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Effect of *Carica papaya* Leaves Extract on the Morphological and Physiological Factors of *Drosophila melanogaster*

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Abstract
Since ancient times plants have been used for the treatment of various diseases, the medicinal importance of these plants has led to the promotion of these plant extracts as an alternative source of remedy. Interestingly, papaya which belongs to the *Caricaceae* family is used as an effective medicine for several diseases across the globe. The main objective of this experiment is to estimate the changes that are caused due to the addition of papaya leaves extract in Drosophila Media This experiment was conducted to determine the effects of papaya leaf extracts on the development, reproduction and behaviour of the model organism *Drosophila melanogaster*. The experiment was carried out with different concentrations of the ethanol leaf extract obtained via Soxhlet apparatus and the bioactivity of the herb on the model organism was measured. After orally administering the flies with different concentrations of the extract in their diets, the flies were processed through various assays. Some of them include dry and wet starvation, heat and UV tolerance. The flies were continuously monitored for their survival and death rates. Their reproduction yield was also noticed. It was found that the extract increased the mortality rate of male flies leading to increased number of females in the subsequent generations. Also, it reduced the production of off springs over generations affecting the reproduction yield.

Keywords
Carica papaya, *Drosophila melanogaster*, botanical insecticide, lifespan, fertility.

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INTRODUCTION:
From centuries, *Carica papaya* has been used as medicine for several diseases and recently many of its beneficiary effects were discovered. *Carica papaya* is a plant that was originated from Southern Mexico in Central America and is also found in the northern parts of South America. *Carica papaya* which belongs to the *Caricaceae* family is a dicotyledonous (the seed has two embryonic leaves or cotyledons) polygamous (contains male and female lowers on the same plant) and diploid (two sets of chromosomes) species. Tropical countries like Bangladesh, India, Indonesia, Sri Lanka, Philippines, West Indies and Malaysia also cultivate papaya. (Abhishek Agarwal et al). People from many generations were dependent upon the herbal and

medicinal plants for their infinite useful properties. Nearly thousands of new drugs have been developed over years from herbals plants. They contain bioactive chemicals also called phytochemical constituents that naturally are present in them and are widely used for their pharmacological purposes. These active components may be present in the roots, stem, bark, leaves, fruits or seeds. They are found to be useful in providing protection or curing various diseases. (Pallavi Singh et al)

The usage of these extracts is found to be increasing day by day. Among the many cheap and easily available methods for estimating the various components in the herbal plants, phytochemical assay is the most effective one. Phytochemicals are chemicals that are non-nutritive and they occur naturally in all herbal and medicinal plants during various metabolic processes. These metabolites are used as medicines and poisons. (Vimal Kishor Singh et al)

Papaya plays a significant role in human lives and fruit is used for edible purposes while its seeds are used for pharmaceutical purposes. Papaya leaves have recently been found to have tumour destroying properties and is also used for treating obesity, arteriosclerosis, high blood pressure and used blood purifier tonic. (Godson E. Wofia et al) It is also used in the treatment of urinary tract infections and is known to have anti-bacterial as well as anti-microbial properties (Yusha'u, M., Onuorah, F. C. et al.,).

C. papaya was used for malaria treatment and now is widely used for treating dengue. There are about 23 major species from the genus *Carica,* among these two species do not belong to the Angiospermae group.

Apart from the uses mentioned above, the papaya (As shown in the figure 1) has a lot of pharmacological properties which include its emmenagogue (increases menstrual flow), analgesic (pain killer), cholagogue (stimulates bile production), cardiotonic, vermifuge (anthelminthic medicine), laxative and febrifuge (fever) properties. Every part of the papaya has a significant commercial role. Nowadays the ruminants are also fed with these leaves in order to reduce the emission of methane and thus minimizing greenhouse gases that contribute for global warming. (Manpreet Kaur et al)

Figure 1: The tender young leaves of *Carica papaya* leaves were used for the experiment.

Among all the parts of the papaya tree, the leaves are the most useful and significant ones. The tender leaves of papaya are consumed orally as spinach. The oral consumption is for treating certain urinary tract infections and gonorrhoea. In cases of severe jaundice, the tender leaves are consumed in the form of paste. The fresh leaves are used as dressing for foul wounds and are also used for healing sores as poultice. Fresh leaves are also used for tonsillitis and are also found to cure asthma when smoked and inhaled. Dried leaves are known to cause abortion when consumed. The papaya leaves are also found to act as diuretic and are hence consumed as tea. They are also known to put an end to cough, respiratory, splenic and liver disorders. They are also proven to be useful in oedema and loss of appetite. Oral diseases like candidosis are also treated with papaya. (Apurva et al).

Phytochemical screening is a process in which the extraction, identification and screening of phytochemicals is done easily for a variety of medicinal and herbal plants. Primary phytochemical screening includes proteins, amino acids, sugar, chlorophyll, etc and secondary constituents include alkaloids, terpenoids, phenols, flavonoids, etc. These phytochemicals act as precursors for the synthesis of

various new drugs. Terpenoids are the oldest biomolecules and are found very diverse in nature. They are abundant in nature and are known for their anti-fungal, anti-inflammatory and anti-bacterial properties. Alkaloids are another group of phytoconstituents and are known to be the best anaesthetic agents used in various surgical practices. The properties of alkaloids include anti-cancer, anti-malaria, antiasthma. Flavonoids are known as anti-oxidants and stimulate several health effects. (S Akhila et al,)

The presence of these phytoconstituents has led to the development of drugs from these plants. Therefore, they have been used for the treatment of numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel thread worms and stimulate reproductive organs. (Vanessa Cristiane wohlenberg et al).

MATERIALS:

Sample Collection:

The papaya leaves were collected from Kanhangad, Kasaragod district- 671532, Kerala, India (as shown in figure 1). The collected plant material was identified and authenticated by botanist, Binu.K.S Mahatma Gandhi University, Kottayam.

Sample preparation:

The fresh leaves were collected, washed with tap water, followed by distilled water. Then the leaves were allowed to shade dried for 10-15 days so that no nutrients and phytocomponents that are present will be lost as shown in the figure 2. The dried sample was then crushed and soaked in 99.9% Ethanol.

Preparation of the extract:

The soaked leaves were then extracted by continuous hot extraction process using Soxhlet apparatus as shown in the figure-3. About 15 grams of the leaves were taken and were soaked in 150 mL of ethanol and then extracted in a Soxhlet apparatus for six to eight hours until complete extraction of the leaves as shown in figure 3. Further the extract was concentrated by evaporation (figure 3).

Phytochemical Analysis:

The various phytochemicals present in the papaya leaf sample like saponins, alkaloids, flavonoids, etc., were estimated. All of these were determined based on the methods of analysis described by Vijayanand S and Ann Steffy (2018).

Figure 2: The image shows the fresh leaves that were obtained after shade drying on the left side and the aqueous extract of *Carica papaya* leaves obtained after hot Soxhlation process to the right side.

Collection of Flies:

The stock population of *Drosophila melanogaster* (wild type) was obtained from The National Centre for Biological Sciences (NCBS) as shown in the figure 3. This starter culture was transferred to culture bottles with standard corn sooji (semolina) media. And the flies wee incubated at 24±2 °C with 12 hours light and 12 hours dark cycles.

Figure 3: Stock flies – wild type (CS-Red eyed) obtained from NCBS (National Center for Biological Sciences), Bangalore, India.

Media Preparation:

For the preparation of 500mL of standard media, about 500mL of distilled water was taken in a container, add 50g jaggery and dissolve completely on boiling. Now 50g of Corn Sooji (Semolina) was added and cooked for about 10 to 15 minutes. In order to solidify the media, 5g of agar was added to it and mixed thoroughly until dissolved. The flame was turned off and the *Carica papaya* leaves extract that was completely dissolved was added to the media. Once the media cools down to 50 degrees Celsius 3.7mL of Propionic acid was added as preservative. Immediately the media was transferred to the vials about 20mL each and then allowed to cool for about 3 to 4 hours. Cotton plugs were made before transferring the flies.

Four different concentrations of the extract that was obtained by Soxhlet method was added to the standard media. The four different concentrations that were included in the experiments are 5mg/mL, 10mg/mL, 20mg/mL and 40mg/mL of the extract was dissolved completely before adding to the media. After the establishment of a stock culture, the flies were transferred to the new glass tubes (50mL) containing the standard media along with the extract. A control was also maintained. The flies were transferred to new vials containing fresh standard media with the extract every three days. 10 flies were maintained in a vial such as 5 males and 5 females respectively as shown in the figure 4.

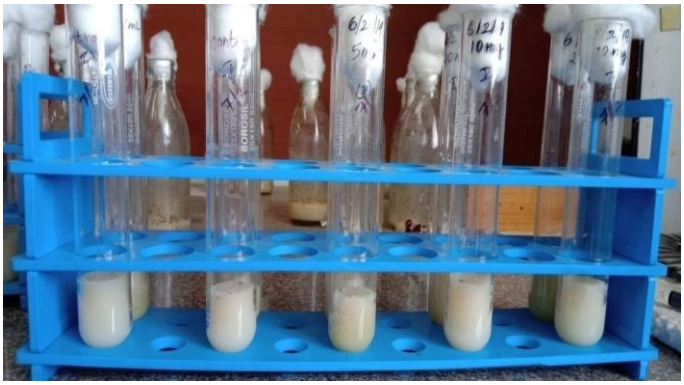

Figure 4: Vials containing the two control and eight experimental batches in the standard media with the different concentrations of the extract.

METHODOLOGY:

The following tests were performed on the flies that were fed with the extract on the 21st day. And the tests were performed on the basis of the procedure explained in (12). The flies were fed with four different concentrations of the extract; 5, 10, 20 and 40 mg/mL. The extract was dissolved with 10% ethanol and then added into the media. Therefore, the control also contained 10% ethanol.

Estimation of Drosophila Life Span:

A total of 30 vials (with 6 control tubes) were loaded with 12 flies of which 6 were males and 6 were females in each vial. Therefore, 180 in total per sex. The flies were transferred every three days to a new

vial containing fresh food. And the number of deaths during each transfer was recorded and noted.

Effect on Wet Starvation Tolerance:

On the 21st day of the feeding, the flies were transferred into an empty vial containing a piece of filter paper that was soaked in distilled water. The number of deaths was recorded after 20 hours of incubation.

Effect on Drosophila Dry Starvation:

On the 21st day of feeding, a specific number of flies were transferred into an empty vial and incubated for 20 hours. The mortality rate was estimated.

Offspring Viability:

The adult flies were transferred every three days and the number of progenies or offspring produced was counted. Therefore, result was calculated in percentage.

Sex Ratio:

The male: female ratio was estimated by finding the longevity i.e. counting the number of flies for every transfer.

Effect On Uv Tolerance:

On the 20th day, the flies were placed in a petri-dish covered by a plastic film and this was then placed on an UV trans-illuminator. The flies were exposed for 6 minutes with a 2-minute suspension. The survival rate is estimated. The data was converted into percentage.

Phytochemical Analysis:

The analysis of the various phytochemicals present in the extract was estimated according to the method given by Vijayanand S and Ann Steffy (2018).

Test for Terpenoids (Salkowski Test):

To 0.5ml of the extract, add 2 ml of chloroform. Then 3 ml of Concentrated H2SO4 was added. A reddish-brown color at the interface indicates the presence of terpenoids.

Test for Flavonoids:

5 ml of dilute ammonia was added to 0.5 ml of the extract. Add 1 ml of Concentrated Sulfuric acid. A yellow color that disappeared on standing indicates the presence of flavonoids.

Test for Saponins:

To 0.5 ml of extract, 5 ml of distilled water was added. The solution was shaken vigorously and observed for froth. The frothing was mixed with three drops of olive oil and shaken vigorously. Observe for the formation of an emulsion, presence of an emulsion indicates the presence of saponins.

Test for Tannins:

About 0.5 ml of the extract was boiled in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride were added. Observe for brownish green or a blue or black color. This indicates the presence of tannins.

Test for Alkaloids:

0.5 ml of the extract was diluted to 10 ml with acidified alcohol and boiled. To 5 ml of this diluted extract, add 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. To this, Mayer's reagent was added. The formation of a cream precipitate was regarded as positive for the presence of alkaloids.

Test for Reducing Sugars (Fehling's Test):

To 0.5 ml of extract in a test tube, Fehling's Solution A and B was added and then placed in boiling water bath. A reddish-brown color indicates the presence of reducing sugars.

Test for Anthraquinones:

0.5 ml of the extract was boiled with 10 ml of sulfuric acid. Add 5 ml of chloroform and shake well. The chloroform layer was pipetted into another test tube. Add 1 ml of 10% dilute ammonia. The resulting solution was observed for color changes as an indication for the presence of anthraquinones.

Test for Cardiac Glycosides (Keller-Killiani Test):

To 0.5 ml of extract which was diluted with 5 ml of distilled water, add 2 ml of glacial acetic acid containing one drop of 0.5% ferric chloride solution. This was mixed with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

Test for Steroids:

2 ml of acetic anhydride was added to 0.5 ml of the extract. To this, add 2 ml of concentrated sulfuric acid. The color changed from violet to blue or green indicates the presence of steroids.

Test for Phenols (Ferric Chloride test):

0.5 ml of extracts was treated with few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

Test for Carbohydrates:

0.5 ml of extracts was dissolved individually in 5 ml of distilled water and 2% anthrone reagent was added followed by concentrated sulfuric acid. A dark green color was obtained. It indicates the presence of carbohydrates.

Tests for Oils and Resins:

The extract was applied on a Whatmanns filter paper. The development of a transparent appearance on the filter paper indicated the presence of oils and resins.

Determination of Total phenolic content (TPC):

The total phenolic content (TPC) of ethanol leaves extract was determined by using Folin-Ciocalteau method (Demiray et al., 2009). Sample absorbance was measured at 650 nm.

RESULTS AND DISCUSSION:

The media containing extract with different concentrations having equal number of both male and female Drosophila were maintained for about 3 generations. This led to an observation in the decline in life span with a death rate showing 3:1 ratio in the death of males over females. Hence, it was observed that male death rate is more than the female. Higher concentrations of extract also led to less production of the offspring with increase in generation. Over the generation's females are able to adapt well than the males. The females were more active than males.

More the concentration of the extract a reduction in the male lifespan was observed. The female lifespan was also affected in the presence of higher concentrations of the extract while the lower concentration didn't show much difference to that of the control. It was also found that the male population gradually decreased over consecutive generations. It was also interesting to note that the extract had a negative impact on the reproduction ability of Drosophila, as the number of offspring over generations also decreased gradually.

Crude leaf extract of *Carica papaya* was extracted using ethanol (solvent). The extract was used in different concentrations in drosophila media for studying its effect on the physiological and morphological functions. The extract was fed to the Drosophila for 3 weeks. Higher concentrations affected the life span of male Drosophila and also the offspring count. For further observations Qualitative phytochemical analysis of *Carica papaya* leaf extract was done. It indicated the presence of the following phytochemicals that include: terpenoids, flavonoids, saponins, tannins, alkaloids, anthraquinones, carbohydrates, cardiac glycosides and steroids**.**

Other tests were also done for checking the presence of phenols, oils and resins but gave negative result. There were additional tests conducted for the confirmation of the effect of the extract on the Drosophila which included:

ESTIMATION OF DROSOPHILA LIFE SPAN:

A total of 30 vials containing 12 flies each having equal number of male and female were transferred every 3 days to a new vial containing particular extract concentration and the number of deaths were noted and is expressed in a graphical form as in figure 5. As expected, sex had effect on life span duration. Females had greater longevity in comparison with males in the experimental group. The male longevity seems to be affected by extract plant. The males, which larval phase fed on diet with plant extract, had a shorter life span than males reared on standard diet in average (12). These results suggest that fertility of female decreased in consequence of shorter male life span in test group. In consequence, females of test group survived longer than those in control group. The figure illustrates the number of deaths of Drosophila with each transfer.

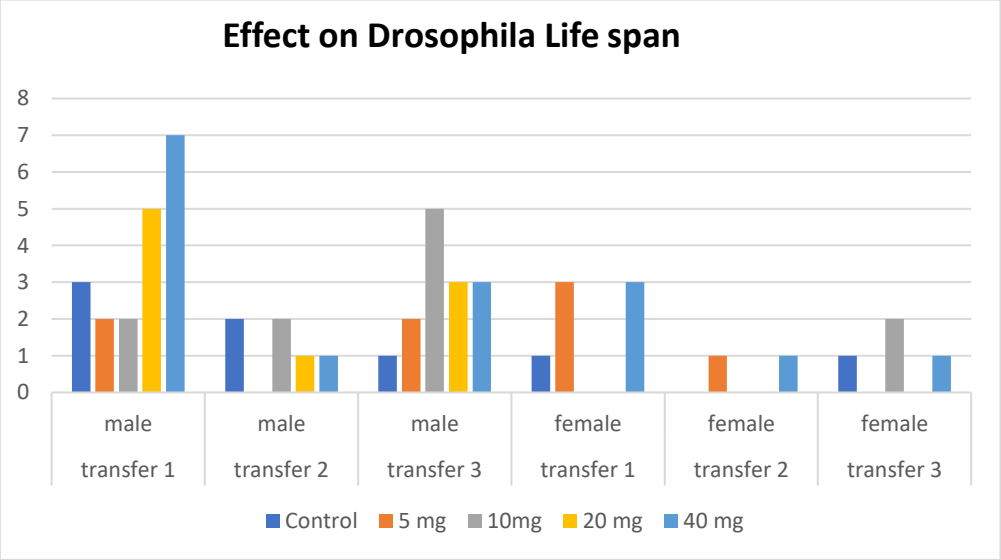

Figure 5: Death of *Drosophila melanogaster* males and females reared on standard media containing extract of *Carica papaya* leaves of four different concentrations.

EFFECTON WET STARVATIONTOLERANCE:
The number of deaths were recorded after 20hrs of incubation in wet condition. As mentioned earlier the extract has a greater advent on males than females and hence the results show the death of more males than females. This is clearly portrayed in the figure 6 where the death of males is found to be more than that of females in the extract treated group. This implies that the treated group males became more vulnerable to environmental stress than females.

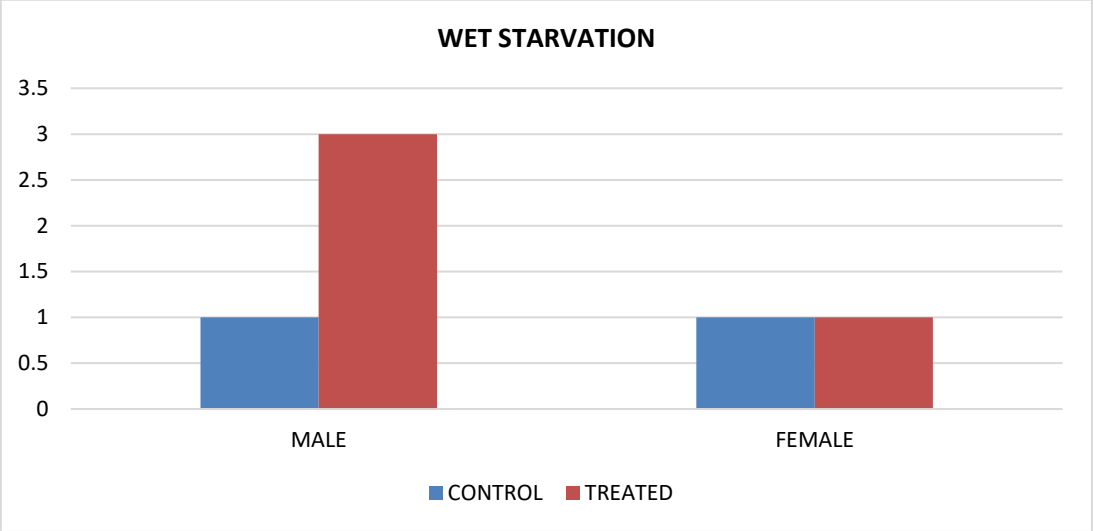

Figure 6: Death of *Drosophila melanogaster* males and females reared on standard media containing extract *Carica papaya* after 20 hours of starvation. And of the control group that were reared without the extract simultaneously.

EFFECT ON DRY STARVATION:
The mortality rate was estimated by incubating the flies from the 21st day to an empty vial for 20hrs.The treated batch flies were all dead while in the control batch ¼ of them still survived. The figure 7 clearly indicates that the extract hs a negative effect on the flies and it makes all of them more vulnerable to the environmental stresses while the control batch had flies that still survived even after a long period of starvation.

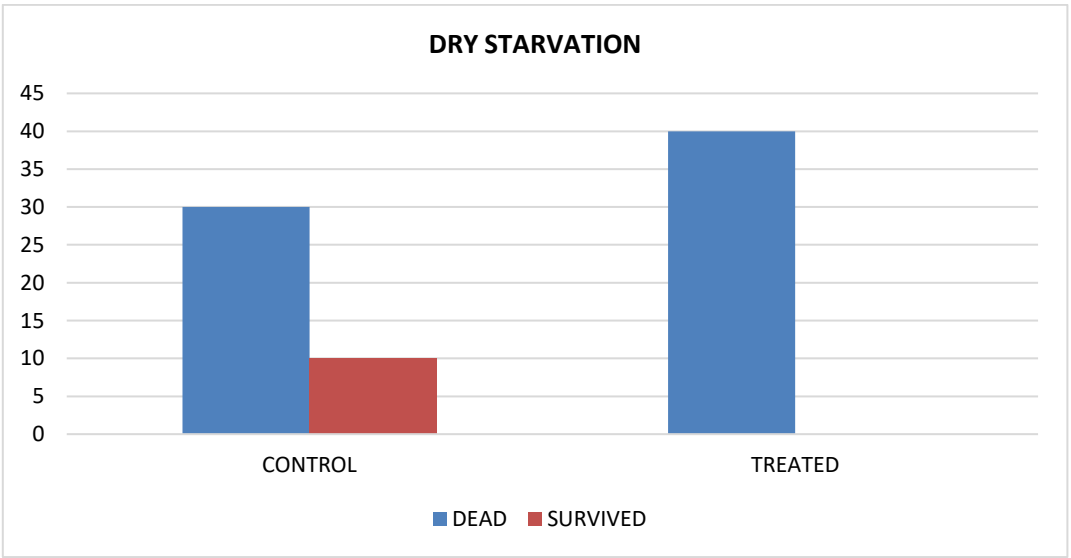

Figure 7: Death of *Drosophila melanogaster* reared on standard media containing extract and standard without the extract (Control batch) *of Carica papaya* after20 hours of starvation.

OFFSPRING VIABILITY:

The offspring count was taken and recorded in percentage after every three days transfer of the adult flies. It was found that the plant extract reduced significant number of offspring production. It thus indicates the existence of negative effect of aqueous extract of *Carica papaya* on reproduction. The low reproduction can be an indicative of toxic effect from a substance. Some secondary plants compounds can reduce fecundity of insects, in both sexes because bioactive compounds can interfere with their reproduction cycle. In this research was not possible determine if males or females, or both had formation of germ cells impaired by plant extract. As shown in figure 8 the number of offspring reduced significantly with the significant increase in the concentration of the leaf extract. The leaves like the fruit possess certain chemicals that inhibit the reproductive ability of the flies or it could simply be because of the decrease in male population.

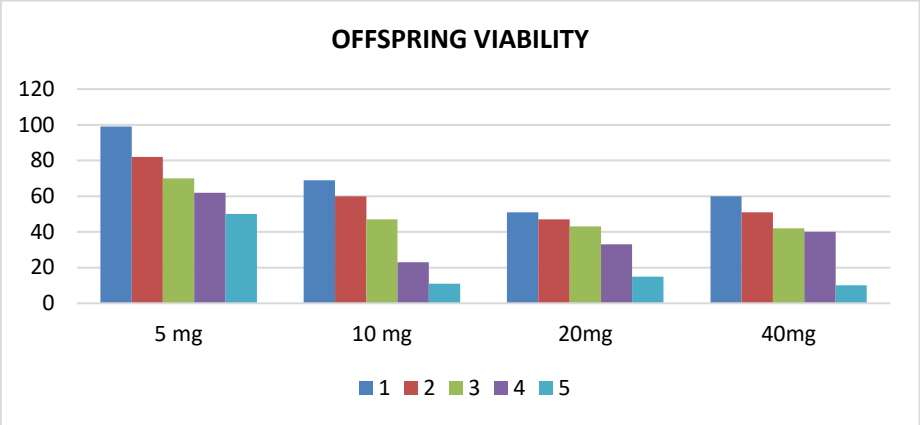

Figure 8: Death of *Drosophila melanogaster* larvae over every transfer that seemed to decrease over time. The numbers represent the transfer of the batch to new vials. And the last transfer has a significant reduction in offspring production.

SEX RATIO:

The male: female ratio was estimated by finding the longevity i.e. counting the number of flies for every transfer. The sex ratio male/female was found to be insignificant for the control group. For experimental group, the sex ratio was different. As hypothesis, males can be more sensible to plant extract, which implies in differences of sexual ratio. As shown in the figure 9, we can conclude that the minute concentration of the leaf extract renders no effect to the female lifespan while a gradual increase can also affect the female longevity. In contrast the presence of minute quantity of the extract can impair the longevity of male females. As the concentration increased the number of deaths for male increases simultaneously.

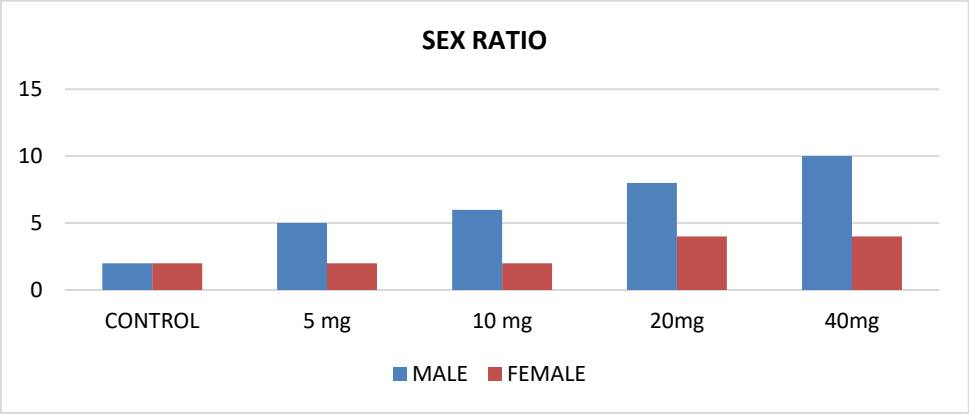

Figure 9: The death rate of both the sexes on the standard medium containing the extracts in four different concentrations and the control set without any extract.

EFFECT OF UV TOLERANCE:

The flies from the 20th day were placed on a Petri dish covered with a plastic film followed by exposure to the UV rays. The flies were exposed for about 6 minutes with a suspension of 2 minutes, the data collected were recorded in percentage. As shown in the figure 10, in contrast to the other tests, this test seemed to show positive effect of the leaf on the flies.

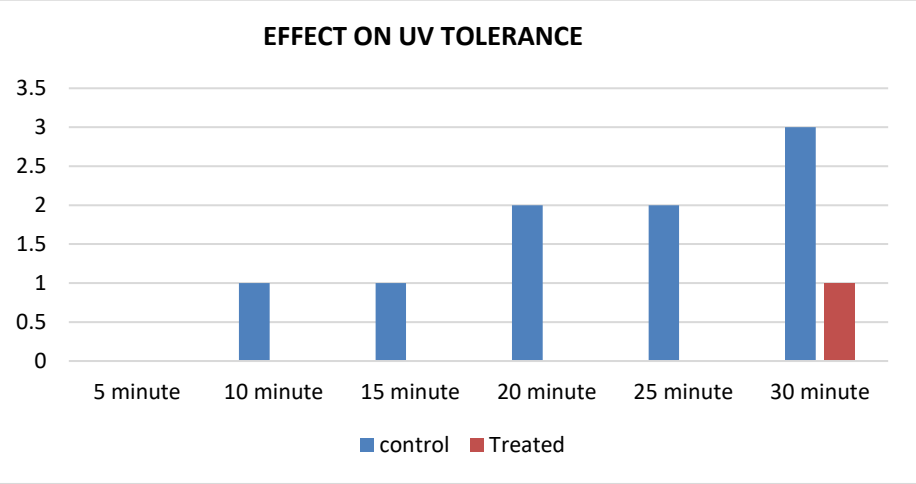

Figure 10: Death of *Drosophila melanogaster* males and females reared on standard containing extract of *Carica papaya* and the control batch after UV exposure.

The phytochemicals that were estimated to be present in the ethanol extracts of *Carica papaya* and that was used for the administration to the flies are mentioned in table 1 as follows.

Table 1: The various phytochemicals that were found to be present in ethanol extract of *Carica papaya* were listed in the table below:

SL NO.	TEST	PRESENT / ABSENT
1	Flavonoids	Present
2	Saponins	Present
3	Tannins	Present
4	Alkaloids	Present
5	Reducing Sugars (Fehling's Test)	Absent
6	Anthraquinones	Present
7	Cardiac Glycosides (Keller-Killiani Test)	Present
8	Steroids	Present
9	for Phenols (Ferric Chloride test)	Absent
10	Carbohydrates	Present
11	for Oils and Resins	Absent
12	For Terpenoids	Present

CONCLUSION:

This paper brings about the use of simple, precise and economical techniques like Soxhlet extraction techniques which helps in the detection, separation and identification of the phytochemical classes of compounds present in herbal plant materials. It has been observed that most active principles present in the leaves are flavonoid, alkaloids, steroids, and tannins. These phyto-constituents may be responsible for various pharmacological actions of this plant part although their specific roles remain to be further studied.

Likewise based on our observations, we conclude that the leaves extract of papaya found to reduce the lifespan of male Drosophila by unknown mechanism which is yet to be investigated. Also, is found to impair the reproductive fitness of the flies. It has been observed that most active principles present in

the leaves are flavonoid, alkaloids, steroids and tannins. These phyto-constituents may be responsible for various pharmacological actions of this plant part although their specific roles remain to be investigated. From our observations, we conclude that the leaves of *Carica papaya* phytochemicals that aided in therapeutic and medicinal uses can also be poisonous or toxic in some cases. In our study, it was found that the leaves extract had a negative impact on the various developmental factors of Drosophila mainly on male Drosophila than females.

In the test, the male population gradually decreased with the increase in exposure to the extract. And over generations the number seemed to reduce by 3 times from the initial generation. The mechanism behind that however is still unknown which is yet to be investigated. Also, it was found that the extract impaired the reproductive fitness of the flies. Not only the life span but the number of viable offspring that were produced was found to be reduced by greater rate. Therefore, the assumption which could be confirmed through this test is that, the reproductive efficiency and the life span of male flies are affected due to the adverse effects of the extract. The actual cause of it is still unknown and yet to be studied.

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