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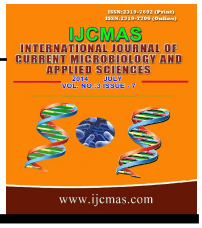


Original Research Article

Effect of *Glycyrrhiza glabra* Root Extract on Behaviour and Fitness of *Drosophila melanogaster* and Vestigial wing Mutant

G.Suchitra[1] and V.Shakunthala[2*]

[1]Department of Zoology Maharani's Science college for Women JLB road Mysore, India
[2]Department of Chronobiology Laboratory Department of Studies in Zoology University of Mysore, Manasagangotri, Mysore-570006 Karnataka, India
**Corresponding author*

A B S T R A C T

The fruit fly *Drosophila* has been extensively important organism not only for genetic, molecular, developmental studies but for also medicinal science, because of similiarility in pharmacological and physiological properties common to humans. Liquorice extracts have been used for more than 60 years in Japan to treat chronic hepatitis, and also have therapeutic benefit against other viruses, including human immunodeficiency viruses (HIV), cytomegalovirus (CMV) and Herpes simplex. Deglcyrrhizinated liquorice (DGL) preparations are useful in treating various types of ulcers, while topical liquorice preparations have been used to sooth and heal skin eruptions such as psoriasis and herpetic lessions and it has aphrodisiac property etc. Thus the present study is focused on the Aphrodisiac property of *Glycyrrhiza glabra* root extract on courtship behaviour and fitness of *D. melanogaster*. To examine this, *D. melanogaster* flies were treated with aqueous root extracts of *Glycyrrhiza glabra* by adult feeding method along with control. Mating latency and copulation duration was observed and the same flies were used to check their egg laying ability and fertility. The result showed that there is decrease in the mating latency and increase in copulation duration in treated groups compared to control and flies have showed increased fecundity and fertility in treated groups compared to control flies.

Keywords

Aphrodisiac; *Glycyrrhiza glabra;* *Drosophila melanogaster*; vestigial wing mutant; mating latency; Copulation Duration; fitness

Introduction

Ayurveda literally 'science of life' is based on twin principles of wholeness and balance. Ayurveda is the most ancient medicinal system and it is 5,000 years old Indian method of healing that includes diet, natural therapies. The current aim of Ayurvedic medicine is to identify a plant with antidoshas and understand the medicinal properties using several new techniques available such as Panchakarma, Rasayana, Bhasmas and Aphrodisiacs. Our current interest of study is to analyze the Aphrodisiac property of *Glycyrrhiza glabra* on behavior and fitness of *Drosophila melanogaster* and vestigial wing mutant.

Aphrodisiac are substances that stimulate or increases sexual desire and performance. According to Sushruta, sexuality and reproduction are so vital in Ayurveda that an entire discipline, known vajikarana, is dedicated in enhancing fertility and rejuvenation of sexual and reproductive energy. Vajikarana therapy improves the function of reproductive organs and vitalizes reproductive tissues, increasing semen count and strengthening their motility and making the eggs more viable for conception. This not only enhances the quality and longevity of individual life, but also the health and vitality of offspring. Infertility is one of the most serious social problems facing advanced nation, although it is generally not well recognized that number of infertile couples is rising in these countries. *Glycyrrhiza glabra,* Linn belongs to the family leguminaceae is a genus of perennial herbs and under shrubs distributed in the subtropical and warm temperature regions of the world. The roots are unearthed in the autumn season. It is grown in India, Spain, Iran, Russia, China and Italy. A number of components have been isolated from licorice, including water soluble, biologically active complex that account 40-50 percent of total dry material weight.

This complex is composed of triterpene, saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances (Obolensteva, et al ;1999). Glycyrrhizin a tritepenoid compound accounts for the sweet taste of licorice root. This compound represents a mixture of potassium calcium magnesium salts of glycyrrhizic acid that varies within a 2-25 percent range (Yamamura, et al; 1992). The isoflavones glabridin and hispaglobridins A and B have significant antioxidant activity (Vaya J et al; 1997) and both glabridin and glabrene possess estrogen like activity (Tamirs, et al 2001). There are numerous reports of aphrodisiac activity attributed to plants (,Tajuddin A.S et al 2004 and Zheng B.L et al; 2000). Phytochemical analysis of *G. glabra* root extract showed that it contains saponin triterpenes (glycyrrhizin, glycyrrhetimic acid and liquirtic acid), flavonoids (liquirtin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch, choline, phytosterols and bitter principles(Snow 1996; Fukai J et al 1998 and Arystanova T and Irismetov Sophekova A 2001). Thus the extract has been used for the treatment of different diseases such as Addison's disease, bronchitis, cough, arthritis, rheumatism, hypoglycemia, inflammatory and allergic conditions(Chatterjee 1996) and gastric ulcer (Alkofahi A and Atta A 1999 and Khayyal M 2001). Cerebral ischemia (Zhan C and Yong J 2006) and antioxidant capacity towards LDL oxidation (Vaya J et al; 1998). The aphrodisiac property is not well studied. In view of this, the effect *G. glabra* root extracts are used to study the behavior and reproductive ability of *Drosophila melanogaster* and vestigial wing mutant

Materials and Methods

Drosophila melanogaster and vestigial wing mutant were obtained from Drosophila stock center university of Mysore, Manasagangotri, Mysore. The isogenic culture of these flies was maintained under standard wheat cream agar media (Hegde S.Nand Krishnamurthy N.B 1979 and Guruprasad B.R et al; 2008). *Glycyrrhiza glabra root* were collected from local panchasara store Mysore, Karnataka. Then they were shade dried and milled into coarse powdered by mechanical grinder. The coarse powder root material was extracted with water by decoction using round bottom flask. The water was evaporated into semisolid mass. The semisolid mass were dried and stored for future and the aqueous

extract of *Glycyrrhiza glabra* were used for present studies (Dhingra D and Sharma A; 2008).

Drosophila melanogaster (Organ K strain) stocks and vestigial wing mutant were used for the present experiment. Then flies were maintained at 22 ± 1^0C and 75% relative humidity in 30 ml culture bottles containing wheat cream agar medium. For treated groups or experimental groups two concentration were taken viz, 1.0mg/100ml (0.01mg/ml) and 2.0 mg/100ml (0.02mg/ml) mixed in wheat cream agar medium mentioned as T1 and T2 respectively. In order to fix the concentrations of root extract of *Glycyrrihiza glabra* LC 50 was determined using log dose probit method. The lethal concentration for this test is 3.0mg/100ml (0.03mg/ml). The sub lethal (Effective concentration) concentration is T1and T2 as mentioned above. Bachelor male and virgin female were collected in the early morning and aged for 3 days and the flies were starved for 8 hours and fed for 40 hours. Sexual behavior was studied in the morning hours between 6.15-7.15 AM. 20 flies of both sexes were used to study Courtship activities (Spieth H.T., Ringo J.N 1983; Chowkamba 2003)

Mating Latency (ML) defined as time between the introduction of males and females into mating chamber and initiation of copulation of each pair and Copulation Duration (CD) defined as time between initiation and termination of copulation of each pair. The terminologies were used as per the description of (Hegde S.N and Krishnamurthy N.B; 1979). Mating latency (ML) and Copulation Duration (CD) were observed. For this observation bachelor male and virgin female were introduced into Elens-Wattiaux mating chamber (5x5cm circular glass chamber with a lid to facilitate easy observation). Mating Latency (ML) and copulation duration (CD) observed flies were used to study the reproductive fitness parameter such as fecundity and fertility. For fecundity test mated females were transferred to vials containing fresh wheat cream agar media and the number of eggs laid during the following ten days was scored using stereomicroscope for both control and T1 and T2 groups. The number of progeny produced from the single mated female was counted. Ten successive changes were made and the total number of flies emerged from each vial both in control and T1 and T2 groups were counted.

Statistical analysis

Mating Latency, copulation duration, fecundity and fertility data were subjected to ANOVA using SPSS software in 11.5 versions.

Results and Discussion

Drosophila melanogaster is one of the most valuable organisms in biological research, particularly in genetics and developmental biology. The fruit fly *Drosophila* is one which stands very close to man in having 60% similarity. The recent genome projects have brought to light that about 13600 human genes have homologs to *D. melanogaster* (Adams M.D 2000). It has large number of induced and spontaneous mutations with a few chromosomes in a small genome. In the last decades numerous researches have focused their attention on understanding many aspects of neurodegenerative diseases, cancers, reverse genetics, forward genetics and so on. Fruit flies have a life cycle that is very different from that of mammals, yet developmental and cellular pathways are conserved**.** Many ayurvedic researches have been done using *Drosophila* as model organism in screening psycho stimulant plant materials.

Mating behaviour of *Drosophila melanogaster* includes series of courtship elements, which consists of various behavioral displays followed by an interchange of different sensory stimuli, courtship latency and copulation duration is an important component of fitness. It is correlated with different components like fecundity and fertility.

In *Drosophila*, successful mating depends on male activity and female receptivity. Courtship latency is one of the parameter, which indicates vigor of male *D. melanogaster*. A male with high vigor reacts quickly in the presence of female while a male with less vigor, reacts slowly. Mating latency has decreased in all treated flies of *D.melanogaster* and vestigial wing mutant were treated with 1.0mg/100ml and 2.0mg/100ml concentration of *G.glabra* root extract (table 1-3). Copulation duration has increased in all treated groups of *D.melanogaster* and vestigial wing mutant compared to control.

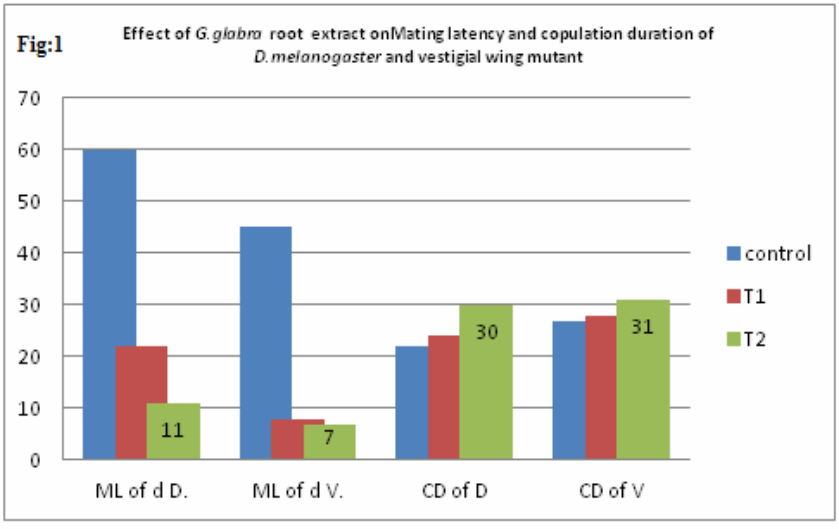
Courtship is a pre requisite for copulation. Copulation duration is the time. It is a quite natural that copulation is severly affected when courtship is affected mutant copulation duration of *D.melanogaster* slightly decrease compare to the vestigial wing mutant. Root extract of *G.glabra* severly affected to mutants compared to wild type. The reason is obvious that *G.glabra* root extract severly affect male behaviour, thus affecting copulation duration. This result agrees with the decreased copulation duration of control groups.

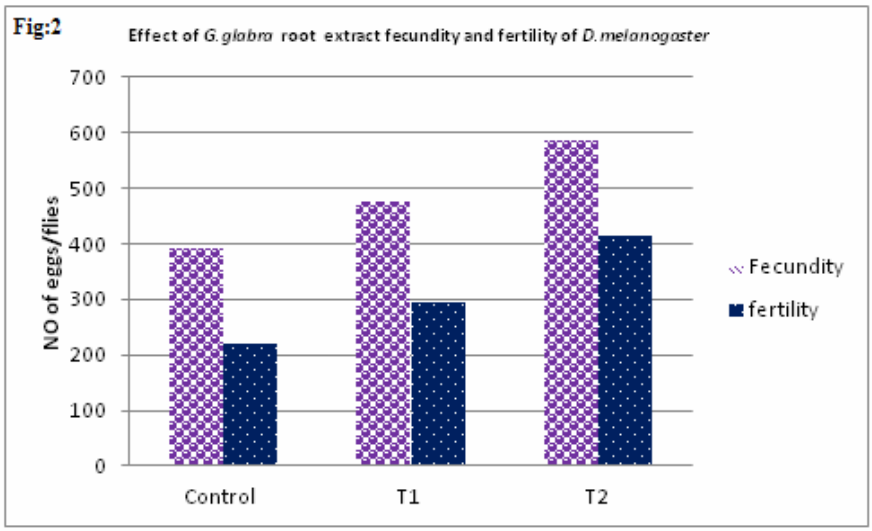
Fecundity is one of the fitness parameter that is used to assess the fitness in the different species of *Drosophila.* It is the most obvious trait that influences the reproductive value of female by her genotype, body size, age and her mate as well as the effects of environmental factors. Estimation of fecundity is important in routine ayurvedic research of various drugs. This gives an insight into the extent of effect on physiological factors, which is expressed in the terms of egg and offspring production. Fast mating males in *D.melanogaster* perform large number of copulations and produce more offspring than the slow. Fast mating will provide the female with an advantage of receiving the largest number of sperms since the male deposits the largest number of sperms in the first male and there is a positive correlation between fast mating. In the present study results showed that there is a significant increase in the number of eggs laid in the treated groups compared to control. The present study indicates that in treated groups fecundity increases may be accounted for the facts that the flies under the influence of root extracts of G.globra. Data obtained from the above results after treatment with different concentration of *G.glabra* root extract showed increased fecundity in treated groups compared to control flies (table 2).

Organism's fertility is a quantitative trait determined by both environmental and genetic components (Clara M.U and Luckiribili L.S 1985; Graves J.L 1993; Partridge L et al; 1987) as shown in the Table (1-3), the *G.glabra* treated flies have shown increased fertility compared to that of control flies. Interestingly, the 1.0mg/100ml and 2.0mg/100ml concentration have significantly effect on *Drosophila melanogaster* and vestigial wing mutant flies. However there is a significant difference in fertility between control and treated groups.

Table.1 Mean ± SE Mating behaviour of *Drosophila melanogaster and vestigial wing mutant* in control and treated groups of *G.glabra* root extract.

Components of mating behavior		Mean ± SE	F ratio	Sig
Drosophila melanogaster **Mating latency**	Control 1.0mg 2.0mg	60.8000± 3.10841 24.7000± 7.69711 12.8000± 2.12812	25.524	0.000
Drosophila melanogaster **copulation duration**	**Control** **1.0mg** **2.0mg**	24.6000± .70238 27.7000± 1.07548 29.9000± .90000	8.646	0.000
Vestigial wing mutant Mating latency	**Control** **1.0 mg** **2.0 mg**	45.1000±6.41257 8.3000±1.90933 8.0000±2.82843	25.876	0.000
Vestigial Wingmutant Copulation Duration	**Control** **1.0 mg** **2.0mg**	25.9000±.76667 27.2000±1.34825 31.4000±1.10755	*6.825*	0.004





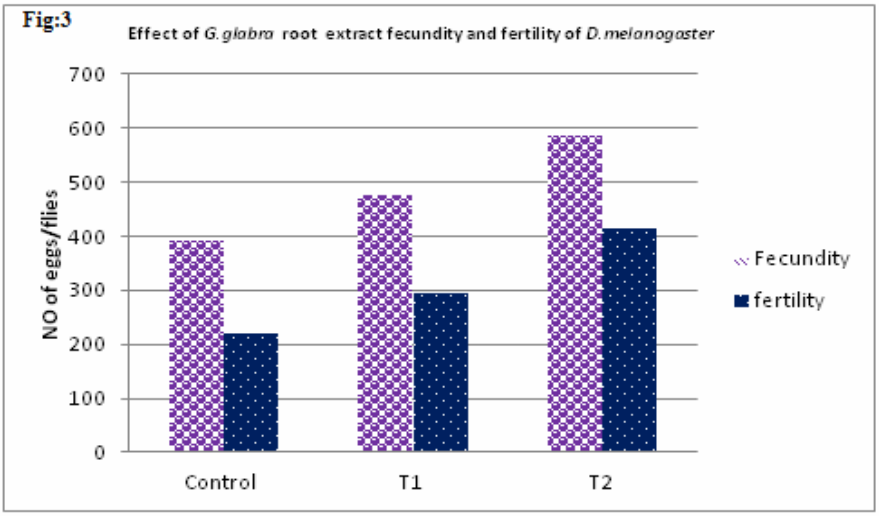


Table.2 Mean ± SE Fecundity of *Drosophila melanogaster and vestigial wing mutant* in control and treated groups of *G.glabra* root extract.

STRAINS	**Fecundity**	**Mean ± SE**	**F**	**Sig**
D.melanogaster (Wild)	Control	391.2000±12.10308	34.453	0.000
	1.0 mg	476.6000±11.14869		
	2.0mg	585.4000±23.54108		
D.melanogaster (Vestigial)	Control	372.2000±12.32414	20.571	0.000
	1.0 mg	458.8000±22.23401		
	2.0mg	517.6000±11.57315		

Table.3 Mean ± SE fertility both control and *G.glabra* root extract treated groups of *D. melanogaster*

STRAINS	**Fertility**	**Mean ± SE**	**F Value**	**Sig**
D.melanogaster(Wild)	Control	119.3000±5.69025	38.395	0.000
	1.0 mg	159.1000±3.80190		
	2.0mg	207.4000±10.25909		
D.melanogaster(Vestigial)	control	127.7000±6.19507	40.208	0.000
	1.0 mg	172.9000±6.46607		
	2.0mg	206.5000±6.03922		

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