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Behavioral and biochemical effects of alcohol in *Drosophila melanogaster*

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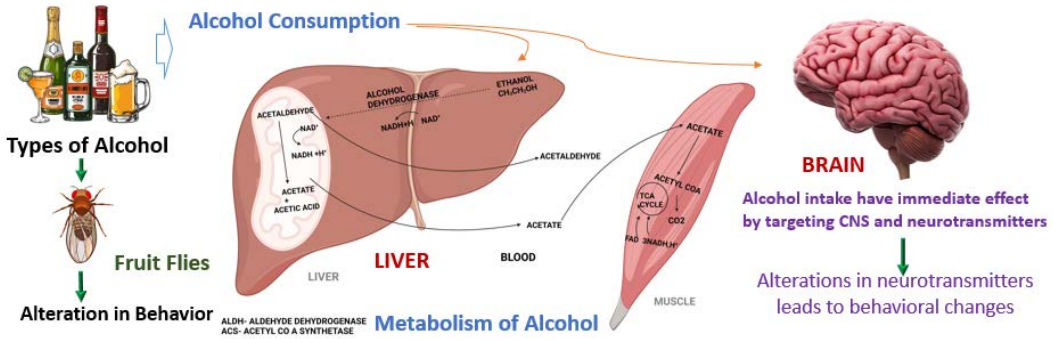
ABSTRACT



Drosophila, known as the fruit fly used in research as they share distinct homology to humans. The effects of alcohol on the fly has been an important area of research over the decades. The components in alcohol impact behavioral, metabolic, and sexual activity in humans. The metabolites of alcohol, acetaldehyde and acetate have been proved to induce carcinogenic effects. In this study, alcohols like distilled ethanol, undistilled ethanol, methanol, and isopropyl alcohol at 10%, 15%, and 25% concentrations were tested. The behavioral activities like RING, larval locomotor activity, sedation and tolerance were observed and the results were compared with the effects of the different alcohols. This study focuses on comparative study in behavior of Drosophila when treated with various concentrations of different alcohols. All the alcohol types showed significant alterations with increase in concentration. Human has natural tolerance towards certain amount of alcohol, however prolonged and increased amount of alcohol exposure increases the sedation and tolerance effect. The amount of protein after acute exposure was also determined. However, this study includes the effect of acute alcohol exposure in Drosophila. Chronic exposure of alcohol might lead to profound effects and induce increased tolerance which may also result in alteration of biochemical process.

Keywords: Alcohol, Drosophila, Behavior, Biochemical, Ethanol

INTRODUCTION

Drosophila is commonly referred to as the fruit fly which grows to a height of 3mm and has a wheel of life of about 2 weeks.[1] It is an animal model whose origin and heredity have been explored and elucidated sufficiently.[2] *Drosophila* has been used as a model organism in genetic studies since 20th century owing to its smaller life cycle and high fecundity rate.[3] *Drosophila* has been employed as a model to study human genetic diseases. The genetic analysis of human and *Drosophila* have been completed and it is clear that about 75% of the genes that are accountable for the disease in humans are present in the fly as well. It is possible to manipulate the genetic system of the *Drosophila* that helps create a large variety of mutants that help study human genetic diseases and also analyse the origin of diseases.[4,5] The structure of mammalian alimentary canal has esophagus, intestine and large intestine which is similar to the structure of *Drosophila* alimentary canal where it has foregut, midgut and hindgut.[6] The association between the consumption of alcohol and *Drosophila* was primarily evaluated based on the environmental and developmental approach. Alcohol(ethanol) is ingested by the fruit flies as a byproduct of fermentation of nutrient medium that contain sugar. *Drosophila* consists of very small chain of alcohol dehydrogenase enzymes that rapidly respond to the alcohol present in the environment and thus it has become more resistant to the toxicity of alcohol and shows greater attraction towards alcohol and also helps to study the behavioral actions.[7]

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Alcohol and types of alcohol

Alcohol consumption has been in practice by humans historically which has influenced nutrition, caste, religion, and also assumed to change the quality of the lives of the people. Alcohol remains a controversial subject in the Indian society and will continue to be in the future.[8,9] Alcohol is mainly composed of ethyl alcohol in its fermented form which is absorbed in the stomach and small intestine. Alcohol absorption takes about 2 to 6 hours. After its absorption, it is distributed throughout the body. Almost 90% of the alcohol consumed is oxidized and the remaining alcohol is unaltered and excreted. There are some factors that affect the absorption of alcohol. If the consumption of alcohol occurs on a regular day to day basis it causes an increase in the rate of tolerance or the ability to resist higher amounts of alcohol. There can also be reverse tolerance where one cannot drink as much alcohol as the previous attempt.[10–12]

The most common types of alcohol that are used in manufacturing alcoholic beverages are ethyl alcohol, methyl alcohol and isopropyl alcohol. The commonly available alcohols in the market are beer, wine and spirit (distilled liquors).[13] Beer and wine are known to be in commercial use for several decades. There are several types of distilled spirits that have are in commercial use like brandy, whiskey, vodka, tequila, gin, and rum. The amount of ethanol present in beer ranges from 2.5 to 10% which is measured by weight. There are several types of beer that are available in the market with alcohol percentage as low as 1%. The ethanol content in case of wine is about 8 to 15%. The composition of ethanol in distilled spirit ranges from 50% to 100%. There is proof that indicates that intake of these alcoholic beverages causes various types of cancer including oral cancer, pharynx, larynx, liver, pancreatic and colorectal cancers.[14–18]

Components of alcohol and their effects on humans

Alcohol is a mixture of components. Higher alcohols are usually alcohols with more than two carbon atoms. Some amount of methanol present in alcoholic beverages with ethanol are produced by pectines. Some of the other components that are present in larger amounts are lead and ethyl carbamate which are also carcinogenic. Ethanol and acetaldehyde are first metabolites and are group 1 carcinogens. Non-alcoholic compounds are known to cause defects in the function of the pancreas, induces type 2 diabetes, osteoporosis, cardiovascular disease and also cancer [19]. Acetaldehyde is a metabolite of ethanol but the production of acetaldehyde other than ethanol metabolism induces risk for the drinkers resulting in the increase of salivary acetaldehyde causing oral cancer.[20] Chronic alcohol abuse will have serious effects on bone resulting in decreased bone mass which will ultimately lead to fragile bones. It also alters the composition of fat that has an impact on the bones. Excess alcohol on a regular basis also influences metabolic processes.[21] Alcoholic beverages also contain alcohols of low molecular weight other than ethanol, aldehydes, esters, low levels of lead, iron, histamines, cobalt, adulterants, tannins, phenols and many other inorganic and organic compounds which have various effects in physiology, behavior, cardiac activity. These congeners are present in varying quantities in different alcoholic beverages and hence it has different effects.[22] Some other compounds that are found in the alcoholic beverages in negligible amounts are amines, ammonia, amino acids, N-nitrosamines. Some phytocompounds such as carotenoids, isoprenoids and certain preservatives like sorbic acid, sulphur dioxide, velcorin, vitamin C, natamycin and hydroxy cinnamic acids which have minor effects are associated with major component ethanol.[23]

Role of alcohol in human physiology and behavior

Alcohol is widely consumed by humans as a psychoactive drug. The effect of alcohol on human behavior and physiological role is diverse but its mechanism of action and how it induces such effects remains inadequately understood.[24] Drug addiction may be considered as a process of disease or abnormal patterns of behavior. Alcohol or drugs do not themselves give rise to a behavior.[25] There are advanced research findings which help in the understanding of how alcohol influences specific mechanisms of behavioral patterns and how it also alters verbal behavior of the person.[26] The taste of alcohol also has an important role in altering the patterns of behavior. The sense of taste is primary in case of preferring different kinds of alcohol for consumption. Various types of alcohols have dissimilar oro-sensations depending on their taste, composition and their components inducing the alcohol disorder or changes in the patterns of the behavior. Many research evidences found out that consumption of alcohol mainly targets the brain resulting in various neurological and behavioral diseases. It is still debatable that over consumption of alcohol is one's lifestyle pattern and decreasing the intake of alcohol may reduce the harmful effects caused by it.[27,28] Yet, they are not sufficient to determine the alterted behavior in humans but they may be one of the causes for it.[29] Alcohol consumption is related to sexuality. It sometimes results in positive effects but it also has many uncommon and abnormal effects on the patterns of sexuality.[30]

Chronic and long-term alcohol exposure have various effects on the liver physiology (Figure 1) typically either directly or indirectly resulting in the structural change of the liver that leads to the formation of hepatic cellular carcinoma in some cases. Their mechanism is however unclear.[31]

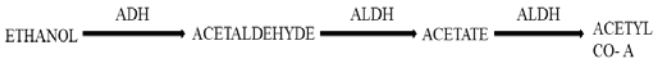


Figure 1. Metabolism of ethanol to acetyl CO-A

Lipid metabolism is associated with liver and it is downregulated by alcohol consumption and is likely to increase the risk of developing many advanced diseases.[32] Acetaldehyde which is a metabolite of ethanol is reactive in nature and can form DNA adducts and also resist the repair mechanism. It is also found that the high level of acetaldehyde causes changes in the activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) that are linked to the risk of hepatocellular carcinoma (HCC) in chronic drinkers.[33] The chronic consumption of alcohol is always related to the alterations in the central and peripheral structure and activity of the nervous system which gradually leads to irreversible and weakening activity.[34] The consumption of alcohol has severe defects ranging from alcohol intoxication to dementia. It is also suggested that alcohol has either direct or indirect effects on the central nervous systems which include intoxication, hangover, cerebellar affective disorder, stroke,

pellagra, encephalopathy and other alcohol-related neural diseases.[35]

Sexual, biochemical and physiological effects of alcohol

Alcohol addiction is one of the more frequent conditions associated with major physical and mental illnesses worldwide. Alcohol consumption has major effects on almost all the systems of the body including the gastrointestinal system, central nervous system, cardiovascular system, deficiency of nutrients and it also alters the hematopoietic system. The major effect of alcohol consumption is on the gastrointestinal system like gastritis, peptic ulcer, cirrhosis of the liver etc. It's effects on the cardiovascular system namely angina, hypertension and heart failure are also noted.[36] Alcohol associated liver disease is the primary effect of alcohol consumption however it can also affect the liver through other related diseases. The chief hepatic alteration due to alcohol consumption is fatty liver or steatosis. Steatosis influenced by alcohol consumption is more rapidly and voluntarily reversible on termination of alcohol consumption. The liver is the primary organ for the metabolism of lipid for the whole organism.[37] Alcohol readily affects the various characteristics of the hepatic flux which will eventually result in the accumulation of lipids.[38] The effect of sexual behavior in association with alcohol differs with gender, characteristics, culture, background, amount of alcohol consumed and duration of consumption. The increase in the percentage of alcohol results in increasing sexual arousal, erectile as well as behavior only to a certain limit but when the intake limit exceeds it results in sexual dysfunction, decreases sexual activity and responses.[39] Several studies suggest that alcohol is related to aggression in sexual behavior. The effects may contribute to a range of behavior and also strategies for sexual activity but its effects related to the consumption of alcohol must be studied. Sexual effect severely affects outcome like forced intercourse, rape attempt and rape execution.[40] Many studies on human and animal models state that over consumption of alcohol will lead to reduction in the quality of semen due to high ROS production.[41] One of the effects of alcohol is hangover which is a condition caused due to many factors like biochemical, neurological and genetics. Many research studies in animal models have explained that hangover is associated with many pathological conditions such as factors of inflammation, change in the neurotransmitter release and also the receptors of neurons, dysregulation of mitochondria, and alcohol metabolites.[42] Ethanol in alcohol is found to play a major role in the metabolism of folate and its bioavailability. It is affected by the intoxication of alcohol which increases the folate excretion in the urine when ethanol ingestion is increased. Ethanol affects the resistance in the synthesis of the reaction that is induced by the methionine synthase enzyme.[43]

MATERIALS AND METHODS

***Drosophila* culture and maintanence**

Drosophila melanogaster (*Canton S*) was used for this eperimental study. They were cultured on corn meal agar medium maintained at 12 hours of light and dark cycle. The flies were transferred into fresh food every 2 days in order to maintain the growth and fecundity. Every vial contained about 30-35 flies. These flies were used to study the behavioral studies and also for protein estimation.

The assays that were performed in this study were (Table 1):

1.RING assay
2. Larval crawling assay
3. Sedation assay
4. Tolerance assay
5. Protein estimation

Alcohol exposure

The types of alcohol used in this study were distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol. 10%, 15% and 25% concentrations were prepared for all alcohol types.

For RING assay andLarval crawling assay, the flies and larvae were exposed to all alcohol types at 10%, 15% and 25%(respectively) mixed in the media. For sedation assay, tolerance assay and protein estimation, the flies were incubated in 10% ,15% and 25% of all alcohol types.

Rapid iterative negative geotaxis assay

Flies demonstrate negatively geotaxis. Adult male flies were collected and anesthetized. It was then acclimatized until they adapted to the environment. 100 male flies were taken and were exposed for each concentration (10%,15% and 25%) of each alcohol type. Negative control (0%) and positive control (benzaldehyde) was also set up in the exposure chamber for about 15 mins. All the flies were exposed after they had been starved for 8 hours. These exposed flies were then transferred to the climbing apparatus.

1. The flies were allowed to settle at the bottom of the falcon tubes by knocking the apparatus on the surface and then the timer was set for 3seconds.

2. 20 trials were performed with a gap of 2 minutes followed by the 4th second.

3. The average height climbed by the number of flies were observed and determined for each concentration of each alcohol type and the difference in the ability to climb after the exposure was statistically analyzed and sorted.[44–46]

Larval crawling assay

Freely moving active 3rd instar larvae were scooped out from a well-bred bottle of flies. These larvae were washed in 1X PBS solution to remove excessive media 4 larvae were selected and exposed to a beaker that contained 5% of sucrose and four different alcohol types of different percentages (10%, 15% and 25%) and negative (0%) and positive control (benzaldehyde) for a time period of 15 minutes. It was then followed by transferring the larva to the petri plate that was already coated with 2% agarose and kept above a graph sheet that has a 0.2cm^2 grid. The larva was observed for a minute and the number of square grids traveled by the larva for 3 trials were determined. For each concentration 100 individual larvae were analyzed and observations were recorded.[47,48]

Sedation assay

100 to 125 adult female flies were sorted out from a well bred bottle of flies(without yeast), for each alcohol type and their concentrations. The following day the flies were then transferred to the exposure chamber of different alcohol types, negative and positive control. The timer was set up immediately. After every 3 minutes, the chamber was tapped on the surface of the bench and

flies were observed for a short duration in order to observe the behavior of the flies. The exposure was cut off once all the flies were sedated. The time taken was noted for each concentration and the results were compared for the effects of each alcohol and categorized.[49,50]

Tolerance assay

Rapid tolerance was determined in this study which was measured after 24 hours (except if indicated otherwise) after sedation was performed. The flies were sedated and transferred to the typical food medium without yeast. The next day the sedated flies were sedated again for the same concentration of different alcohol types and the time taken by the flies during the second exposure was noted and the results of each concentration and type of alcohol were tabulated and analyzed.[49,51]

Protein estimation

100 to 125 adult flies were collected and exposed to all the alcohol types of different concentrations (10%,15% and 25%), negative control and positive control. They were then homogenized using 100µl of homogenization buffer initially and 400µl of the buffer was added after thorough homogenization of flies. The sample was then spun at 2500 X g for 1 minute. 5µl of the supernatant was taken and mixed with 500µl of the Bradford reagent. It was incubated at room temperature for 5 minutes. It's optical density was measured at 595nm and the concentration of protein was determined using standards. The protein quantification was done and tabulated, compared with different alcohol and its concentration. Triplicates were done for each alcohol concentration.[52]

RESULTS

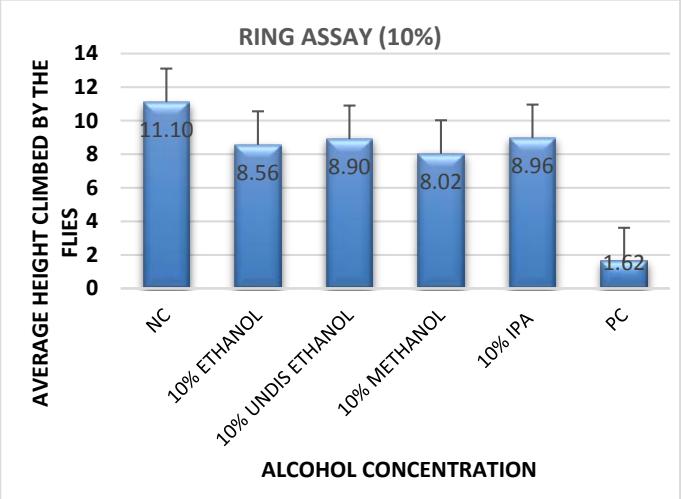
Ring assay

In this assay, apart from measuring the height climbed by the adult flies, the postural regain of the flies once they were knocked off on the benchtop was also observed. It was also observed that the flies were attracted towards alcohol. For each group about 100 male flies were sorted and the height climbed by each fly was determined. The number of flies were kept constant for repeated trials. 0% alcohol (n=100)

Table 1 Exposure time

S. No	ASSAY	FLY STAGE	NO. OF FLIES	ALCOHOL CONCENTRATION	EXPOSURE TIME
1.	RING assay	Adult fly (Male)	100	0%(NC),10%,15%,25% and PC	15 mins
2.	Larval crawling assay	3rd Instar Larva	100	0%(NC),10%,15%,25% and PC	15 mins
3.	Sedation assay	Adult fly (Female)	100	0%(NC),10%,15%,25% and PC	Exposed until the flies were sedated
4.	Tolerance assay	Adult fly (Female)	100	0%(NC),10%,15%,25% and PC	Exposed until the flies were sedated
5.	Protein estimation	Adult Flies	100	0%(NC),10%,15%,25% and PC	20 mins

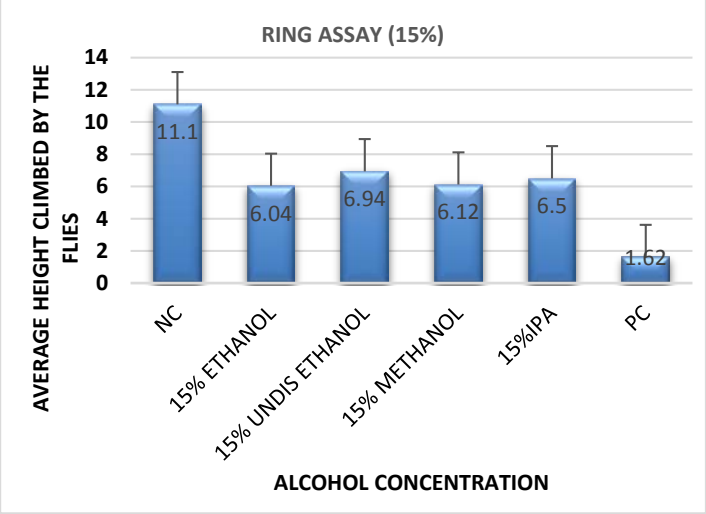
Young adult male flies were sorted and they were exposed to 0% alcohol, they climbed to an average height of 11.1 cm which was the maximum height flies could climb in the designed set up. The number of flies that remained at the bottom of the tube was determined to be a value of 0.



10% of alcohol (n=100 each alcohol):

Figure 2. RING Assay (10%)

Initially, there was an increase in the ability of the flies to climb a certain height but it slowed down after first few trials. The flies started to lose control in their posture resulting in decrease in height climbed. The number of flies settled at the bottom increased during the last few trials. An average height climbed by the flies was 8.56cm, 8.9cm, 8.02cm and 8.96cm respectively (Figure 2).

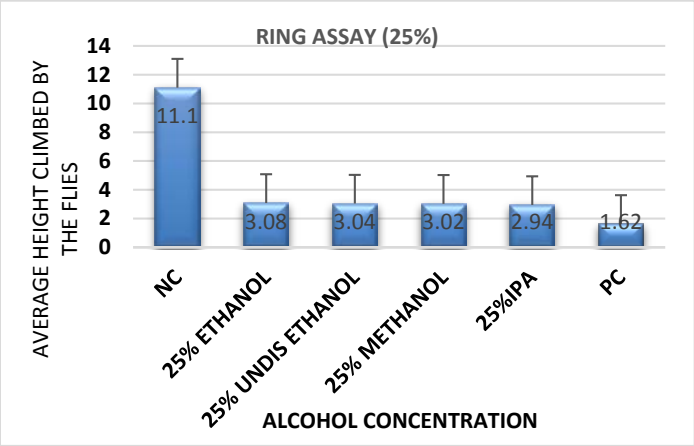


15% of alcohol (n=100 each alcohol):

Figure 3. RING Assay (15%)

The flies during the 2nd and 3rd trial were hyperactive and were able to reach maximum heights but after few trials the flies were desensitized and struggled to climb a certain height. During the last few trials there were certain number of flies at the bottom of the

tube without postural activity. The average height climbed was measured to be 6.04cm, 6.94cm, 6.12cm and 6.5cm respectively (Figure 3).



25% of alcohol (n=100 each alcohol):

Figure 4. RING Assay (25%)

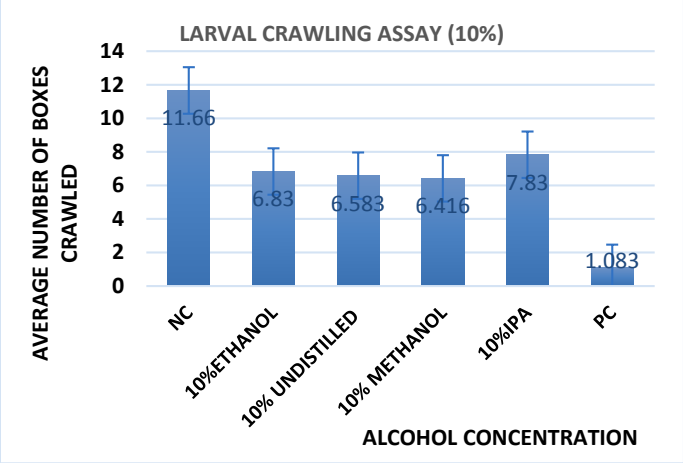
The average height climbed was found to be 3.08cm, 3.04cm 3.02cm and 2.94cm respectively. The flies showed some increased biphasic movement over the first few trials after which the ability of the flies to climb gradually decreased. There were significant number of flies which lost their postural control were found still at the bottom of the set up (Figure 4).

The results from this study suggested that there is a significant decrease in the ability of the fly to climb with increasing concentration. After alcohol exposure, some also showed hyperactivity followed by a reduction in their ability to climb compared to the negative and positive control after 10 trials for each concentration. At the highest concentration, the climbing height was at its lowest.

Larval crawling assay

3rd instar larvae were sorted out for this assay. In this stage, the larvae were able to metabolize alcohol.

0% alcohol (n=100)



10% alcohol (n=100)

Figure 5. Larval crawling assay (10%)

The larvae were exposed to 0% alcohol which exhibit their natural peristaltic movement. They had a constant movement throughout the trial. The larvae showed head lifting, turning and crawling. The average number of boxes crawled by the larvae in the negative control was determined to be 11.66 cm^2.

The larvae showed consistent peristaltic movement during the first two trials. Later, the larvae showed decrease in their movement with numerous head lifts, turning and sometimes no movement. The average number of boxes crawled by the larvae in the 10% of distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol were determined to be 6.83cm^2, 6.583cm^2, 6.416cm^2 and 7.83cm^2 respectively (Figure 5)

15% alcohol (n=100)

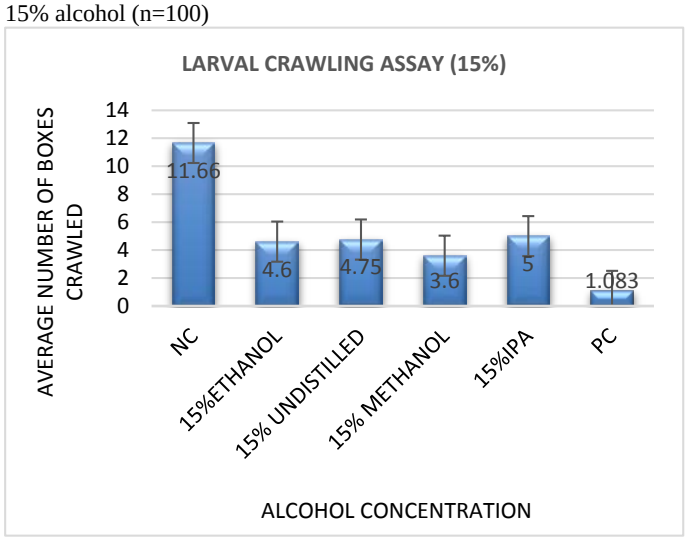
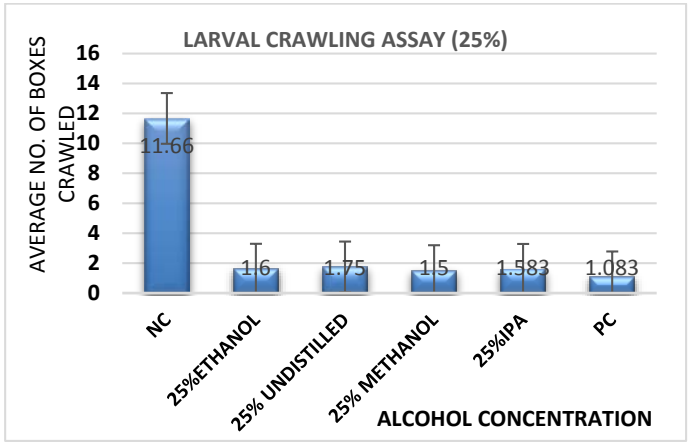


Figure 6. Larval crawling assay (15%)

These larvae did not observable peristaltic movements, however it crawled after trials over a few boxes with numerous turning head lifts and inconsistent peristatic movements. The average number of boxes crawled by the larvae exposed to 15% of distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol were found to be 4.6cm^2, 4.75cm^2, 3.6cm^2 and 5cm^2 respectively (Figure 6).



25% alcohol (n=100)

Figure:7 Larval crawling assay (25%)

These larvae did not show any peristaltic movement and their movement was still. Many numbers of turns was noted and these larvae stopped and stayed still for few seconds before continuing their movement. The number of boxes crawled by the larvae that are exposed to 25% of distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol were calculated to be 1.6cm^2, 1.75cm^2, 1.5cm^2 and 1.583cm^2 respectively (figure 7).

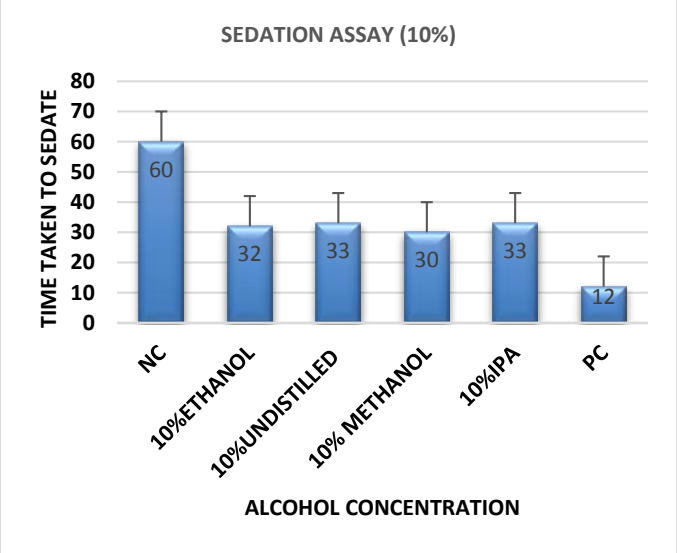
From the results of the larval crawling assay, it can be clearly noted that the ability of the larva to crawl decreased with increasing alcohol concentration. The larvae should demonstrate normal peristaltic movement. With the exposure to alcohol, the larval seemed to be in a confused state with multiple head lifts and turning in the same place and the larvae did not show any movement for a short duration. It was observed that the lowest number of boxes crawled was in its highest concentration.

Sedation assay

100 to 125 number of flies of the same sex were sorted out a day before, and were transferred to food vial without yeast. The next day these flies were exposed to the alcohol types and percentages and the time taken for the flies to get sedated was noted.

0% alcohol (n=100):

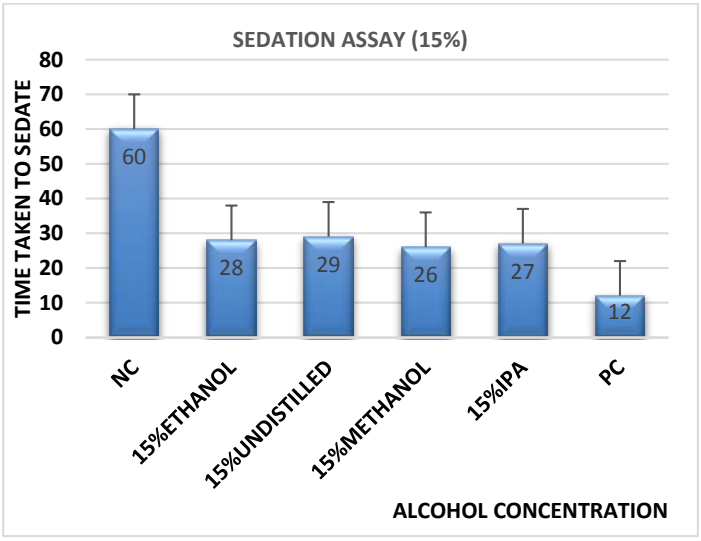
The flies were constantly active, climbing, jumping in the vials but after 60 minutes, the fly's ability to climb and jump slowed down and it became hypoactive.



10% alcohol (n=100):

Figure 8. Sedation assay (10%)

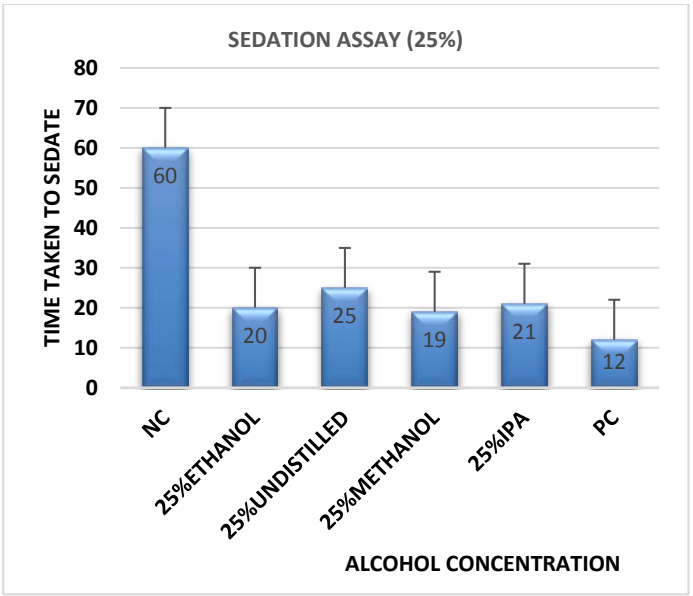
The flies that were exposed to 10% of the alcohol were hyperactive with numerous jumping and climbing. Later these flies slowed down in their ability to climb and jump and they were hypoactive. After 30 minutes, the flies started to startle did not show any movement and sedated. The time taken for the flies that were exposed to distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol were at 32 min, 33min, 30 min and 34min respectively (Figure 8).



15% alcohol (n=100):

Figure 9. Sedation assay (15%)

The flies were initially hyperactive with normal postural control for few trials. Later the movement of the flies slowed down and became hypoactive resulting in the loss of movement in the flies. The average time taken for the flies to sedate after being exposed to distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol were 28 min, 29min, 26 min and 27min which is comparatively lower than 10% alcohol (Figure 9).



25% alcohol (n=100):

Figure 10. Sedation assay (25%)

The exposed flies lacked their behavioral control ultimately becoming hypoactive leading to the loss of the movement and became still and desensitized. The time taken for the flies to get sedated when exposed to distilled ethanol, undistilled ethanol, methanol, and isopropyl alcohol was calculated to be 20min, 25min, 19min and 21min respectively which clearly indicates that when the

concentration increases the time taken for the flies to sedate decreases (Figure 10).

The sedation assay clearly suggested that the time taken for the flies to sedate gradually reduced with increasing concentration. After the alcohol exposure, the flies were too wild and were hyperactive but it significantly reduced upon continuous exposure and the flies were startled and then sedated. Flies exposed to the highest concentration of 25% took least time of 20 minutes to sedate.

Tolerance assay

In this study, acute tolerance was determined with the flies that were sorted out for the sedation assay were kept in the regular food vial(without yeast) for a period of 24 hours. The next day after sedation, the flies were exposed to the same concentration of the same alcohol type and the time taken was calculated.

0% alcohol (n=100)

The flies were again exposed to 0% alcohol 24 hours after sedation were observed to have normal postural behavior and movement. Later, it was observed that there is a decrease in their activity and flies started to settle at the bottom of the chamber.

10% alcohol (n=100):

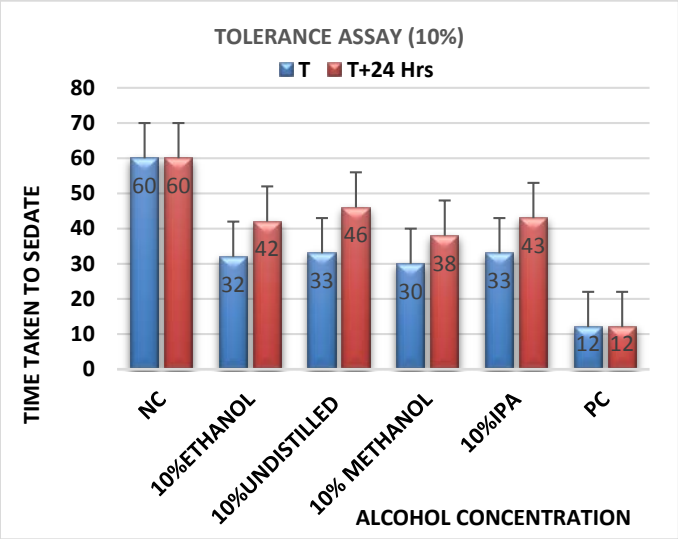


Figure 11. Tolerance assay (10%)

The flies that were sedated a day earlier were sorted and they were again treated with the same alcohol type for the tolerance assay. The flies were hyperactive and it took more time comparing to the previous day in order to get sedated. The time taken for the flies to get sedated after being treated with 10% of distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol was calculated to be 42min, 46min, 38min and 43min respectively (Figure 11).

The flies that were segregated for sedation and were maintained in corn meal agar without yeast. After 24 hours, those flies were treated again with the same alcohol type. The flies were active and had normal postural behavior. It was observed to be hyperactive and it took sometime even after being exposed to alcohol. The time taken for the flies to get sedated after 24 hours when treated with 15% of distilled ethanol, undistilled ethanol, methanol and isopropyl alcohol was observed to be 37min, 36min, 35min, and 34mins respectively (Figure 12).

15% alcohol (n=100):

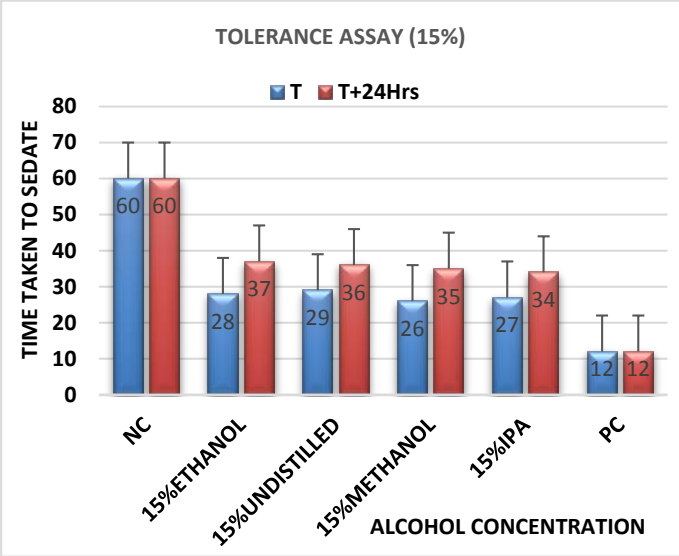
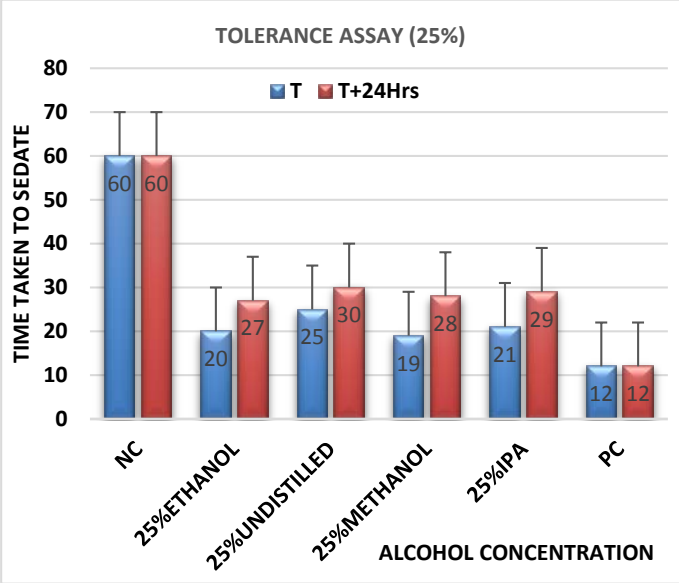


Figure:12 Tolerance assay (15%)



25% alcohol (n=100)

Figure 13. Tolerance assay (25%)

The flies were treated after 24 hours and these flies were normal in their behavior and the time taken for the flies to get sedated after the exposure to the different concentrations and alcohol types was observed to be 27min, 30min, 28min and 29min respectively which clearly states that the time taken with increases with increasing concentration. Comparatively time taken to get sedated after 24 hours also increased when exposed to same alcohol concentration (Figure 13).

From these observations, it was clearly evident that initially, the flies have taken time to get sedated with lower time period for the increasing concentrations but when the same flies were exposed after 24 hours to the same concentration and alcohol type, flies were hyperactive and did not seem to be affected by alcohol which then evolved slowly towards their confusions leading to sedation at an increased time period comparatively which indicated that the flies have significantly gained tolerance towards acute alcohol exposure.

Protein estimation

The estimation of protein helps us to understand about the effect of alcohol on the concentration of protein. In this study, the changes in the concentration of protein in the flies after it is treated with the four different alcohol types and their concentration for few minutes (acute exposure).

0% alcohol concentration (n=100):

The flies were treated with alcohol types and concentrations for the specific time period of 20 minutes and they were homogenized. The concentration of the protein was calculated by Bradford method.

Acute alcohol exposure showed a minor increase in the concentration of protein. From the results obtained, there is only a marginal increase in the protein concentration. The highest alcohol concentration exhibited a marked increase in protein concentration compared to negative and positive control. However, the determination of the protein type cannot be determined by this method and the results obtained for acute exposure contradictory to the earlier studies with chronic alcohol exposure (Figures 14,15,16,17)

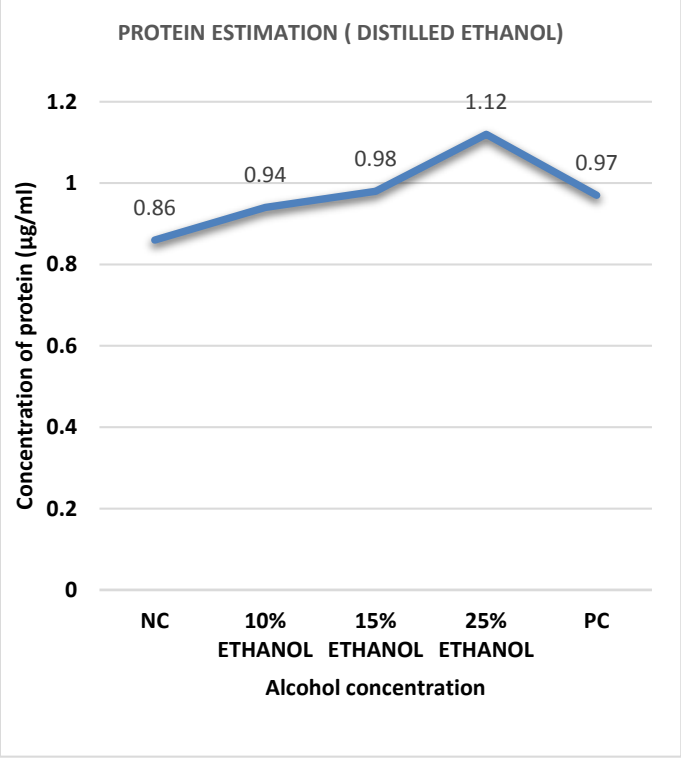


Figure 14. Protein estimation (Ethanol)

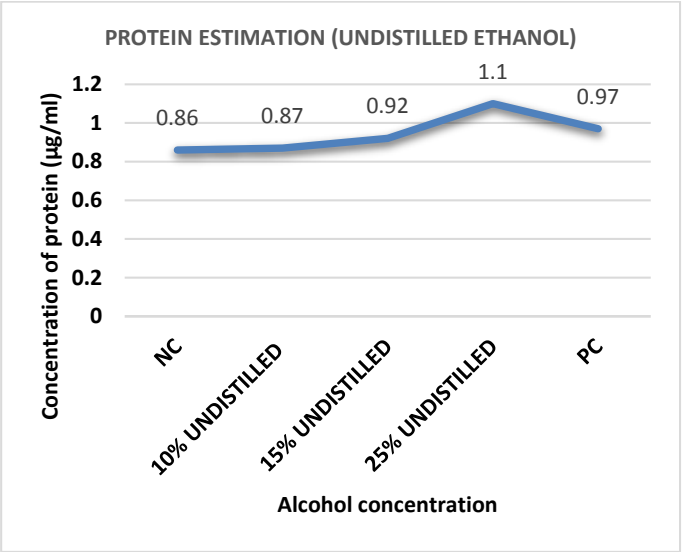


Figure 15. Protein estimation (Undistilled ethanol)

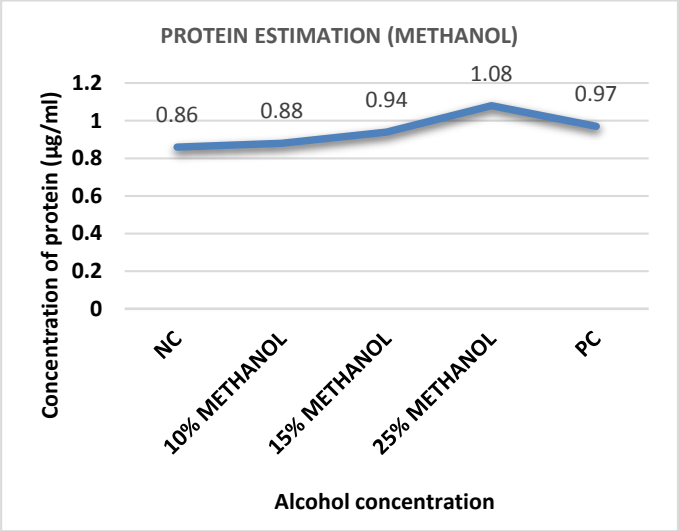


Figure 16. Protein estimation (Methanol)

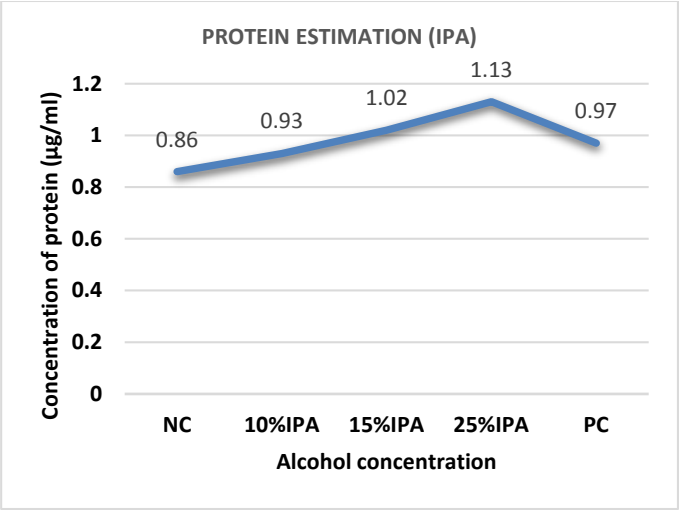


Figure 17. Protein estimation (Iso Propyl Alcohol)

DISCUSSION

Mammalian species are inter-related in many biological and life processes which requires a co-ordinated circuit in order to amplify the sensory inputs and their respective motor outputs. Alcohol intoxication have similar effects in case of living beings which includes alteration in behavioural patterns, resistance or decrease in their metabolic activity.

With the results obtained from this study, we can suggest that an increase in the concentration of all the four alcohol types will have a significant decrease in the behavioral pattern- locomotion, sedation.

Alcohol induced behavior in humans and other species can be recapitulated by *Drosophila* to a higher extent, including behavioral and neurobiological changes. The olfactory ethanol preferences are controlled by TbH in VNC and mushroom bodies of the flies [53]. One of the major alcohols induced neurobiological activity is known to be biphasic behavior, by means of stimulation of the nerves followed by the regression. Alcohol affects the dopaminergic system, GABA, and 5HT in flies by stimulating it at the initial stage and gradually decreasing it over a time period thus showing a biphasic behavior. The results obtained are in concordance with previous studies [54].

Further, in larval crawling assay, initially there was an increase in locomotion of the larvae at the 10% alcohol concentration which gradually reduced in the third and fourth trial due to its sedating effects. With 15% and 25% concentrations, marked difference in their locomotive ability was observed. Larval peristaltic movement is the result of coordinated contraction and relaxation of the muscles along the body that is controlled by central pattern generators which are present on the thoracic and abdominal segments of the VNC along with the neurons that are either excitatory or inhibitory [45]. Octopamine (OA) and Tyramine (TA) oppositely regulate the larval locomotion in which the OA is stimulated in the neuro muscular junction causing increased locomotion in case of hunger and TA stimulation reduces the locomotion in the larvae. This is in relation with this assay where alcohol preferences induce the larval locomotion and decreases after ethanol exposure. Therefore, the results determined are in concordance with the previous studies [55].

Alcohol induced sedation takes place normally in flies by a specific G protein coupled receptor called the dopamine/ecdysteroid receptor. DA does not have a direct impact on sedation through DopEcR but it has an indirect effect on other behaviors through DopEcR.[56] Also, Cp1 present in the cortex glia of the CNS in the flies regulate the sedation induced by alcohol. In this assay, the flies which are subjected to alcohol were hyperactive initially, followed by sedative behavior. The results also demonstrated that increased alcohol concentration took lesser time to sedate. The results obtained are in concordance with previous studies.[50]

Regular consumption of alcohol leads to tolerance which leads to alterations in the alcohol associated behaviors. It is a well-known fact that natural tolerance towards alcohol is present in flies as alcohol is the one of the components (as a part of its diet) necessary for its growth and development. It was demonstrated that alcohol related behavioral changes is essentially influenced by $GABA_B$ resulting in tolerance and addiction. Alcohol intoxication have direct increase in the production of GABA that are responsible for the alterations in the behavior. The results obtained from this assay suggested that the time taken for the flies to lose its posture after 24 hours of alcohol treatment resulting in the development of tolerance towards alcohol. The results obtained are correlated with the previous studies [57]

Protein estimation is an important biomolecule marker and necessary to study about various effects in the synthesis of protein. In this study after acute exposure of alcohol there was no significant increase in the amount of protein but only a marginal difference with increasing concentration of alcohol. Only the total amount of protein was determined in this study. Certain findings reported that with chronic exposure there is a decrease in the synthesis of protein. Here only acute exposure to alcohol was given to flies and the amount of protein was determined hence the results obtained are contradictory.[58,59]

CONCLUSION

In conclusion, this study compare the effects of different alcohol types that are commercially available at different concentrations in fruit fly. From the data obtained it was observed that different types of alcohol produce similar effects due to behavioral and biochemical studies. Some of the findings need to be analysed in case of chronic alcohol exposure in order to compare the actual effects of alcohol and their changes in the phenotype and genotype for future perspectives.

ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1.	ADH	Alcohol Dehydrogenase
2.	ALDH	Aldehyde Dehydrogenase
3.	HCC	Hepatocellular Carcinoma
4.	IPA	Isopropyl Alcohol
5.	RING	Rapid Iterative Negative Geotaxis
6.	PBS	Phosphate Buffer Saline
7.	NaOH	Sodium Hydroxide
8.	Na_2SO_4	Sodium Sulphate
9.	VNC	Ventral Nerve Cord
10.	OA	Octopamine
11.	TA	Tyramine

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CONFLICT OF INTEREST

The authors do not have conflict of interest for publication of this work as this study was not supported by any funding agency.

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