura, 1985).

Statistical analysis of the data on toxicity brings out several important points. The $\chi^2$ test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep slope values. The steepness of the slope line indicates that there is a large increase in the mortality of fish with a relatively small increase in the concentration of the toxicant. The slope is, thus, an index of the susceptibility of the target animal to the tested material used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of the toxicological mechanism, yet it is a useful
Table 2. Toxicity (LC_{10, 50, 90} values) of the Apigenin extracted from leaves of J. gossypifolia at different time intervals to the fish Channa punctatus and Swiss albino mice Mus musculus.

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Channa punctatus</th>
<th>Mus musculus</th>
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<tbody>
<tr>
<td></td>
<td>Effective dose (mg/L)</td>
<td>Slope value</td>
</tr>
<tr>
<td>24h</td>
<td>LC_{10} = 49.805 (28.147-57.342)</td>
<td>6.879±2.384</td>
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<tr>
<td></td>
<td>LC_{50} = 76.485 (67.505-117.594)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LC_{90} = 117.455 (90.318-432.273)</td>
<td>-</td>
</tr>
<tr>
<td>48h</td>
<td>LC_{10} = 45.554 (26.272-52.956)</td>
<td>7.246±2.296</td>
</tr>
<tr>
<td></td>
<td>LC_{50} = 68.454 (61.362-85.383)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LC_{90} = 102.665 (83.475-236.361)</td>
<td>-</td>
</tr>
<tr>
<td>72h</td>
<td>LC_{10} = 38.344 (16.148-46.847)</td>
<td>6.981±2.310</td>
</tr>
<tr>
<td></td>
<td>LC_{50} = 58.515 (49.074-66.295)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LC_{90} = 89.298 (74.882-186.850)</td>
<td>-</td>
</tr>
<tr>
<td>96h</td>
<td>LC_{10} = 36.677 (15.732-44.790)</td>
<td>8.057±2.623</td>
</tr>
<tr>
<td></td>
<td>LC_{50} = 52.900 (41.456-58.492)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LC_{90} = 76.300 (66.946-124.651)</td>
<td>-</td>
</tr>
</tbody>
</table>

- Values could not be calculated because no further mortality after 24 h.
- There was no mortality in control groups
- Mortality was determined at every 24h.
- Regress coefficient showed that there was significant (P<0.05) negative regression between exposure time and different LC values.
- Values given in parenthesis are LCL (Lower confidence limit) and UCL (Upper confidence limit) at 95% confidence limits.

Parameter for such a study. Since the LC_{50} of both tested materials of J. gossypifolia lay within the 95% confidence limits, it is obvious that in replicate tests of random samples, the concentration response lines would fall within the same range (Rand et al., 1988; Yadav and Singh, 2006).

In case of mice, both tested materials from J. gossypifolia was found to have significant (P<0.05) nociceptive effect at all the doses tested. Although, the inhibitory effect in mice was dose-dependent, the percentage inhibition at a dose of 200 mg/kg body weight of crude latex and 1450 mg/kg body weight of apigenin was found to be highest in comparison to other doses. Morbidity increased with increasing doses in mice.

Conclusion

J. gossypifolia is classified as a medicinal plant and slightly toxic. The crude latex and compound apigenin has been shown to possess anti-coagulant activity (Oduola et al., 2005) and anti-cancerous activity respectively (Kupchan et al., 1970). However, since death occurred just after convulsions, it is postulated that the extract killed the mice by the action on the nervous system. However, because of genetic variation in response to drugs by different species, it is difficult to directly translate the results of this study to other animal species or to man (Ogwal-Okeng et al., 2003). This has been recognized as a limitation in this study. Never the less, in view of the above findings, patients receiving larger doses, or under-going prolonged medication with plant parts, should have cancerous (Kupchan et al., 1970) and coagulant functions (Oduola et al., 2005) evaluation regularly.

Findings of the study indicates that the above both tested material of J. gossypifolia at higher doses have potent piscicidal activity as well as muricidal activity. So these crude latex/compound cannot be used directly in...
water bodies or for medicinal purpose, without knowing their structural activity relationship with non-target organisms.

Apigenin is less toxicity than the crude latex. Higher toxicity of crude latex may be due to the synergistic action of several compounds present in latex powder (Horsten et al., 1996; Auvin-Guette et al., 1997). Data emerged from this study will be helpful in the development of eco-friendly drugs and pharmaceutical compounds for other purposes.

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