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ORIGINAL ARTICLE

Toxicological effect of *Ricinus communis* aqueous leaf extract on the locomotor activities in *Drosophila melanogaster*

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ABSTRACT
Plants have been used as a medicinal source since time immemorial, in both traditional and folk medicinal system as a treatment for various diseases due to the presence of various bioactive compounds of therapeutic value. In the present study, an effort has been made to investigate the various phytochemicals present in the aqueous leaf extract of Ricinus communis and to determine its effect on the locomotor activities in Drosophila melanogaster by using the dipping method. The phytochemical analysis revealed the presence of proteins, carbohydrates, phenols, tannins, saponins, flavonoids, glycosides and steroids. The results of the locomotor assay conducted on the flies that had emerged from the treated 3rd instar larvae on day 7 using the negative geotaxis test were expressed in the form of number of flies that were able to climb the 6cm mark in 6 seconds (Mean ± SEM) and Performance Index(PI) was calculated for the same. The Mean ± SEM obtained for 25%, 50% 75% and control were 5 ± 0.179399, 6.066667 ± 0.158537, 5.033333 ± 0.242038 and 5.833333 ± 0.182621 respectively and the performance indices obtained were 0.625, 0.758333, 0.629167 and 0.729167 respectively. Statistical analysis was done using ANOVA which revealed that the decreased locomotor activities in Drosophila melanogaster induced by both 25% and 75% leaf infusions were significant (p<0.05). This preliminary study paves the way for future studies to explain the exact mechanisms underlying the action of the extract at different concentrations.

Keywords: *Drosophila melanogaster, Ricinus communis, locomotory deficits, aqueous leaf extract, dipping method, phytochemical analysis*

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INTRODUCTION

Plants have been used since time immemorial in both traditional and folk medicine due to the presence of certain bioactive compounds of therapeutic value which renders it suitable to treat several disease conditions [1, 2]. These biomolecules include alkaloids, terpenoids, steroids, saponins, flavonoids, tannins, glycosides etc [3]. These plant derived compounds have been given importance in the field of agriculture, human therapy etc increasing its value in the field of scientific research and is now also gaining economic importance. Despite these beneficial effects, there is substantial evidence suggesting toxicity of these plant extracts and its derivatives which makes it important to evaluate these plant extracts for its harmful effects before utilization [4, 5]. Recently, several weed plants have been characterized for their insecticidal activities on many organisms and their phytochemical and pharmacological value has tremendously increased in the recent past [6].*Ricinus communis* a perennial herb belonging to the family Euphorbiaceae commonly called "castor plant" is a poisonous weed widely distributed in Asia, South Africa, Brazil and Russia [7]. It is an evergreen shrub which can grow up to a height of 5 m with reddish or green leaves and round deep red color fruit. It is known to possess therapeutic properties like anticancer, antidiabetic, antimicrobial, antifertility, hepatoprotective, antioxidant, antifilarial, antiasthmatic etc [3] The leaf, root and seed oil of this plant is commonly used to

treat ailments like inflammation and liver disorders. Apart from this, due to the presence of many active biomolecules, it is extensively being researched for its insecticidal, antifeedant and repellant activities. [8]*Drosophila* is a genus of small flies 2-4mm long with color varying from pale yellow to reddish brown to black, commonly called fruit flies [9] belonging to the family Drosophilidae [10]. These are dipteran insects extensively used as a model to conduct many neurodegenerative [2, 11], biological, toxicological and biomedical studies as it is cost effective, easy to culture, has rapid generation time, short life cycle and approximately 75% of genes that cause diseases in humans have functional homolog in fruit flies [4, 9, 12]. For more than 100 years it has been used as a model organism in the field of biology as it meets the standard of the European Centre for the Validation of Alternative Methods (ECVAM): Reduction, Refinement and Replacement (3Rs) of laboratory animal usage [13].The life cycle of *Drosophila* is short i.e., 10-12 days at 25ºC. It is a holometabolous insect undergoing 4 different stages of development i.e., egg, larvae (3 instars), pupa and adult. Each of these stages serves as an opportunity to conduct various studies. The fecundity of a single fertile female is about 100 eggs per day and the embryogenesis lasts only for 24 hours. The larvae of *Drosophila melanogaster* are considered to be voracious feeders [9, 14, 15].

In the present study an effort has been made analyse the toxic effect of *Ricinus communis* aqueous leaf extract on the locomotor activity in *Drosophila melanogaster.*

MATERIALS AND METHODS

Collection and preparation of *Ricinus communis* leaf extract

The leaves of *R.communis* were collected from natural population growing in wasteland of Mysuru city in the month of April 2021. To make cold aqueous leaf extract, these leaves were washed with running tap water twice to remove all the dust particles, cut into small pieces and air dried for 2 days; post which they were oven dried at 30 degrees for 20 minutes. These dried leaves were subjected to mechanical grinder to obtain a fine powder which was passed through a sieve [6, 16]. A total of 50g of leaf powder was obtained.

Cold extraction and filtration of aqueous plant sample:

The aqueous extract of dried leaves was made using distilled water. The powdered plant sample of about 50 grams was taken and mixed with 500ml distilled water (1:10 w/v ratio) in a 1L sterile beaker covered with silver foil and was subjected to magnetic stirring at 250rpm for 24hrs. This mixture was subjected to filtration twice using muslin cloth followed by filtration using Whatman filter paper twice. The final obtained crude extract served as stock and from this 25%, 50% and 75% solutions were prepared for.

Phytochemical screening of *Ricinus communis* leaves:

Phytochemical screening of *R.communis* cold aqueous leaf extract was carried out to determine the presence of several metabolites using the meyhod described by Yadav and Argarwala [5].

Collection and rearing of *D.melanogaster*:

The wild type flies stock of *D. melanogaster* were obtained from NCBS (National Center for Biological Sciences) Drosophila fly base.. The flies were reared in culture bottles which were 15cm in length and 4 cm in width at 28 ± 2º celsius temperature and 60% relative humidity.

Preparation of culture media:

To 500ml of distilled water, 50g of jaggery was added and stirred until it had dissolved completely. To this, 50g of suji previously mixed with 5g of Agar-Agar was added slowly with constant stirring to avoid lump formation. Once the slurry was formed 3.5ml of propionic acid was added and stirred for a minute. This slurry was poured into dry culture bottles and covered with a wet cloth overnight. The next day all the moisture content built up in the bottles were removed using glass rod wrapped with blotting paper. Prior to introduction of flies, to each of these culture bottles with media, 3 granules of dried yeast was added.

Experimental design:

A total of 180 3rd instar larvae of *D.melanogaster* were used in this experiment. Larvae used for each concentration were divided into 3 groups of 15 each along with a 4th group used as control with 15 larvae as well. The dipping method was used to treat the larvae [7, 17–19]. Fifteen 3rd instar larvae were treated by dipping them in 25%, 50% and 75% aqueous extracts of *Ricinus communis* for 60 seconds in a petri dish. The treated larvae were then transferred to culture bottles with rearing media prepared as mentioned above. For control, larvae were treated with water. The negative geotaxis test was conducted to assess the effect of *Ricinus communis* aqueous leaf extract on the locomotor activities in *Drosophila melanogaster* which had developed from treated larvae on day 7.

Negative geotaxis test :

The negative geotaxis test was conducted on day 7 with the flies that had emerged from treated larvae. 8 flies were taken for both "extract exposed" and "control" in each replicate and subjected to brief anesthesia for about 2-5 minutes and transferred to a culture bottle with a marking at 6cm. A recovery period of about 30 minutes was provided post which the flies were gently tapped to the bottom of the bottle. After 6 seconds the number of flies that crossed the 6cm mark was noted down along with the flies that had remained below the 6cm mark. This experiment was repeated 10 times at 1 min intervals. The results were expressed in the form of number of flies that were able to cross the 6cm mark in 6 seconds (Mean ±SEM). The Performance Index was calculated for the same using the formula $1/2[(\boldsymbol{n}_{tot} + \boldsymbol{n}_{top} - \boldsymbol{n}_{bot})/\boldsymbol{n}_{tot}]$. Statistical analysis of the performance indices was conducted using ANOVA[11, 13]

RESULTS AND DISCUSSION

Many different methods for phytochemical extraction are adapted. The effectiveness of the extraction methods employed depends on a variety of factors including the chemical nature of the phytochemicals present, particle size of the same, the solvent used etc [13]. The present phytochemical analysis of *Ricinus communis* leaves was conducted using cold extraction method which confirmed the presence of Proteins, Carbohydrates, Phenols, Tannins, Flavonoids, Saponins, Glycosides and Steroids as represented in Table 1. These phytocompounds were found to be responsible for the antibacterial activity of *Ricinus communis* leaf extract [3]Usage of plant extracts in medicine is common, but there is upcoming evidence on the potential toxicity of these plant extracts. Therefore it has become important to check the safety of these plant extracts. There is evidence for the potential insecticidal activity of *Ricinus communis* leaf extracts against *Musca domestica* where dipping method was used to evaluate the toxicity of *Ricinus communis* leaf extract on 3rd instar larvae of *Musca domestica*[7]. The studies revealed that the leaf extract caused larval mortality and developmental anamolies in the life cycle of the fly. It prolonged the pre-pupation period and adult emergence period. A study by Aouinty et al., [20] on the toxicity showed that the aqueous leaf extract of *Ricinus communis* was the most effective extract against *Culex pipiens* larvae which suggests that the aqueous extract of *Ricinus communis* is a potential insecticide. Both the essential oil and leaf extracts of *Ricinus communis* have been found to be toxic against *S.oryzae* and *T. castaneum* where it induced adult mortality and also inhibited the development of eggs, larvae and pupae in *S.oryzae* [21]. These insecticidal properties could be attributed the various biomolecules present in the leaf extract of *Ricinus communis.* It was also found that the total phenolic, condensed tannins and falvonoid content in the leaf extract of *Ricinus communis* was more than that of what was found in the roots [22]. Even if the concentrations at which the extracts brought about mortality was high it was found that at lower concentrations it induced certain locomotor deficits. The results obtained for negative geotaxis test conducted are presented in Table 2, 3, 4 and 5. The number of flies that were able to cross the 6cm mark in 6 secs were expressed in the form of Mean ±SEM. The value obtained for control was 5.8333 ± 0.182621, 25 % leaf extract was 5 ± 0.1793, 50% leaf extract was 6.066 ± 0.1585 and 75 % leaf extract was 5.0333 ± 0.2420. Table 6 represents the Performance Index calculated for the same and Figure 5 is the graphical representation. The Performance Index was reduced by 10.41% for the flies that had developed from larvae treated with 25% *Ricinus communis* leaf extract and by 10.01% for the flies that had developed from larvae treated with 75% *Ricinus communis* leaf extract. The statistical analysis showed that this decrease in locomotor ability for both these concentrations was significant ($p<0.05$). In an analogous study, *E. unifloria* leaf derived essential oil was able to induce mortality and locomotor deficits along with signs of oxidative stress in *Drosophila* [2]. These results are similar to the results obtained in the present study. However our assessments were not able to indicate that this effect was concentration dependant as seen in the previous studies. No linear trend was observed. There was slight raise in the Performance Index of flies whose larvae were treated with 50% leaf extract. Among the three concentrations, 25% was found to be the most effective.These neurological deficits which cause locomotor impairments have been attributed to the inhibition of the activity of acetylcholinesterase. Acetylcholine is an excitatory central nervous system transmitter involved in the signal transfer in the synapses which undergoes hydrolysis to give choline and acetyl group once it gets delivered at the synapse. This reaction is catalysed by Acetylcholinesterase. It also acts as a neuromodulator within the CNS [23–25]. Many plant extracts have been explored to identify the bioactive compounds present in them which could affect the acetylcholinesterase activity. For example, the bark powders of *Salix alba* (L.) was shown to have significant anti acetylcholinesterase activity [26]. In a study conducted on the Anticholinesterase property of plants from north eastern Brazil, *Citrus limonum, Ricinus communis* and *Swnna occidentalis* were found to be the most promising in terms of anticholinesterase activity [27].

Ricinus communis aqueous leaf extract in the present study showed significant results in terms of locomotory deficits when compared to that of previous work on *Drosophila melanogaster*'s climbing assay

on treatment with different other plant extracts. In a study done by Alexander et al., [13] on *Drosophila melanogaster* using cold aqueous leaf extract of *Mangifera indica* where the negative geotaxis test was conducted on flies treated with the leaf extract, showed only a slight and non significant decrease in both the climbing assay and acetylcholinesterase activity. Similarly a study on toxicity of *Azadirachta indica* hydroethanolic leaf extract in adult *Drosophila melanogaster* byTochukwu et al., [4] also revealed that the leaf extract was not able to induce any significant decrease in the climbing properties and acetylcholinesterase activity. Therefore in comparison to these pervious works, it could be concluded that *Ricinus communis* aqueous leaf extract serves as an excellent source for insecticidal biocompounds. Since the activity of inhibition of acetylcholinesterase activity has been recognized to be a human biological marker of pesticide poisoning and an indicator of poor locomotor activity [13], the decrease in locomotor activities of *Drosophila melanogaster* in the present study could be attributed to the anti acetylcholinesterase activity of *Ricinus commmunis* aqueous leaf extract.

Figure 1: Drying of leaves Figure 2: Dried leaves

Figure 3: Experimental setup of trial bottles Figure 4: Culture bottles for Negative Geotaxis

Figure 5 : Graph representing the Performance Index

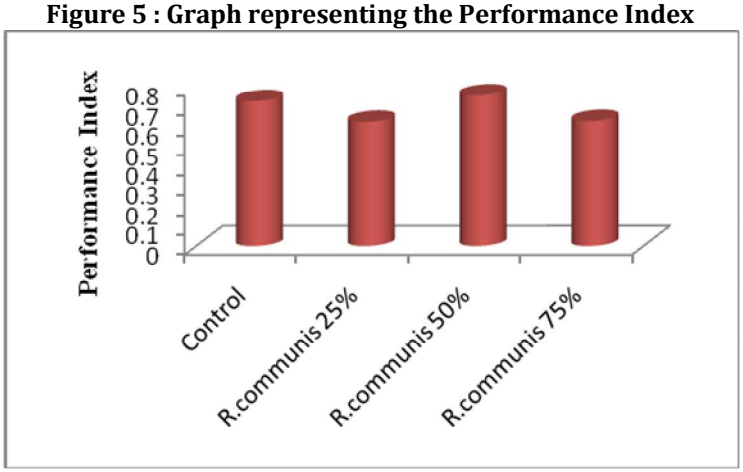

Table 1: Phytochemical analysis of *Ricinus communis* aqueous leaf exract

Plant	*Ricinus communis*
Proteins	Present
Carbohydrates	Present
Phenols/ Tanninns	Present
Flavonoids	Present
Saponins	Present
Glycosides	Present
Steroids	Present

Table 2 : Results of negative geotaxis test for control

Trial	Flies above 6 cm mark (n_{top})			Mean
1	6	5	5	5.33333
2	6	5	6	5.66667
3	5	6	6	5.66667
4	5	7	5	5.66667
5	5	6	7	6
6	5	8	6	6.33333
7	6	7	5	6
8	5	8	4	5.66667
9	6	7	4	5.66667
10	6	7	6	6.33333

Table 3 : Results of negative geotaxis test for 25% leaf extract

Trial	Flies above 6 cm mark (n_{top})			Mean
1	4	5	5	4.66667
2	5	5	4	4.66667
3	5	7	5	5.66667
4	5	7	5	5.66667
5	5	5	4	4.66667
6	4	5	5	4.66667
7	5	6	5	5.33333
8	5	7	5	5.66667
9	6	5	2	4.33333
10	4	5	5	4.66667

Table 4 : Results of negative geotaxis test for 50% leaf extract

Trial	Flies above 6 cm mark (n_{top})			Mean
1	5	4	7	5.333333
2	7	6	6	6.333333
3	5	4	6	5
4	6	7	7	6.666667
5	6	7	6	6.333333
6	6	7	6	6.333333
7	6	6	6	6
8	7	5	6	6
9	6	7	7	6.666667
10	5	6	7	6

Table 5 : Results of negative geotaxis test for 75% leaf extract

Trial	Flies above 6 cm mark (n_{top})			Mean
1	7	7	3	5.666667
2	7	5	4	5.333333
3	6	5	7	6
4	4	3	5	4
5	7	5	5	5.666667
6	5	5	4	4.666667
7	6	4	6	5.333333
8	4	3	5	4
9	6	3	4	4.333333
10	7	5	4	5.333333

Table 6 : Performance Index for negative geotaxis assay conducted

Trial	Performance Index
Control	0.72925
25%	0.625
50%	0.758333333
75%	0.629166667

CONCLUSION

After evaluating the results of toxic activity of the aqueous leaf extracts of *R.communis* against*D. melanogaster'*s locomotor abilities*,* it can be concluded that the leaf extract of *R.communis* has certain bioactive compounds in it which are capable of inducing locomotor deficits in the adult flies of *D. melanogaster* whose larvaehad been treated with the plant extract*.* Since these leaf extracts are used in traditional medicine, it is very important to screen them for toxicity.It can also be suggested that further investigation is needed to detect the specific compound which is responsible for showing this sort of effect. This preliminary study paves the way for future studies to explain the exact mechanisms underlying the action of the extract at different concentrations.

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COMPETING INTERESTS

The authors state that no competing interest exists.

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