

Original Article

FECUNDITY STUDIES OF ANDROGRAPHIS PANICULATA ON DROSOPHILA MELANOGASTER

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ABSTRACT

Objective: *Andrographis paniculata* is widely used in Asia for various medicinal purposes. The plant has a major bioactive chemical constituent Andrographolide, which exhibits various essential pharmacological properties. Recently, to enhance immunity against viral fevers especially dengue viral fever during monsoon season, Tamil Nadu state government has advised rural health centres to provide a tea or kashayam made from a mixture containing this plant leaf along with selected plant roots and leaves. However, there is concern among the general public population that this plant extract may have a negative impact on potency. This study is being done to investigate the toxic effects of *Andrographis paniculata* on fecundity and physiological properties of *Drosophila melanogaster*.

Methods: The flies were treated and mated in pure and mixed form of *Andrographis paniculata* separately.

Results: The results were obtained in the form of egg count, which was compared to control and the conclusion was obtained that *Andrographis paniculata* might affect the fecundity and at the same time, it was observed that the developmental span from an egg to an adult fly increased upon treatment in successive generations. More importantly, the effect of pure form plant on the fecundity was found to be significant, while that of the mixed form was not. On mating a treated male with an untreated female and an untreated male with a treated female when the treatment is being discontinued, it can be very well seen that there is no effect on the fecundity of the flies.

Conclusion: Since the continuity of the treatment played a major role on the effect of fecundity, highlighting the importance of the period of treatment and the exposure time of the compound on the fly system, therefore this research suggests that the conception of tea or kashayam extracted from mixed plant content could not have the same effect as the similar concentration of pure *Andrographis paniculata* and it does not have any acute effect on the fecundity when being consumed over a short period of time in *Drosophila melanogaster* model.

Keywords: Andrographolide, Acute effect, Continuity, Developmental span, Fecundity, Nilevembu

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INTRODUCTION

Since time immemorial, herbal remedies have been utilized in the majority of the human races as a source of medication [1, 2]. Majority of the world's population still depends upon medicinal herbs for basic health care needs [3-5]. Although phytochemicals are a significant part of food source and they're usually thought of as natural and comparatively harmless, phyto-preparations don't seem to be completely free from toxicity [6, 29]. Presently very little data is accessible on the fruitful effects of popular medicinal plants. There has been growing interest over the safety of natural products in clinical use and on the analysis of its effects on the system has been thought of as a part of the safety studies of widely used medicative plants [7].

During the past few decades, the autochthonal or ancient system of medicine has gained importance in the field of medicine. In most of the developing countries, majority people depend on traditional practitioners, who in turn are dependent on medicinal plants, to meet their primary healthcare needs [3, 5, 6]. In both underdeveloped and developed countries plant-based medicines still play an important role in the primary healthcare because of their reduced side effects and their availability in the nature [3, 10, 30]. The amount and kind of food consumed are a fundamental determinant of human wellbeing [11]. Overall transformation for the change of patient wellbeing is picking up energy; henceforth the subject of drug safety turns out to be considerably more conspicuous in the present day situation. Development of restorative plants with research facility produced species is being endeavoured based on concoction creation and is probably going to be utilized as a part of the expanded way for a business reason. These progressions may have a significant effect on the wellbeing and viability of the Ayurveda medicines in the market.

Andrographis paniculata [Burm. F] Nees, is also known as "King of Bitters" belonging to family Acanthaceae is usually used in Ayurveda

and homeopathy medicine as well as in tribal medicine in India, Southeast Asia and tropical and subtropical Asia, West Indies (including Jamaica, Barbados, and the Bahamas), and elsewhere within Europe. In India, *Andrographis paniculata* (*A. paniculata*) is known as "Kalmegh"; in China as "Chuan-Xin-Lian"; in Thailand as "Fah Tha Lai"; in Malaysia as "Hempedu bumi"; in Japan as "Senshinren"; and in Scandinavian nations as "green chiretta" [12].

The plant is primarily found in the plains throughout India from Himachal Pradesh to Assam and Mizoram, West Bengal, and all over South India. For centuries the aerial parts, roots and whole plant of *A. paniculata* have been used in Asia as a traditional medicine for the treatment of various ailments. The major chemical constituents present in *A. paniculata* are andrographolide, neoandrographolide, isoandrographolide, deoxyandrographolide, homoandrographolide, andrographin, panicolin, 14-deoxy-11, 12-didehydroandrographide, chlorogenic acid, myristic acid, andrographiside, andropanoside, etc. [13, 28]. The variety of doses that can be administered are as follows: powder [1-3 g], juice [5-10 ml], boiling [20-40 ml], and liquid [0.5-1 ml] [14]. Some proprietary medicines of *A. paniculata* also are accessible in the market to cure numerous diseases. The plant possesses a broad range of various pharmacological properties like antimalarial, [13, 15, 31, 33], anti-inflammatory [13, 16, 17], anthelmintic [18], antihyperglycemic [19], antihepatitic [13, 17, 20], antioxidant [13, 17, 21], anticancer [12, 13], antipyretic [22], and antitumor, antidiabetic [16, 17, 27], immunomodulatory [13, 16, 17], sex hormone modulatory [17], hepatoprotective activity [17, 32] and insecticidal activities [17]. Because of the impressive variety of biological activities exhibited by *A. paniculata*, researchers attempted numerous times to structurally modify Andrographolide. Various *A. paniculata* derivatives have emerged in recent times and their pharmacological activities have been evaluated thoroughly by researchers. However, very fewer studies have comprehensively analysed *A. paniculata* and its derivatives. We are

performing this study to understand the effect of *Andrographis paniculata* on the fecundity of the flies and therefore get a better understanding on how it gets affected. This will help us in addressing the general concern of the public on whether the plant has a negative effect on the potency and consequently whether it can be consumed for treating various ailments.

MATERIALS AND METHODS

Preparation of food

A powder of the leaves of *Andrographis paniculata* and a powdered mixture of *Andrographis paniculata* and various other medicinal plant parts were obtained commercially from a local vendor Deva

Dry Fish Shop, Andhra Pradesh. The treatment food was prepared with the contents mentioned in table 1. Two treatment groups were made namely 'Pure' and 'Mixed' in which the pure *A. paniculata* leaf powder and the mixed powder consisting of various medicinal plant parts along with *A. paniculata* plant parts were added respectively in the treatment groups.

Fly strain

The Oregon K strain of *Drosophila melanogaster* was used as an animal model for our studies and was obtained from National Institute of Health and Neurosciences (NIMHANS), Bangalore. They were stored in a sterile environment inside an incubator at a constant temperature of 25 °C.

Table 1: Contents used for the preparation of food for the treatment of *Drosophila melanogaster*

Ingredients	Per 1000 ml
Cornflour	80 g
D-glucose	20 g
Sugar	40 g
Agar	8 g
Yeast	15 g
Propionic acid	10 ml
Ops (orthophosphoric acid)	1 ml
Tego	1 g methylparaben in 5 ml ethanol
<i>Andrographis paniculata</i> powder	According to dosage

Treatment

The male and female flies were subjected to treatment separately for 2 w with pure and mixed *Andrographis paniculata* leaf powder added to their food in a sterile environment at 25°.

Chemicals and reagents

Agar-agar type I was procured from HiMedia, Mumbai. D-glucose, yeast extract powder were purchased from SRL Chemicals, Mumbai. Propionic acid was purchased from Merck Life Sciences, Mumbai while methyl para hydroxy benzoate was purchased from Rankem, New

Delhi. Orthophosphoric acid was procured from Thermo Fischer Scientific, Mumbai. All the chemicals used were of analytical grade.

RESULTS

Studies have shown that Andrographolide (a phytoconstituent of *A. Paniculata*) induced toxicity is not relatively sex-specific. Studies on mice models have confirmed that Andrographolide has no observed adverse effects on male Wistar rats and their fertility [23], but this study is being done to check whether continuity of the treatment has any effect on the fecundity of the fly and to check its effect on the different stages of the life cycle.

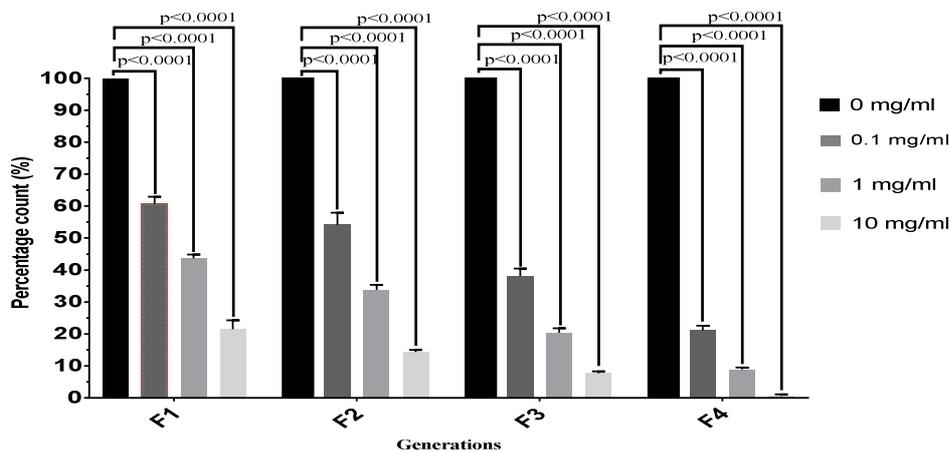


Fig. 1: Graph showing egg count of flies treated with pure *Andrographis paniculata* leaf powder in generations F1-F4 expressed in terms of a percentage along with p values, n=3, the data is presented as mean±SD

In our study, we found a significant decrease in the number of eggs laid by *Drosophila melanogaster* which had been treated for two weeks and then mated in the same treatment food to avoid discontinuous treatment. Mating of a treated male fly with a treated female fly showed a lesser egg count which indicates that *A. paniculata* could be selectively affecting the flies. For the concentration of 0.1 mg/ml [fig. 1, fig. 2] the mating of F1 generation showed a decrease of 10.90% for pure *A. paniculata* and 14.49% for

mixed *A. paniculata* when compared to the control i.e. 0 mg/ml concentration in the F2 generation egg count. Further, it showed a decrease of 29.59% and 44.92% in the F3 and F4 generations respectively for pure *A. paniculata* and a decrease of 19.49% and 32.63% in the F3 and F4 generations respectively for mixed *A. paniculata*. A decrease of 39.22% for pure *A. paniculata* and 23.75% for mixed *A. paniculata* was observed in the F1 generation egg count when compared to the control.

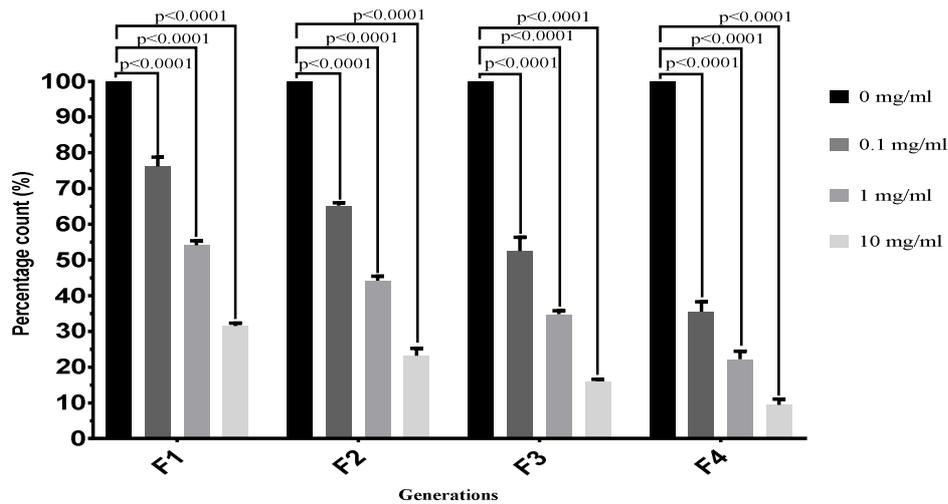


Fig. 2: Graph showing egg count of flies treated with mixed *Andrographis paniculata* leaf powder in generations F1-F4 expressed in terms of percentage along with p values, n=3, The data is presented as mean±SD

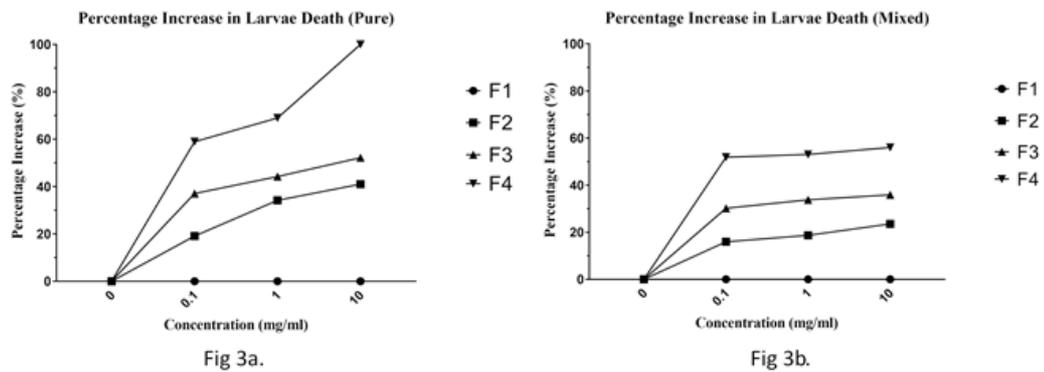


Fig. 3: Graph of percentage increase in larvae death for pure (fig. 3a.) and mixed (fig. 3b.) *Andrographis paniculata* with respect to generation F1-F4 expressed in percentage

For 1 mg/ml [fig. 1, fig. 2] concentration of the pure and mixed treatment groups of *A. Paniculata*, the decrease was found to be 22.78% and 18.36% respectively for the F2 generation when compared to the control. There was also a further decrease of 39.34% and 56.75% in the egg count of F3 and F4 generation respectively for the flies treated with 1 mg/ml concentration of pure *A. paniculata*, and a decrease of 21.25% and 36.50% in F3 and F4 generation of flies respectively, treated in 1 mg/ml concentration of mixed *A. paniculata*. A decrease of 57.06% for pure *A. paniculata* and 46.73% for mixed *A. paniculata* was observed in the F1 generation egg count when compared to the F0. For 10 mg/ml [fig. 1, fig. 2] concentration a decrease of 36.33% for pure *A. paniculata* and 26.31% for mixed *A. paniculata* was observed in the F2 generation egg count. Furthermore, it showed a decrease of 46.15% and 92.85% in the F3 and F4 generations respectively for pure *A. paniculata* and a decrease of 30.95% and 41.37% in the F3 and F4 generations respectively for mixed *A. paniculata*. A decrease of 78.57% for pure *A. paniculata* and 68.68% for mixed *A. paniculata* was observed in the F1 generation egg count when compared to the F0.

Also, we found a notable increase in the percentage death of larvae in *Drosophila melanogaster* which developed from the eggs laid by the flies. For 0.1 mg/ml [fig. 3] concentration of the pure and mixed, the increase was found to be 19.09% and 15.94% respectively for the F2 generation. Also, a further increase of 37.07% and 58.92% in larval death of F3 and F4 generations respectively for pure *A. paniculata*, and an increase of 30.17% and 51.85% in F3 and F4 generation of larvae for mixed *A. paniculata*. For 1 mg/ml [fig. 3] concentration of F1 generation larvae, it showed an increase of

34.17% for pure *A. paniculata* and 18.68% for mixed *A. paniculata* in the F2 generation larvae death. Further, it showed an increase of 44.23% and 68.96% in the F3 and F4 generations respectively for pure *A. paniculata*, and an increase of 33.78% and 53.06% in the F3 and F4 generations respectively for mixed *A. paniculata*. For 10 mg/ml [fig. 3] concentration an increase of 41.02% for pure *A. paniculata* and 23.52% for mixed *A. paniculata* was observed in the F2 generation larvae death. Furthermore, it showed an increase of 52.17% and 100% in the F3 and F4 generations respectively for pure *A. paniculata*, and an increase of 35.89% and 56% in the F3 and F4 generations respectively for mixed *A. paniculata*.

On the other hand, we observed that there was no decrease in the egg count when we mated treated male flies with untreated female flies [fig. 4a, fig. 4c] and treated female flies with untreated male flies [fig. 4b, fig. 4d]. The treatment was continued for 2 w and then discontinued for a period of time before they were mated. This was done for only 1 generation since we did not observe any decrease in the egg count after treatment. We did not continue with the next generations since there was no chronic effect on the fecundity of either male or female *Drosophila melanogaster* after they were separately treated and mated with an untreated fly of the opposite sex.

The flies were transferred back to normal untreated food where they were allowed to feed and mate. The results obtained indicate that the continuity of the treatment played a major role; also giving us a clear understanding as to how the leaf powder might affect the fecundity of the flies when being treated continuously for over a period of time.

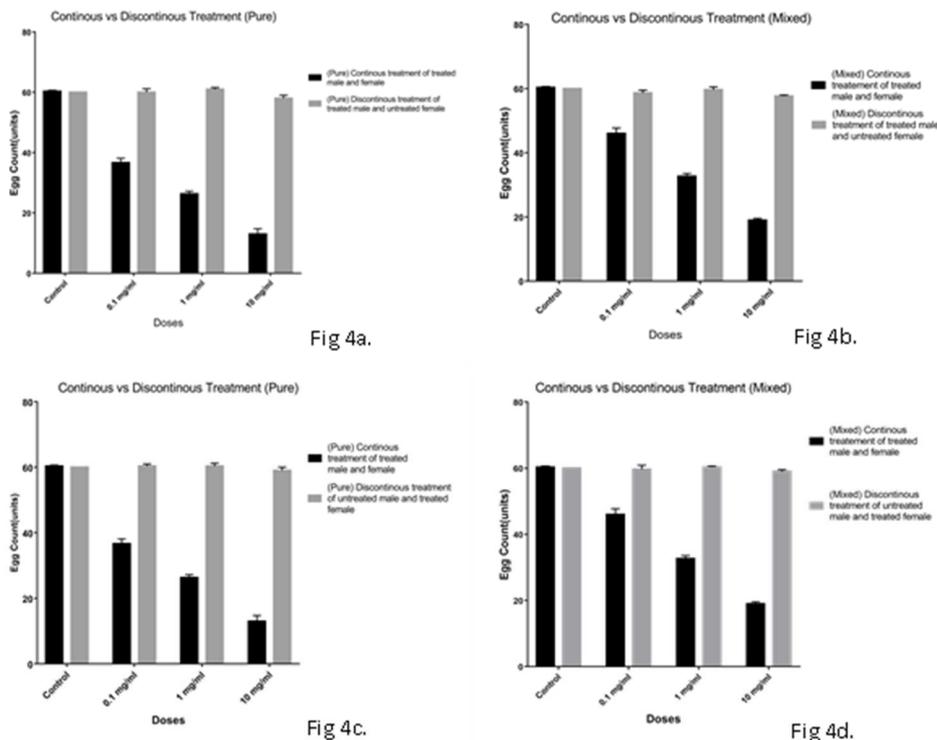


Fig. 4: Graph of egg count for continuous treatment of treated male and female in comparison with discontinuous treatment of 4a.treated male with pure *Andrographis paniculata* and untreated female, 4b, treated male with mixed *Andrographis paniculata* and untreated female, 4c.untreated male and treated female pure *Andrographis paniculata*, 4d, untreated male and treated female with mixed *Andrographis paniculata*, the data is presented as mean± SD where n=3

DISCUSSION

From our study, we have observed that the fecundity of the healthy *Drosophila melanogaster* flies have been affected after the treatment of the flies using *Andrographis paniculata* leaf powder. It can be safely concluded, from the results obtained that the egg counts and the larvae count that have been recorded for 4 generations have significantly decreased. There was a very significant decrease in the egg count and larvae count of flies treated with the pure form of *Andrographis paniculata* than the flies treated with the mixed form of *Andrographis paniculata*. The obtained results were as follows because of the presence of only 11.1% of *Andrographis paniculata* in the mixed form whereas the pure form consisted of 100% of *Andrographis paniculata*.

It was also observed that there was no effect on the fecundity when the flies were mated after treatment was discontinued. When a treated male and an untreated female or a treated female and an untreated male were mated in normal food which had no trace of *Andrographis paniculata*, there was no reduction in the egg count. Therefore it can be inferred that there was no effect on the fecundity of the flies due to discontinuous treatment. Fecundity was only affected when the *Andrographis paniculata* leaf powder was being consumed throughout the life span of the flies. From the data obtained, it can also be seen that the *Andrographis paniculata* leaf powder had a more rigorous effect on the adult and larval stages of the fly which was continuously treated with *Andrographis paniculata* leaf powder rather than on adults who were just treated for a short span of time and discontinued. It can be seen from the data that the larval stage had more adverse effects when compared to the adult stage. Due to the comparison study between the continuous and discontinuous treatment of flies, we can finally observe that the fecundity was being affected because of the continuous exposure to the flies. This can be confirmed from the mating data of continuous treatment of treated male and female and discontinuous treatment of treated male and untreated female and untreated male and treated female.

There was another significant development that was observed specifically in the 10 mg/ml concentration treated flies. The life span of the flies, which is 8-9 d for normal *Drosophila melanogaster*,

gradually increased up till 18 d in the F4 generation of the 10 mg/ml treated flies. The advised dose by various commercially available powdered form of *Andrographis paniculata* mixtures was to be 5-10 g of powder mixed along with water per day [24, 25]. To confirm that, the fly dose in mg/ml was converted to its human equivalent dose in mg/kg to check the amount of leaf powder safe for human consumption [Equation 1, 2, 3] [26]. It was found that 0.1 mg/ml dose was equal to 0.177 mg/kg of human equivalent dose. This dose when being administered to a human will be equivalent to 10 g which is considered safe by various commercial vendors [24]. Therefore, 1 mg/ml and 10 mg/ml doses will be equivalent to 100 g and 1000 g respectively which might be lethal to the human system and might in turn, have a negative impact on the kidneys and liver.

$$BSA (m^2) = \frac{\sqrt{\text{height (cm)} \times \text{weight (kg)}}}{60} \quad (1)$$

$$Km = \frac{\text{weight (kg)}}{BSA (m^2)} \quad (2)$$

$$\text{Human equivalent dose (mg/kg)} = \text{fly dose (mg/kg)} \times \frac{\text{fly } Km}{\text{human } Km} \quad (3)$$

CONCLUSION

From the results it can be safely inferred that the active compound Andrographolide present in *Andrographis paniculata* does not have any major effect on the potency or fecundity since we did not observe any permanent damage that was caused to the fly and therefore comes to a conclusion that's it is safe for consumption during treatment of various ailments however a continuous long term consumption might have negative effect on the fecundity of *Drosophila melanogaster*.

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AUTHORS CONTRIBUTIONS

Hindol Nag-Author, Research and experimentation, Suneeti Madhavan-Research and experimentation, Abhinav Chatterjee-Research and experimentation, Pallavi Dan-Research and experimentation, Mahasweta Bhattacharya-Research and experimentation, Zaid Ali Nawaz-Research and experimentation, Sahabudeen Sheik Mohideen-Corresponding Author, Research and experimentation.

CONFLICTS OF INTERESTS

Declared none

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