ROLE OF HERBAL EXTRACTS ON REPRODUCTION AND BEHAVIOUR OF *DROSOPHILA MELANOGASTER*

Dissertation Report:

Submitted in partial fulfillment of the requirements for the degree of

Masters of Science in Zoology (HONS)

Submitted by

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CERTIFICATE

This is to certify that **SWATI SINHA** (11506094), have completed dissertation report entitled **"ROLE OF HERBAL EXTRACTS ON REPRODUCTION AND BEHAVIOUR OF *DROSOPHILA MELANOGASTER*"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of her report has ever submitted for any other degree at any university.

The dissertation is fit for submission and the partial fulfillment of the conditions for the award of **Master of Science (HONS.) in Zoology.**

Date

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DECLARATION

I hereby declare that the project **entitled "ROLE OF HERBAL EXTRACTS ON REPRODUCTION AND BEHAVIOUR OF *DROSOPHILA MELANOGASTER*"** is an authentic record of my own work. The work has been carried out at School of Biosciences and Biotechnology, Lovely Professional University, Phagwara, Punjab under the guidance of **Dr. MahendraPratap Singh**, Assistant Professor, School of Biosciences and Biotechnology, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree Master of Science in Zoology.

Swati Sinha

Date

I certify that the above statement made by the student is correct to the best of my knowledge and believe.

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ACKNOWLEDGEMENT

All the praises and thanks are for the most omnipotent, gracious, ubiquitous, merciful GOD, who blessed me with his grace and mercy to complete my dissertation project.

I would like to express my gratitude to all those who gave me the opportunity to complete this dissertation report entitled **"ROLE OF HERBAL EXTRACTS ON REPRODUCTION AND BEHAVIOUR OF *DROSOPHILA MELANOGASTER*".** I want to thank the School of Biosciences and Biotechnology of Lovely Professional University for giving me permission to commence the dissertation, to do the necessary research work and to use departmental facilities. I furthermore want to thank **Dr. Mahendra Pratap Singh,** who gave and confirmed the permission and encouraged me to go ahead with my project.

I am deeply indebted to my supervisor, **Dr. Mahendra Pratap Singh,** whose help, stimulating suggestions and encouragement helped me in research and writing of this dissertation report.

Thank You

SWATI SINHA(11506094)

LIST OF ABBREVIATIONS

DMSO	Dimethyl sulfoxide
EF	*Eryngium foetidum*
CYP	Cypermethrin
TPC	Total phenolic content
TFC	Total flavanoid content
DPPH	1, 1-Diphenyl-2-picryl-hydrazyl
QE	Quercetin
ppm	Parts per million

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that affects the physical, psychological and functional status of an individual. It is the second most common neurodegenerative disease worldwide (This disorder is common in individual from 50-65 years of age (Martilla*et al.*, 1976). This disorder belongs to a group of conditions called motor system disorder that is caused due to the loss of dopamine producing brain cells. The most obvious symptoms of this disorder are shaking, slow movements and difficulty with walking. Dementia and depression also occurs commonly in people suffering from PD (Sveinbjornsdottir*et al.*, 2016). Bradykinesia is another symptom of PD, which is characterized by slow movements.

Current therapies are aimed at symptom management. . There is no permanent cure to PD but there are a few drugs that slow down the progression of this disorder. Dopamine agonists are used for example bromocriptine, pergolide, lisuride etc. that cures dyskinesia (Fahn, 2008). However, there are risks involved in using such drugs. It can cause leg and ankle edema, impulse control disorders like hyper-sexuality (Fahn, 2008; Rascol*et al*., 2011). It also causes hallucinations and confusions in elderly patients (Fahn, 2008). Another drug used is Amantadine, but its effects tend to reduce over time and it can also induce hallucinations and ankle edema (Fahn, 2008). Levodopa is also used as a treatment but it also has side effects causing dyskinesias and stiffness of the body (NCCCC, ed 2006).

Combination of several herbs has been used by the traditional Indian medicine system to cure PD like *Bacopamonnieri, Cantellaasiatica*(Mishra, 2003; Williamson, 2002). It has been studied that oxidative stress is also one of the major causes for the progression of PD (V. Mu˜noz-Soriano*et al*., 2011). It has also been studied that Flavonoids can reduce the oxidative stress in neurons

(Naoi*et al*., 2001; Nurko*et al*., 2010; Long *et al*., 2009). It has been found that *Eryngium foetidum* contains flavonoids (Swargiary*et al*., 2016). EF is a traditional plant and it is widely distributed worldwide. In India it is commonly found in the North Eastern region (Swargiary A. *et al*., 2016). In Tripura it is locally called as "Owarphakeitom" by the bishnupriyamanipuris. In Assam it is locally called as "Gongardhundia" (Swargiari*et al*., 2016). *Eryngiun foetidum* is a perennial herb belonging to family Apiaceae. It is commonly called as the Mexican coriander. It has been extensively used as a medicinal plant in most of the tropical regions (Paul *et al*., 2011). It has been traditionally used as antimalarial, curing hypertension, in burns, in cases like infertility, curing headache and fever too. It has been also studied as anthelminthic, anticonvulsant, anti-inflammatory and antibacterial (Paul *et al.,* 2011). In the current study we sought to find out the effect of the extract of *Eryngium foetidum* on the locomotion of *Drosophila melanogaster* (fruit fly). Cypermethrin has been used as a test chemical to induce neurotoxicity at a dose of 0.02ppm on a non-target organism i.e. *Drosophila* (Mukhopadhyay*et al*., 2002)

The extract of EF was used to study the emergence of the *Drosophila* and has been used as an alternative to animal model organism in various studies because it is time effective, cost effective and it provides easy way to evaluate the efficacy of potential therapeutics (Pandey and Nichols, 2011). It has gene homology of about 75% with human genome.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Eryngium foetidum is a biennial tropical herb, which grows best in moist and open banks. It belongs to the family Apiaceae. Its common names include Mexican coriander, spirit weed, bandhania and shadobeni (Duke*et al*., 2009). In Assam it is called as Gongardhundia (Swargiary*et al*., 2016). In Tropical America and the West Indies it is used as food and medicine (Duke *et al*., 2009). In Northeastern region of India it is used as a food (Prasad *et al.,* 2008). It is used for garnishing as it has a strong flavor and is also used as a salad in a Northeast India. Essential oil extracted from this plant has great international market value (Ignacimuthu*et al.,* 1999). Traditionally it is used for curing fever, headache, infertility, malaria, diarrhea etc. Despite many traditional uses, only a few studies have been done on this plant.

PHYTOCHEMISTRY

The aerial part of the plant is rich in calcium, iron, carotene, vitamin A, B and C and essential oils (Ramcharan *et al*., 1999; Munsell *et al*., 1950). In the study conducted by Ramcharan *et al.* (1999) showed that the fresh leaves contains 85% water, 3.3% protein, 0.02% iron, 6.5% carbohydrate, 1.7% ash, 0.06% phosphorous. It has been found that the major constituent of the essential oil is E-2 dodecenal also called Eryngial (Lo *et al*., 1991). Eryngial is an alkenal and it was found in various amounts in the extract of the leaves of EF from different countries. In EF from Malaysia it was 59.7% (Chowdhury *et al.,* 2007), in India it was 45.9% (Bagchi *et al.,* 2005), in western Nepal it was 58.1% (Thakuri *et al.,* 2006). Hill *et al*. in the year 2004 conducted an experiment that showed that Eryngial inhibited human cytochrome P450 2E1 activity. This study also suggested that consuming this extract containing Eryngial could inhibit drug metabolism (Hill *et al*., 2004 and Paul *et al.,* 2010).

ANTHELMINTIC ACTIVITY

In a study conducted by Forbes (2002) elicited *Eryngium foetidum* was effective against a parasite *Strongyloides stercoralis.* This parasite is highly infective and it infects via skin penetration. The disease is epidemic in the Caribbean region (Forbes *et al*., 2002). Among the 25 Jamaican medicinal plants used, *Eryngium foetidum* was the most effective against the third stage larva of *Strongyloides stercoralis.* The extract of EF was more effective than either its methanol–water or dichloromethane extract. The main anthelmintic compound was found to be Eryngial (Forbes *et al.,* 2002).

ANTICONVULSANT ACTIVITY

An ethnographic survey was done and 14 plants were collected that claimed to be the traditional cure to malaria. Anti-plasmodium screening showed that the extracts from the leaves of *Eryngium foetidum* was effective against the plasmodium (Roumy *et al.,* 2007). It has been studied that it was also used as a traditional cure against fits (sudden violent attack of a disease e.g. epilepsy) (Roumy *et al.,* 2007). An experiment was done on the rats to see the effects of EF on epilepsy. It was found that the aqueous extract of the leaves and stem of EF when imparted intra-peritoneal to the rats, showed equal effectiveness against epilepsy as Phenobarbitone (Nsour *et al*., 2000). Epilepsy is a neurological disorder that causes convulsions, muscle spasm and loss of consciousness. It was also found that the extracts inhibited writhing induced by acetic acid in mice (Saenz *et al*., 1997). Erdem *et al*., 2015, list different components of the Eryngium foetidum in a paper.

ANTIBACTERIAL ACTIVITY

In a study done by Kubo *et al*., 2004, it was found that the Eryngial showed potent activity against *Salmonella choleraesuis* at all growth stages. Weak activity was shown against *Heliobacte*r species at a concentration of 1mg/ml methanol. In a study done by Guevara and his coworkers suggests that the extract from EF was the most effective against *Erwinia* genus of Enterobacteriaceae (Guevara *et al.,* 2007).

ANTI- INFLAMMATORY AND ANALGESIC ACTIVITY

The dry residue of the decoction of the plant was given orally and it inhibited the carrageenan-induced edema in rat paw (Saenz *et al.,* 1997) and also exhibited topical anti-inflammatory activity as it inhibited swelling of mouse ear induced by 12-O-tetradecanoylphorbol acetate (Saenz *et al.,* 1997). *Eryngium foetidum* showed a significant anti-inflammatory activity, when it was administered orally at doses of 250 and 500 mg/kg against carrageenan-induced rat-paw edema (Saenz *et al*., 1997). The extract of EF also showed inhibition of abdominal writhings induced by acetic acid. Hence, the study showed that extracts of EF contains peripheral and central analgesic properties (Saenz et al., 1997). An important study by Garcia *et al.*(1999) showed that the Stigmasterol and hexane was active in reducing the edema in the ear of the rat induced by12-0-tetradecanoylphorbol acetate (Garcia *et al.,* 1999). Stigmasterol showed 41% inhibition at a concentration of 1mg/ear.

ANTI CARCINOGENIC ACTIVITY

In a study conducted by Kamonwan *et al,* the effect of the extract of EF was observed. Colitis induced colorectal carcinogenesis by azoxymethane (AOM) and dextran sulfate sodium (DSS), 39 ICR male mice were studied. It was found that EF at a concentration of 3.2% in their

diet showed preventive effect on colorectal carcinogenesis via pro inflammatory cytokine, COX-2 (Promtes *et al.,* 2016). It was also observed that the mice, which were fed by 3.2%, EF had less body weight than the control (Promtes *et al.,* 2016).

OTHERS

There are many ethno medicinal claims of EF. The paste from the leaves and stem is applied on the forehead as remedy for headache (Kagyung *et al*., 2010). EF decoction prepared from leaves of the plant is used to cure common cold in babies. The baby is bathed in small amount of decoction and a little amount of the decoction is drunk (Sofie Ruysschaert *et al.,* 2009).T o cure fever in child, the leaves and the roots of the EF plant is used. It is mixed with blauwsel and oil of *Cocosnucifera* and the body of the baby is rubbed with it (Sofie-Ruysschaert *et al*., 2009).

It is also used as an ethno-medicinal plant for the treatment of a number of ailments such as fever, chills, stomach ache, asthma, arthritis, snake bites, scorpion stings, diarrhea, malaria and epilepsy (Singh *et al.,* 2014). North East Institute of Science and Technology (NEIST) in Jorhat, a unit of CSIR has already formulated a drug for the treatment of arthritis and skin disease in which the essential oil of EF is on of the main component (Singh *et al.,* 2014). An extract rich in Eryngial has been patented for the treatment of parasites in human (Forbes *et al*., 2002). Yagi *et al.,* 2006 has obtained a Japanese patent for using the extracts of EF as skin whitening agent out of the four plants used.

Ethno-medicinal claims show that EF is used for menstrual pain, remove placenta and shorten labor (Lans C, 2007). However, more data is needed. The detailed ethno-medicinal uses of EF are listed in a study done by Paul *et al*., 2011.

OBJECTIVES

OBJECTIVE

- To examine antioxidant properties by total phenolic content (TPC), total flavonoid content (TFC), DPPH scavenging activity of *Eryngium foetidum.*
- To study the effects of cypermethrin on the reproduction (fecundity) and developmental of *Drosophila melanogaster.*
- To study the effects of cypermethrin on the movement (locomotion) of *Drosophila melanogaster.*
- To investigate the potential of *Eryngium foetidum* on fecundity, emergence and locomotory response in Drosophila.

SCOPE OF THE STUDY

SCOPE OF THE STUDY

The experiment focuses on the effects of the extracts of *Eryngium foetidum* on the behaviour of *Drosophila melanogaster* to examine the effect on the locomotion, its geotaxis behaviour and the memory. The herb used in this experiment is *Eryngium foetidum*.. Traditionally Eryngium foetidum has been used in treating certain conditions like epilepsy, arthritis, infertility, headache and fever. Despite many traditional uses, only a few studies have been done on this plant. Only a few pharmacological studies have been conducted recently on this plant. This area is yet to explored. It has been studied that EF has flavonoids in it, which reduces oxidative stress. Hence use of such herbal extracts could possibly have an effect on the neural control of *Drosophila* and their memory and this study can be extended further to cure disorders like Parkinson's disease and other disorders involving neural complications. Scope of this study was to enlighten the use of these nutraceuticals for its advantages to prevent/cure human related neurodegenerative diseases, which needs further investigation and can be explored by promoting their properties so that they can also be used against genotoxicity and further more.

MATERIALS & METHODS

MATERIALS AND METHODS

FOR THE FOOD OF *DROSOPHILA MELANOGSTER*

Agar, sugar, maize powder, yeast granules (baker's yeast), methyl benzoate, 90% ethanol, propionic acid.

Test Chemical

Cypermethrin, analytical grade

Other Chemicals

Folin-Ciocalteu reagent (FCR), sodium carbonate, methanol, aluminium chloride, potassium acetate, gallic acid, quercetin, DPPH (1, 1-Diphenyl-2-picryl-hydrazyl), ascorbic acid, DMSO (dimethyl sulfoxide), diethyl ether

EQUIPMENTS REQUIRED:

Digital balance, UV visible Spectrophotometer (Systronic), vortex, heating mantle, centrifuge (REMI), B.O.D incubator (REMI) and pH meter

PLANT MATERIAL (Herbal product)

The plant material was collected from Kailashahar region of Tripura in February 2017 and was identified by plant taxonomist Dr. Arbeen Ahmad Bhatt, assistant professor, Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab. The collected plant material was air dried in darkness at room temperature (20^{o}C). The dried leaves were cut and stored in airtight container until needed.

PREPARATION OF PLANT EXTRACTS

For boiled herbal extract

One gram of dried leaves of EF was weighed and mixed with 10 ml of distilled water. It was then boiled for 10 min. After 24 hours (hrs) the infusion was filtered with the muslin cloth. The infusion was then centrifuged at 4000 rpm for 10 min at room temperature (22^{o}C). The pallet was removed and the supernatant was used for the assays. The supernatant was kept in sterile tubes stored in refrigerator at 4˚C for further use and different assays were carried out.

For non boiled/soaked herbal product

The above method was followed but the leaves were not boiled. Instead, the leaves were immersed in normal distilled water and incubated for 24 hrs.

DETERMINATION OF TOTAL PHENOLIC CONTENT IN THE PLANT EXTRACTS

The total phenolic content of the extract was followed by Folin-Ciocalteu method (Baba and Malik, 2015) in this method 500µl of the extract was made up to 3ml using 2.5ml of distilled water and was mixed thoroughly with 5ml of 0.2N Folin reagent for 5 min. Then add 2ml of 7.5% (W/V) sodium carbonate. The mixture was allowed to stand for 30 min in dark. The absorbance wasmeasured at 650nm. The samples were measured in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g of extract.

Table 1- Procedure of phenolic assay for sample extract

Sample extract	Vol. of extract (µL)	Vol. of dH2O (µL)	0.2N folin reagent (mL)	Incubation time (min)	Vol. of 75% Na_2CO_3 (mL)	Storage time in dark (min)
Fennel	500	2500	5	5	2	30
Fenugreek	500	2500	5	5	2	30

Table 2- Method of preparing standard for gallic acid

Test tubes	Vol. OF stock solution (µL)	Vol. of dH2O (µL)	0.2N folin reagent (mL)	Incubation time (min)	Vol. of 75% Na_2CO_3 (mL)	Storage time in dark (min)
1	20	2980	5	5	2	30
2	40	2960	5	5	2	30
3	60	2940	5	5	2	30
4	80	2920	5	5	2	30
5	100	2000	5	5	2	30

DETERMINATION OF FLAVONOID CONCENTRATIONS IN THE PLANT EXTRACTS

The total flavonoid content was explained by aluminium chloride (AlCl3) colorimetric method. (Baba and Malik, 2014). One ml crude extract was mixed with 1ml of methanol. Then 0.1ml of 10% aluminium chloride was added to the mixture. Again, 0.1ml of 1M potassium acetate was mixed and incubated for 30 min in room temperature. The absorbance was measured at 510nm. The samples were measured in triplicates for each analysis and the mean value of absorbance was obtained. The procedure was repeated for the standard solution of quercetin and the calibration line was constructed. The total flavonoid content was calculated from the calibration curve, and the results were expressed as mg of quercetin equivalent per g of extract.

EVALUATION OF ANTIOXIDANT ACTIVITY

For this test ascorbic acid was used as standard (Hinneburg*et.al*, 2005). DPPH acts as a scavenging agent. 4mg of DPPH was mixed into 100ml of methanol after 15 minutes check the absorbance at 520nm. After 1hr the absorbance was measured at 520nm. The absorbance should be between 0.78-0.98 for better control sample. Dilute the mixture with methanol until the desired absorbance is obtained. For the stock solution take 2ml of DPPH in each test tube and add 25µl and 50µl of the extract. Measure the absorbance after 30 min. For measuring the standard, take 10mg ascorbic acid in 1ml-distilled water. Take out 20µl from this mixture and add 980µl of distilled water. This would be the stock solution for the standard. Take 25µl and 50µl of the stock solution and add 2ml DPPH in each test tube. Measure the absorbance at 520nm.

For calculating the scavenging activity,

$$\% \text{ Scavenging activity} = \frac{A_{520}(\text{control}) - A_{520}(\text{sample})}{A_{520}(\text{control})} \times 100$$

REARING OF *DROSOPHILA MELANOGASTER*

Drosophila melanogaster has been used as a model organism because it is easy to rear and have a short life span. Moreover gene homology is about 75% with humans.

Table 3- Components for culture media for 1 unit food

Components	**Quantity**
Water	**360 ml**
Maize powder	17gms
Sugar	15gms
Agar	1.5 gms
Yeast	6gms
Methyl benzoate	1gm
Propanoicacid(CDH,India,Cat#029688)	1ml

For preparing 1 unit of food, boil 200 ml of water in a glass beaker. Add 2g of agar and allow it to boil. In a separate beaker take 100ml of water and add 17gms of maize powder and 15gms of sugar into it and mix it properly. Now add this mixture to the agar solution and give it a good

stir. Keep boiling this mixture in medium heat until the mixture thickens. In a separate beaker take 60ml of water and add 6gms of yeast to it. Add this mixture to the heated solution of maize and sugar. Prepare a solution of methyl benzoate by adding 1g of it to 2-3ml of 90% ethanol. Pour this solution to the culture media and stir properly. At the end when the culture media attains a perfect consistency, add 1ml of propionic acid and stop the flame. Pour the media into test tubes and remove the moisture with the help of tissue paper. Keep the media in the B.O.D. chamber. This media is used for culturing *D. melanogaster*.

TREATMENT PROTOCOL

TREATMENT GROUPS:

Second instar larva was used for the experiment

Group1- Control (untreated)

Group 2: 0.1%) *Eryngium foetidum* (EF)

Group3- Cypermethrin treated (CYP)

Group4- CYP + 0.1%) *Eryngium foetidum* (EF)

CYPERMETHRIN TREATMENT

Stock concentration of 2000 ppm was prepared by taking 8µl of 25% cypermethrin 1ml of DMSO – This becomes the stock 1, from stock 1 take 100µl and add 900µl of DMSO. This makes 200ppm. This becomes stock 2, from stock 2 take 100µl and add 900µl of DMSO. This makes 20ppm. This becomes stock 3, from stock 3 take 100µl and add 900µl of DMSO. This makes 2ppm. This becomes stock 4. Finally, 0.02 ppm was prepared by adding 1ml of stock

solution 4 per 100ml food (Gupta *et al.,* 2010). So, for 10ml of food, 100µl of cypermethrin was used. In each test tube 10ml of food was poured. 3 such tubes were subjected for the experiment.

CYPERMETHRIN + *ERYNGIUM FOETIDUM*

For 10ml of food, 100µl of CYP and 100µl of EF (0.1%) was used. In each test tube 10ml of food was used (Hosamani *et al.,* 2009). 3 such tubes were subjected for the experiment. It was screened in order to govern whether the treatment has any effect on the emergence of experimental larva and the locomotory behaviour of the flies.

ERYNGIUM FOETIDUM

For 10 ml of food, add 100µl of EF extract. Each test tube had 10ml of food and 3 such tubes were prepared and third instar larva was fed on that food.

NEGATIVE GEOTAXIS ASSAY

This assay was performed according to the climbing assay mentioned in Sharma *et al.,*(2012). Adult flies were kept in the treated food for 24 hrs. After 24hrs the assay was performed in triplets. 20 flies were subjected for the experiment at a time. A transparent measuring cylinder was used for the assay (16cm length, 2cm diameter). Flies were tapped and the measuring cylinder was positioned vertically. The number of flies moving above 10cm within 20sec was noted down. This procedure was again repeated twice. Performance index (PI) was also calculated for each experiment as;

$$\mathbf{PI = \tfrac{1}{2}[(n_{tot} + n_{top} - n_{bot})/n_{tot}]}$$

(n_{top}) = number of flies above 10cm

(n_{bot}) = number of flies below 10cm

(n_{tot}) = total number of flies

Emergence of flies

Newly eclosed second larvae of wild-type *Drosophila* were transferred to normal or control food, food mixed with EF, cypermethrin (CYP) contaminated food and CYP+EF food (20 larvae per vial/tube and 3 tubes per group). The number of flies emerging from different groups was counted until all the flies emerged from control and treated tubes. The emergence of the flies in different groups was investigated as mentioned earlier (Gayathri and Krishnamurthy, 1981)

Reproductive capacity (fecundity) of flies

For this experiment, we followed a method explained by Singh *et al*. (2009) with minor some modifications. Freshly hatched second instar larvae were transferred to control/untreated food, food mixed with EF, CYP and CYP+EF mixed food. Larvae were allowed to grow on normal and contaminated food throughout their development. Virgin male and female flies of wild type *Drosophila* emerging from normal and food mixed with test chemical and herbal extract. For each treatment group, 5 pairs of flies in 5 individual vials were taken and they were transferred to fresh vials everyday for the next 10 days. The number of eggs laid during this period was recorded. From analysis, total fecundity (total no. of eggs laid in 10 days), mean daily egg laid by a female (total no. of eggs laid/female/day) was recorded.

STATISTICAL ANALYSIS

The statistical comparison was performed by independent/unpaired t-test using online Graph Pad software (Prism 7). If the *p*-value were 0.05 or less, then the data would be considered significant statistically.

RESULT & DISCUSSION

RESULT AND DISCUSSIONS

TOTAL PHENOLIC CONTENT (TPC)

Phenolic compounds have redox properties, which allow them to act as antioxidants (M.A. Subrattee *et al.,* 2005) and its free radical scavenging activity is provided by the hydroxyl group present in it. The total phenolic content (TPC) of *Eryngium foetidum* was determined by Folin-ciocalteu's assay by using Gallic acid as the standard phenolic compound.

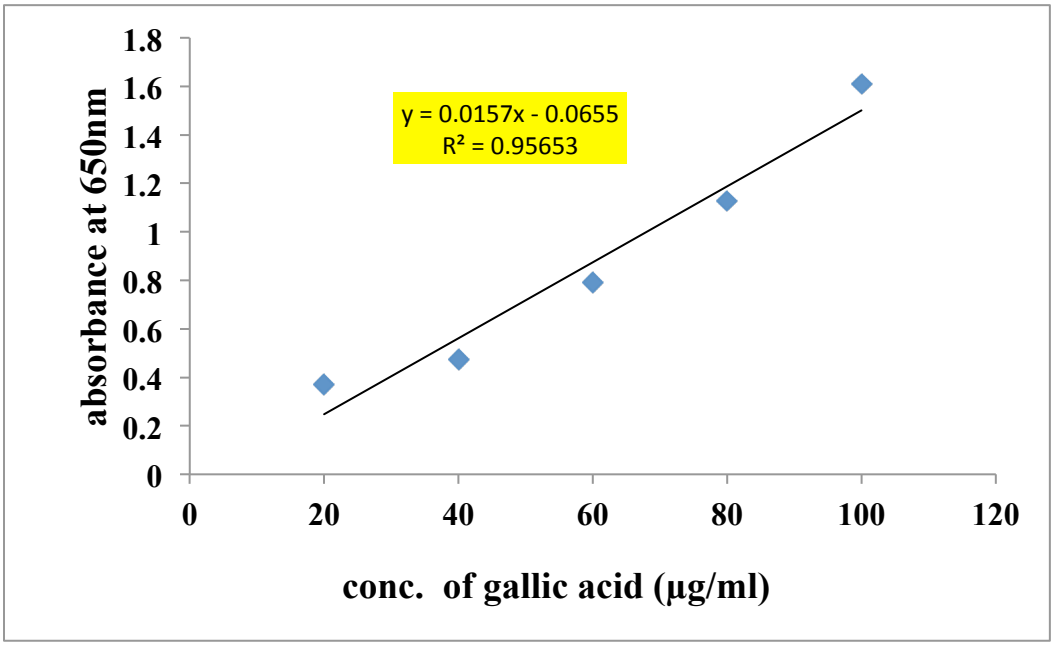

Fig 1- Standard calibration curve of total phenolic content

Table 4- Total phenolic content (Mean ± SD,n =3)

Sample	**Volume of samples (µl)**	**TPC (mg/GAE/g)**
EF (boiled)	500µl	35.3 ± 0.13
EF (unboiled)	500µl	28.1 ± 0.75

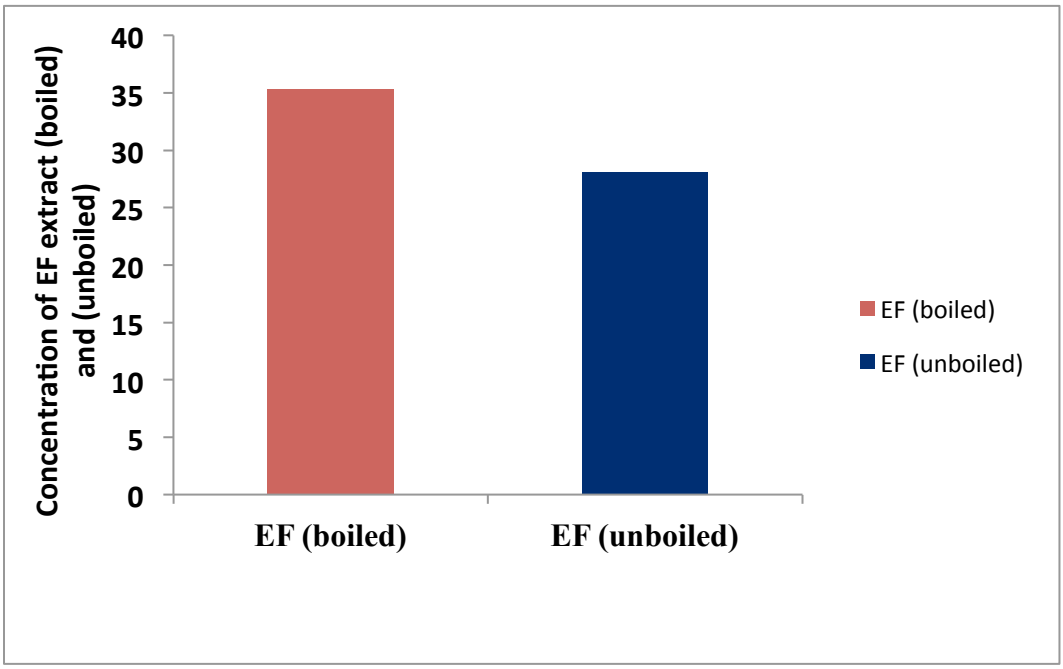

Fig2- Total phenolic content in the extracts of EF (boiled) and (unboiled)

A standard curve of Gallic acid in range of (20-100 µg/ml) with a coefficient of determination (R^2) value was equal to 0.956 (as shown in fig 1). The total phenolic content (TPC) was observed for the extract of EF boiled and unboiled as shown in fig 2. The phenolic content was observed to be 35.3 ± 0.13 and 28.1 ± 0.75 mg/GAE/g for EF boiled and unboiled respectively. From the table, we can say that the TPC of boiled EF extracts is higher than the unboiled one.

TOTAL FLAVANOID CONTENT (TFC)

Flavonoids are plant secondary metabolites and antioxidant property is contributed through free OH groups and these parameters were comparable with previous finding (S. Geetha *et al.,* 2003; K. Shimoi *et al.,* 1996).

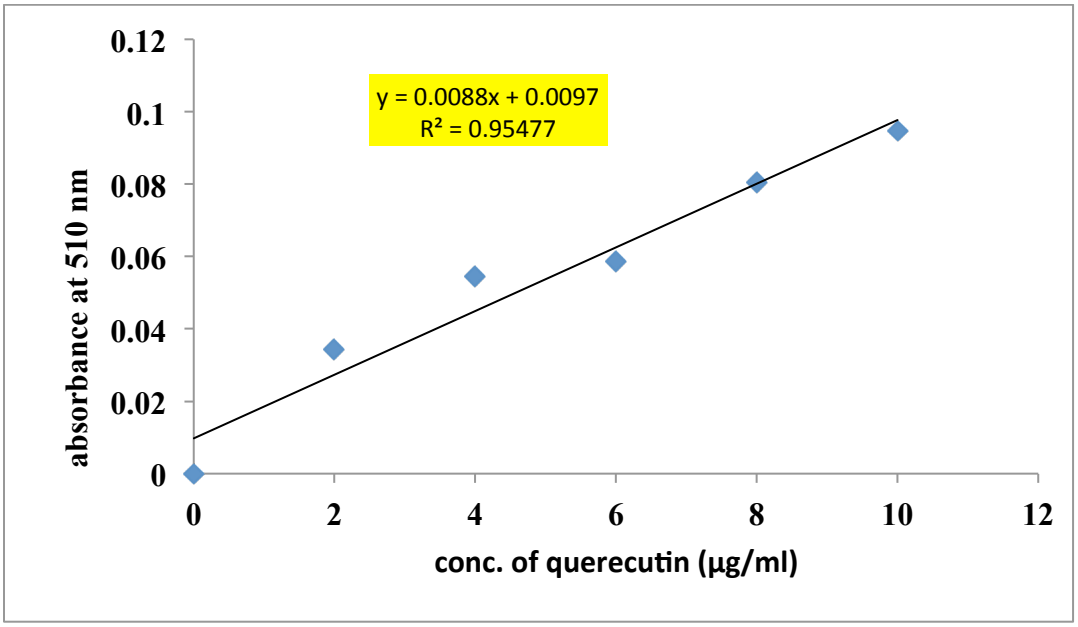

Fig3- Standard calibration curve of total flavonoid content

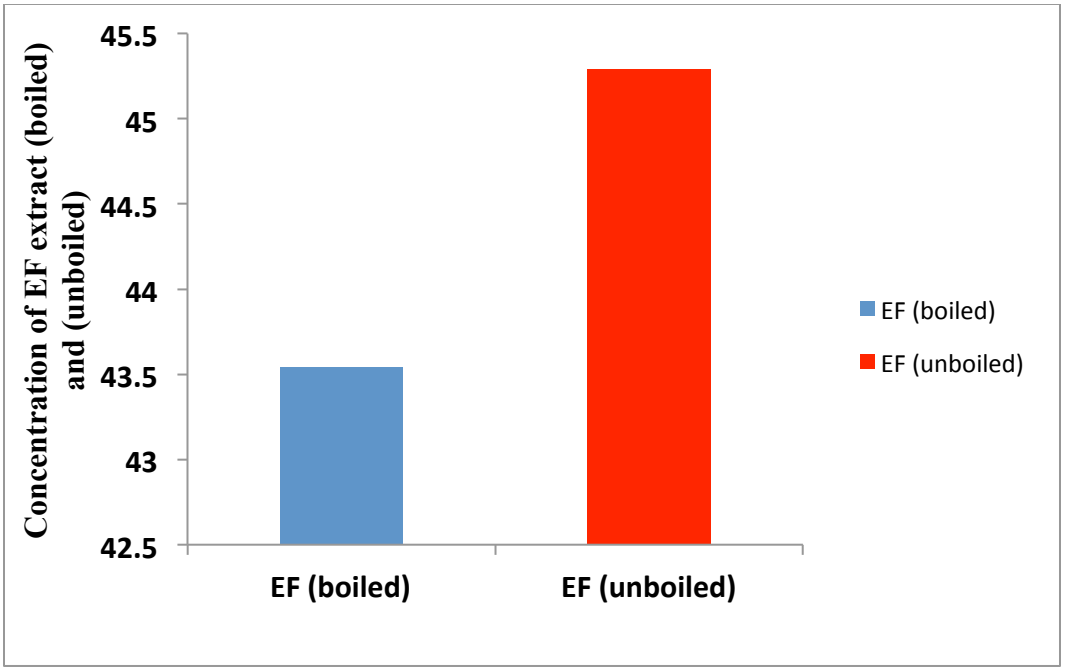

Fig4- Total flavonoid content in the extracts of EF (boiled) and (unboiled)

Table 5- Total flavonoid content (Mean ± SD, n =3)

Sample	**Volume of sample (µl)**	**TFC (mg/QE/g)**
EF (boiled)	1000µl	43.5 ± 1.6
EF (unboiled)	1000µl	45.2 ± 0.76

The total flavonoid content (TFC) of *Eryngium foetidum* was determined by using Quercetin as the standard. A standard curve of Quercetin in the range of 2-10µg/ml) with a coefficient of determination (R^2) value was equal to 0.954 (Fig 3).

The total flavonoid content (TFC) was observed for the extract of EF boiled and unboiled shown in Fig 2. The phenolic content was observed to be 43.5 ± 1.6 and 45.2 ± 0.76 mg/QE/g for EF boiled and unboiled respectively. From the table, we can say that the TFC of unboiled EF extracts is higher than the boiled one.

Result of DPPH ASSAY

DPPH is an oxidizing radical that can be reduced by the antioxidants. Ascorbic acid was used as standard at concentration of 5-10 µg/ml. The scavenging activity in EF boiled and unboiled at 1.25 and 2.50mg/ml was found to be 1.578947 ± 0.003 and 4.736842 ± 0.013 respectively. We observed that the % scavenging activity of EF increased when the concentration of EF extracts was increased from 1.25mg/ml to 2.5mg/ml, shown in Fig 5. Previous studies found that green tea has the % inhibition activity of 80.3 % in methanolic extract (Taheri *et al.,* 2012).

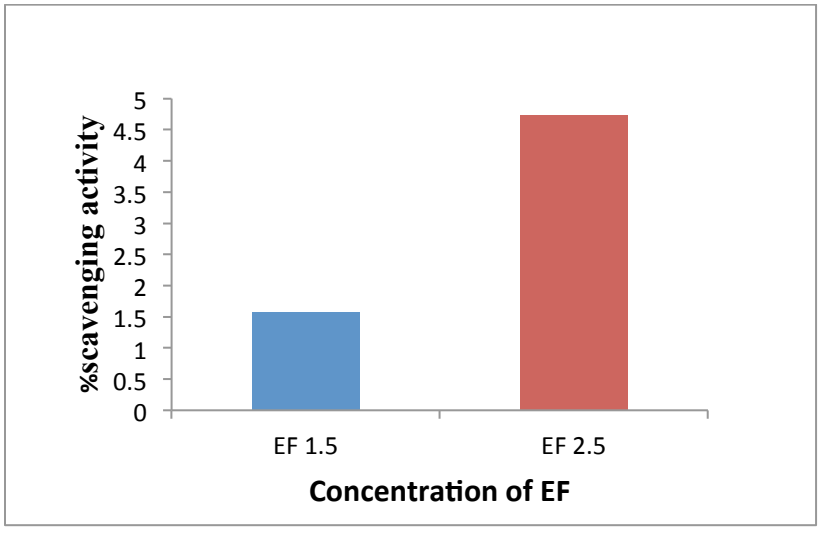

Figure 5: Scavenging activity of *Eryngium foetidum*

Table 6: Antioxidant activity of the *Eryngium foetidum*

Conc. of sample EF boiled(mg/ml)	%age of Scavenging activity
1.25	1.578947 ± 0.003
2.5	4.736842 ± 0.013

CLIMBING CAPACITY OF *DROSOPHILA MELANOGASTER* EXPOSED TO CYPERMETHRIN AND MIXTURE OF CYP+EF

Negative geotaxis assay was performed according to the treatment protocol per triplicates for 24 hrs. Flies were fed on the treated food for 24 hrs. After 24 hrs the floes were assayed for the climbing assay.

Table 7: % no. of flies crossed above 10 cm mark after exposure to cypermethrin and CYP+EF for 24 hrs

SAMPLE	**% OF FLIES CROSSED ABOVE 10CM**
CONTROL 1	75
CONTROL 2	70
CONTROL 3	90
CYP 1	50
CYP 2	40
CYP 3	35
EF (0.1%) 1	85
EF (0.1%) 2	100
EF (0.1%) 3	100

CYP + EF (0.1%) 1	75
CYP + EF (0.1%) 2	85
CYP + EF (0.1%) 3	60

From the above table it was found that the average % of flies climbing above 10cm for the Control is78.3, for CYP was 41.6, for EF it was 95, for CYP + EF it was 73.3.

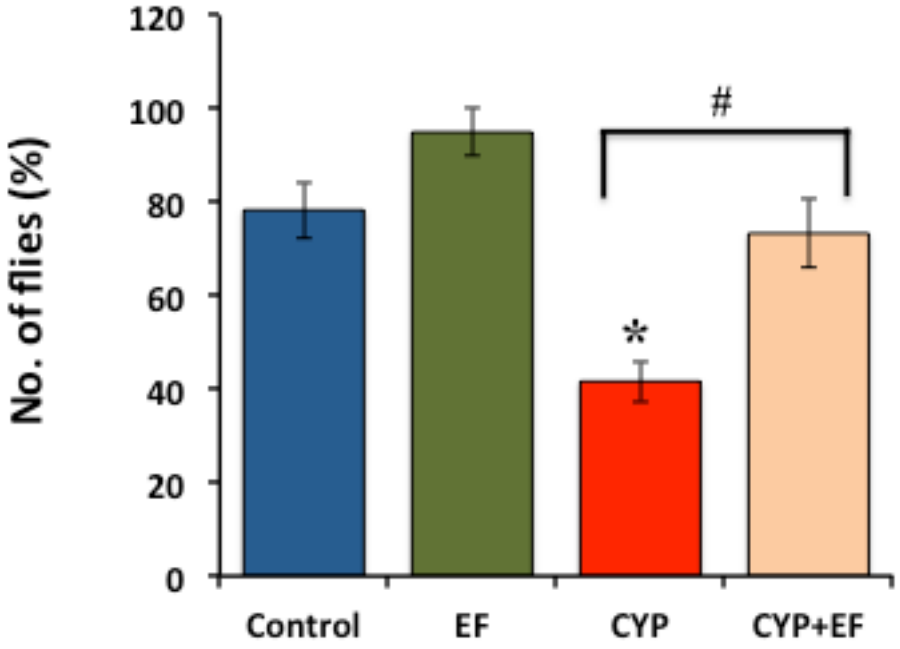

Figure 6: Climbing activity of *D. melanogaster* flies exposed CYP AND CYP+EF mixed food and number of flies was calculated who have crossed above 10 cm mark; Significance ascribed as **P*< 0.05 as compared to control or EF; # ascribed as P<0.05, CYP vs CYP+EF.

It was observed that when EF was used, it improved the movement of the cypermethrin treated flies. EF was able to somehow decrease the effect of cypermethrin. This shows that EF has some positive effect on the motor neurons which controls the locomotion of the flies which is

ultimately controlled by the brain and related to memory. Similar pattern was observed by Jansen and his colleagues (2014)in their experiment when they used *Bacopa monnieri* on the PINK1 mutant *Drosophila melanogaster*. The climbing activity was improved by using the herb *Bacopa monnieri* (Jansen *et al*., 2014). In a study done by Jimenez-Del-Rio *et al.* also found that cannabinoid CP55, 940 improved the locomotory behaviour of *Drosophila*. It restored the negative geotaxis of the flies exposed to paraquat (Jimenez-Del-Rio *et al.,* 2008).

Table 8: % no. of flies below 10 cm after exposure of 24 hrs of treatment

SAMPLE	**% OF FLIES BELOW 10CM**
CONTROL 1	25
CONTROL 2	30
CONTROL 3	10
CYP 1	50
CYP 2	60
CYP 3	65
EF (0.1%) 1	15
EF (0.1%) 2	0
EF (0.1%) 3	0
CYP + EF (0.1%) 1	25
CYP + EF (0.1%) 2	15
CYP + EF (0.1%) 3	40

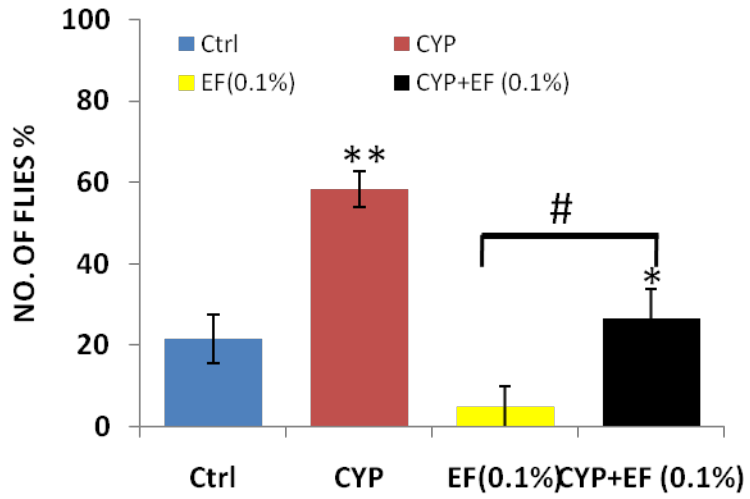

Figure 7: Climbing activity of *D. melanogaster* flies exposed CYP AND CYP+EF mixed food and total number of flies were calculated (below 10 cm mark) Significance ascribed as *P< 0.05 and **P<0.01 as compared to control; # ascribed as P<0.05, CYP vs CYP+EF.

FECUNDITY TEST

Table 9: Effect of cypermethrin on reproduction (egg laying capacity) in *Drosophila melanogaster*

Group	Total Fecundity	Mean daily egg laying/female /10 days
Control (Untreated)	1092	21.84±1.52
EF Control	1128	22.56±1.51
Cypermethrin (CYP)	344	6.88±1.03 $^{a**,b**, c*}$
CYP+EF	747	14.94±0.79 ^{a*,b*}

a= compared to control, b= compared to EF, c= CYP compared to CYP+EF

Five pairs of flies were subjected for the fecundity test in each petri dish. The number of eggs laid was counted using magnifying lense. It was observed that the fecundity was maximum when treated with EF. Fecundity was the least when flies were treated with cypermethrin. Similar kind of effect was observed by LI Xiu Ian and his colleagues when they found that fecundity of *Drosophila melanogaster* was improved by 42.98% by using 2.5% of *Portulaca oleracea* (Xiu-lan *et al.,* 2007). In another study done by Dwiwedi *et al. (2012)* it was found that the use of a traditional ayurvedic formulation called Amlaki Rasayana (AR) improved fecundity in *Drosophila* (Dwiwedi *et al.,* 2012). Liu *et al*. (2012) found that the use of *Cynomorium songaricum* improved the fecundity in *Drosophila*. In a study done by Navrotskaya *et al*. (2012) they found that Berberine, an isoquinoline alkaloid improved locomotory behaviour (vertical climbing) in wild type *Drosophila melanogaster* (Navrotskaya, *et al.,* 2012).

EMERGENCE

For this assay, second instar larva was used. 20 larvae were transferred to the treated and non-treated food. The assay was conducted in triplicates of control, cypermethrin treated, EF treated and CYP + EF treated food. The number of flies emerging was calculated as below (Table 10).

Table 10: Number of total flies emerging on 9th, 10th and 11th day

SAMPLE	**9TH DAY**	**10TH DAY**	**11TH DAY**
CONTROL 1	5	16	19
CONTROL 2	1	16	18
CONTROL 3	5	12	15
CYP 1	2	12	13

CYP 2	4	14	15
CYP 3	1	10	11
EF 1	7	16	20
EF 2	2	15	18
EF 3	9	19	20
CYP + EF 1	1	12	17
CYP + EF 2	3	12	15
CYP + EF 3	4	16	17

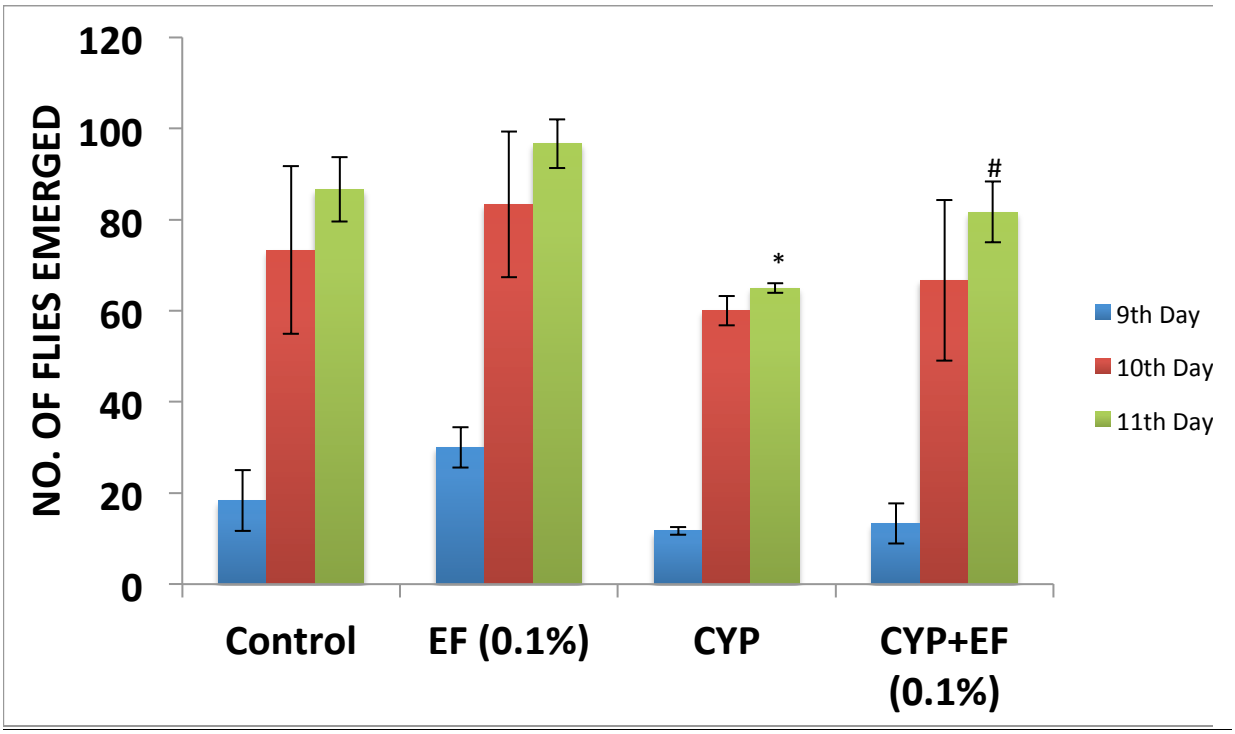

Fig 8: Number of flies emerged on 9th, 10th and 11th day, *P<0.05 as compared to EF (0.1%); reduced emergence and #P<0.05 as compared to CYP+EF (increased emergence)

CONCLUSION

CONCLUSION

The presence of phytochemicals such as total phenolics, alkaloids, and flavonoids provides some scientific evidence for the biological activities and also accounts for the pharmacological use of the plants. Phytoconstituents having antioxidant properties are widely used for protective and therapeutic treatment to various neurodegenerative disease based symptoms. We have used *Drosophila melanogaster* as the model organism. Although, fruit flies are completely unrelated to humans, their fundamental cellular processes as well as many genes are conserved between both organisms. Flies are capable of performing complex motor behaviours such as walking, climbing, and flying and their brain is complex enough to make these behaviours relevant to humans. They have rapid growth and reproduction, and the fact that it is cheap and easy to maintain in the laboratory is features that make Drosophila an ideal model system.

Prophylactic antiepileptic is used to prevent new seizures in patients that undergo surgery. But its effect does not last long. There are several other drugs that are used to cope up with the epilepsy but they have many side effects too. In this experiment we have tried herbs to cure PD. These herbs are cheap and have many ethno medicinal claims. PD is a neurodegenerative disease and affects the motor functions too. Cypermethrin is a neurotoxin. It hampers the motor function of the nerves. The flies treated with cypermethrin showed slow movement in the above experiment. Only 41.6 % of the cypermethrin- treated flies were able to climb above 10cm. whereas, 95% of the EF treated flies able to climb above. Among CYP+EF treated flies, 73.3% were able to climb above 10cm. This shows that the locomotory action was improved as compared to CYP treated flies. When the flies were treated with EF extracts their locomotory behaviour was improved.

Similarly, the fecundity was also improved when EF extracts were used. The total number of eggs laid per day was increased from 6.88±1.03 in CYP-treated to 14.94±0.79 in CYP + EF.

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