IJHSR International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Effect of Ethanol on Alkaline Phosphatase (ALP) Activity in Drosophila Melanogaster (Fruit Fly)

Elsheikh A. M. Obied

School of Health Sciences, Ahfad University for Women, Omdurman, Sudan.
Faculty of Pharmacy, Sudan International University, Khartoum, Sudan.

Received: 22/02/2016 *Revised: 14/03/2016* *Accepted: 15/03/2016*

ABSTRACT

Drosophila is usually found in fermenting fruit. This environment makes the fruit fly drosophila encounters high level of ethanol. Drosophila provides a model system for the examination of many developmental and cellular processes, which are preserved with mammals, including humans. This research study was carried out to investigate the role of ethanol on ALP activity in Drosophila. A wild type of Drosophila strains was obtained. A set of techniques were carried out to determine the role of ethanol on ALP activity in Drosophila. The finding showed that the rises in alcohol cause a significant reduction in ALP activity. The flies also showed a significant increase preference with ethanol concentration. Flies demonstrate increase in activity on exposure to a low to medium alcohol doses, whereas high doses can cause sedation. The results of this research evidence that alcohol ethanol regulates ALP activity in Drosophila by causing a reduction in ALP activity. Certainly, the ALP activity is reduced in Drosophila with increased, as a consequence of the exposure of ethanol, heat stress, time of exposure, or with the combinations of ethanol exposure and heat stress. It has been concluded that ethanol could reduce the activity of ALP in Drosophila, particularly under heat stress.

Keywords: ethanol, ALP activity, drosophila.

INTRODUCTION

Alcoholism causes worldwide social, economic and human health problems. [1] Although mammalian studies have offered major understanding of the molecular mechanisms underlying the consumption of alcohol, [2,3] organisms such as Drosophila melanogaster has been recognized as a model for understanding the genetic role in the behavioural responses to alcohol. [4,5] The behavioural responses to ethanol such as hyperactivity, and tolerance, are preserved among flies and mammals, [3,6,7]because are underlying molecular mechanisms. [3] Both flies and mammals showed locomotor stimulation at low doses and full sedation at higher doses. [8] Yet few studies have examined the self-administration of ethanol in Drosophila. [3] Flies showed tolerance on irregular exposure of ethanol, and they seem to prefer ethanol over other types of alcohols. [8] They attracted to the smell of ethanol that partly mediate ethanol preference, however, are dislike the ethanol taste. [3] Guarnieri and Heberlein [8] found that ethanol regulates the behaviours and mechanisms underlying Drosophila adaptation to long term exposure to ethanol. Other research studies found that ethanol cause a reduction in alkaline phosphatase activity (ALP). [9-11]

The effect of alcohol on the nervous system

Ethanol affects the nervous system directly, and indirectly. The behavioural results of ethanol action on brain neurochemistry, and the neurochemical consequences themselves, are extremely dose and time related. [12] Genetic factor and

gender play a vital role in the metabolism and behavioural action of ethanol, and dose of ethanol produces a feeling of pleasure, activation, and decrease of anxiety in some humans and animals can have unpleasant stimuli causes change in behaviour, sedative, or having no effect in others. [12]

Foetal alcohol spectrum disorder describes a variety of effects that can occur in a person exposed to alcohol in utero. [13] Too much prenatal alcohol exposure, severely affects the physical and neurobehavioral development of the child. The studies of autopsy and brain imaging show decrease and defectives on the whole size and shape of the brain, particularly in structures such as the cerebellum, basal ganglia, and corpus callosum. [14] Too much prenatal alcohol exposure severely affects the physical and neurobehavioral development of the child. The studies of autopsy and brain imaging show decrease and defectives on the whole size and shape of the brain, particularly in structures such as the cerebellum, basal ganglia, and corpus callosum. [14] A variety of neuropsychological insufficiencies were found in children prenatally exposed to alcohol, including insufficiencies in visuospatial functioning, verbal and non-verbal learning, attention, and executive functioning. These children also show a range of behavioural difficulties, which affect their every day functioning. [14]

Memory blackouts were primarily characterized in heavy drinking alcohol, however can be found with social drinking. [15] Alcohol consumption can also cause confusion, loss of balance and muscular co-ordination and loss of social inhibition, and in some situations violence, putting an individual at risk of traumatic injury. 13 Cerebral atrophy, specifically involving the frontal cortex, has been found in patients suffer from alcoholism. [13] The aetiology of cerebellar degeneration is multifactorial; however old age, inadequate nutrition and alcohol are significant factors. [13,16]

The most common effect of heavy alcohol consumption is a toxic polyneuropathy. It results from insufficient nutrition, specifically lack of thiamine and other vitamins B. Furthermore, there is a direct neurotoxin effect of ethanol. [17] Myopathic features, such as gait disturbances, cramps, local pain and decreased muscle mass can also found. This myopathy is found up to approximately 50% of alcohol abusers. [13,18] The indirect effect such as a lack of vitamin B1 includes Wernicke's encephalopathy and Korsakoff psychosis (or alcohol amnesic disorder). [13]

Drosophila as a model for drug addiction

Drosophila melanogaster is one of the most worldwide studied organisms in biology, and it provides a model system for the examination of many developmental and cellular processes, which are preserved with mammals, including humans. [19] Drosophila has a relatively complicated nervous system (about 300,000 neurons) and is able of much complex behaviour. [20] Flies are simple and cheap to take care of in the laboratory and their life cycle is only about two weeks. The main benefit of flies is the simplicity and extent with which they can be manipulated genetically. [21] Furthermore, the Drosophila euchromatic sequence analysis showed a high level of molecular similarity between flies and mammals. For instance, Drosophila has most main neurotransmitters, molecules implicated in the release and recycling of synaptic vesicle, receptors and channels for neurotransmission, and signal transduction mechanisms implicated in the function of neural in mammals. [21] But there are some distinguished differences. For instance, flies use acetylcholine rather than glutamate as the main excitatory central nervous systems neurotransmitter, and glutamate rather than acetylcholine at the neuromuscular junction. Additionally, flies have noradrenaline deficiency with octopamine carrying out noradrenaline many functions. [21,22] Significantly, genes involved directly or indirectly in the action of abused drugs are, in the large part, preserved. [21] Also multiple techniques occur to change the role of particular population of nervous system

cells, therefore permitting the definition of the neuroanatomical Loci, which regulate behaviours of interest. [21,23,24] The availability of these powerful tools has been used in the study of behaviours induced by abused drugs, including nicotine, cocaine, and alcohol in Drosophila. [8,21]As a result, the molecular genetics, neurochemical and neuroanatomical bases for responses to drugs of abuse in Drosophila are starting to be understood, showing a big amount of mechanistic preservation with mammalian system. [21]

In mammalian models, low doses of ethanol stimulate locomotion, while high doses reduce it. The acute stimulant effect of ethanol has been proposed to be a sign of its rewarding effects. In Drosophila, the exposure of ethanol rapidly potentiates locomotor activity in two phase's dose and time dependent manner. [7]

Alkaline phosphatase

ALP consists of a group of enzyme, which catalyse the hydrolysis of phosphate esters in alkaline environment, generating the organic radical and inorganic phosphate. [25] ALP has many isoenzymes, and it is mostly obtained from the liver, bones and in smaller quantity from placenta, kidneys, leukocytes and intestines. [25]

In this research project, a synthetic substrate; p-nitrophenylphosphate, will be used that is dephosphorylated by ALP resulting in p-nitrophenol (Figure 1), which is yellow, absorbing light maximally at 405 nm. [26]

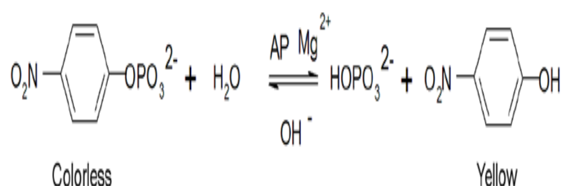

Fig 1: the reaction between ALP and p-nitrophenylphosphate. [26]

It has been found that the consumption of alcohol increases the level of ALP produced by the liver. One study observed the increased level of ALP in alcoholic patients, led to liver damage, increased permeability and hepatocytes necrosis. [27] Another observed study suggested that moderate ethanol intake has modest effects on the liver, include slightly increase in ALP activity. [28]

Sukhanova *et al.* [29] studies the effect of heat stress on ALP activity in two lines of Drosophila virilis different in the stress response of the dopamine system. It was showed that individuals of line 101, responding to stress by an increase in dopamine content respond by a reduction in the activity of ALP under the heat effect.

The sensitivity of alcohol in Drosophila

The usual home of Drosophila includes fermenting plants that frequently have high levels of alcohol (3 or more %). As a result, flies are resistant to alcohol toxic effects and can metabolise alcohol very well for use as source of energy or as a starting material (e.g. substrate) for the manufacture of lipids. [20,30] For examining if a specific Drosophila strain such as wild types is resistant to alcohol toxic effects, research studies added alcohol to the culture medium serving as the flies' food. [20,30] This result showed that Drosophila strains separated from the wild are different in their resistance to alcohol. [20,31,32] Furthermore, research studies showed that they can be rapidly and considerably increase a Drosophila resistance to alcohol in the laboratory. For instance, resistant strains were obtained by selectively breeding flies that survived exposure to high alcohol levels in their food. [20,33]

When exposed to alcohol vapour, Drosophila shows a lot of behaviours like acute intoxication in mammals such as damaged motor control. [20] To determine the effects of alcohol on locomotion, investigators placed flies into a small chamber that is covered with grid lines. Locomotor behaviour is determined with counting the number of lines of the grid crossed as a function of time. [20] Inside a few minutes of exposure, the flies begin to be hyperactive and confused and then uncoordinated and sedated. After about 20 minutes of exposure, they become motionless, however recovered 5 to 10

minutes after alcohol has been withdrawn. [20]

Research studies showed that in rodents, alcohol locomotor stimulating effects are adapted by nerve cell systems using dopamine. [20,34] To explore a potential role for dopamine in alcohol responses in Drosophila, investigators examined flies with severely decreased levels of dopamine for alcohol induced alters in locomotor behaviour using the experiment explained above. In these flies, alcohol ability to induce locomotor activation was significantly decreased; however, alcohol induced sedation was normal. [20,35] These data proposed that dopaminergic systems can play a similar role in the responses of rodents and Drosophila to acute exposure of alcohol. [20]

Aim

The aim of this research project was to assess the effect of ethanol on ALP activity in Drosophila.

The research hypothesis was that ethanol could reduce the activity of ALP in Drosophila, particularly under heat stress.

The Null hypothesis, ethanol could not show any reduction in the activity of ALP in Drosophila under heat stress.

In this research project, a set of techniques were carried out to determine the effect of ethanol on ALP activity in Drosophila.

MATERIALS AND METHODS

Drosophila strain

The strains of Drosophila melanogaster used in this research project were wild types. The strains were obtained from University of East London genetic laboratory.

The measurements of ALP activity

Procedure-1

The flies were homogenized on ice in 0.3 ml (300µl) 0.1M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. The enzyme activity in the supernatant was determined using α-naphthylphosphate as substrate and fast blue RR salt as stain. [36] After centrifugation, 50ul supernatant was transferred to 96-microwell plate to which 0.5 ml (50µl), 0.1M Tris-buffer pH 8.68, and 0.15 ml (150µl) of 10 ml reaction mixture were added (1ml α-naphthylphosphate, 1ml fast blue RR salt, 230 µl 10% MnCl, 230 µl 10% MgCl, 1ml polyvinylpyrrolidone, 1ml NaCl, 5.54 ml 0.1M Tris-buffer pH 8.68). [36] The incubation was carried out in room temperature at the dark for 25 minutes, over a period of 40 minutes, and the reaction was linear with time. [36] 3 ml of ice cold distilled water was added to stop the reaction. The absorbance product was read at 470 nm with an UV-vis microplate spectrophotometer.

Procedure-2

The flies were homogenized on ice in 0.3 ml (300µl) 0.1M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. The enzyme activity in the supernatant was determined using p-nitrophenyl phosphate. 2.5ml 10mM P-nitrophenyl phosphate stock solution, 0.04 ml of 0.02M $MgCl_2$, 7.46 ml 1M Tris phosphate buffer, pH 8.68 was prepared to make a reaction mixture of 10ml. After centrifugation, 50ul supernatant was transferred to 96-microwell plate to which 0.5 ml (50µl), 0.1M Tris-buffer pH 8.68, and 0.15 ml (150µl) of 10 ml reaction mixture were added. The incubation was carried out in room temperature at the dark for 25 minutes, over a period of 40 minutes, and the reaction was linear with time (Bogomolova *et al.,* 2010). 3 ml of ice cold distilled water was added to stop the reaction. The absorbance product was read at 405 nm with an UV-vis microplate spectrophotometer.

Measurement of different concentrations of the pure ALP

Different concentrations 1mM and 1µM of 15 U/mg p-nitrophenyl phosphate were prepared. 50 µl p-nitrophenyl phosphate stock solution (1mM and 1µM), 50 µl 0.1M Tris-buffer pH 8.68, and 150 µl reaction mixture (1mM p-nitrophenyl phosphate, 0.1M Tris-buffer pH 8.68, and 0.02M $MgCl_2$) were transferred to each 96-

microwell plates. Each concentration was measured at 10 minutes time intervals. The absorbance product was read at 470 nm with an UV-vis microplate spectrophotometer.

Measurement of different numbers of flies

5, 10, and 15 flies were homogenized in 0.3 ml (300µl) 0.1M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. The enzyme activity in the supernatant was determined using p-nitrophenyl phosphate. After centrifugation, 50ul supernatant was transferred to 96-microwell plates to which 0.5 ml (50µl) 0.1M Tris-buffer pH 8.68, and 0.15 ml (150µl) of 10 ml reaction mixture (2.5 ml 10mM p-nitrophenyl phosphate, 7.46 ml 0.1M Tis-phosphate buffer, pH 8.68, and 0.04 ml 10mM $MgCl_2$) were added to each plate. The incubation was carried out in room temperature at the dark for 25 minutes, over a period of 40 minutes, and the reaction was linear with time. The absorbance product was read at 405 nm with an UV-vis microplate spectrophotometer.

Temperature measurements

10 flies each were placed under 25^0C, 30^0C, and 37^0C temperature for 30 minutes. Flies were homogenised using 300µl 0.1 M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. 50µl supernatant, 50µl 0.1M Tris-phosphate buffer, pH 8.68, and 150µl reaction mixture (2.5 ml 10mM p-nitrophenyl phosphate, 7.46 ml 0.1M Tis-phosphate buffer, pH 8.68, and 0.04 ml 10mM $MgCl_2$) were transferred to 96-microwell plate. The absorbance product was read at 405 nm with an UV-vis microplate spectrophotometer.

Ethanol concentration measurements

Alcohol ethanol concentrations ranges 10%, 30%, 50%, and 80% were prepared. Each of the 10 flies was exposed to 2ml (2000µl) 10%, 30%, 50%, 80% ethanol respectively. The flies of the same genotype were exposed to ethanol vapours. The flies were exposed for 30 minutes. 10 flies for each concentration were homogenized in 0.3 ml (300µl) 0.1M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. After centrifugation, 50ul supernatant of each ethanol concentration was transferred to 96-microwell plates to which 50µl 0.1M Tris-phosphate buffer, pH 8.68, and 150µl 2.5mM reaction mixture (0.1M Tris-phosphate buffer, pH 8.68, 10mM p-nitrophenyl phosphate, and 10mM $MgCl_2$). The time waiting after adding the reaction mixture was 10 minutes. The absorbance product was read at 405 nm with an UV-vis microplate spectrophotometer.

Ethanol and temperature measurements

Alcohol ethanol concentrations ranges 0% 10%, 30%, and 50% were prepared. 10 flies each have been placed in 8 tubes and each of the 8 tubes was marked with two sets of temperature (e.g. 0% 25^0C, 0% 37^0C, 10% 25^0C, and 10% 37^0C.....). The flies were exposed to ethanol for 20 minutes. Then the flies were exposed to 25^0C and 37^0C temperatures respectively for 30 minutes. The flies for each alcohol concentration were homogenized in 0.3 ml (300µl) 0.1M Tris-phosphate buffer, pH 8.68. The homogenates were centrifuged for 5 minutes at 13,000 rpm. After centrifugation, 50ul supernatant of each ethanol concentration was transferred to 96-microwell plates to which 50µl 0.1M Tris-phosphate buffer, pH 8.68, and 150µl 2.5mM reaction mixture (0.1M Tris-phosphate buffer, pH 8.68, 10mM p-nitrophenyl phosphate, and 10mM $MgCl_2$). The absorbance product was read at 405 nm with an UV-vis microplate spectrophotometer.

Statistical analysis

GraphPad prism software was used to produce the results. Column statistics (one-sample t-test) was used to examine the significance of the differences between the data sets.

RESULTS

Comparison of assay techniques for ALP with different substrates concentrations and time courses

These assays have been carried out to set up the p-nitrophenyl phosphate concentrations that can be used in the research methods to determine the activity of ALP. And what are the correct p-nitrophenyl phosphate concentrations that can be used in this research study.

Fig 2 and 3 (procedure-1 and procedure-2) show the results of measurement of different p-nitrophenyl phosphate concentrations (150µl, 100µl, and 50µl). In fig 2, The P values of different substrate concentrations showed that there is no significant effect on ALP activity. In contrast to fig 2, fig 3 P values of different p-nitrophenyl phosphate concentrations also showed no significant effect on ALP activity. However, in this experiment the significant p value was not required. Fig 2 shows that there was a reduction in 100µl and 50µl concentrations compared with 150µl concentration. When the p-nitrophenyl phosphate concentrations decreased the activity of ALP is also decreased. By contrast, fig 3 shows that when the p-nitrophenyl phosphate concentrations increased the activity of ALP is also increased.

Fig 4 P values show no significant effect on ALP activity with different enzyme concentrations (1mM and 1µM) and different time courses (10min and 20min). However, when 1mM concentrations was compared with 1µM concentration, there was a reduction in ALP activity in 10min time 1µM concentration compared with 1mM concentration in 10min , and there was also a reduction in ALP activity in 20min 1µM concentration compared with 1mM concentration in 20min. The different concentrations of the pure enzyme were used to validate the research assays.

Optimisation of measurement in different numbers of flies

Different numbers of flies have been collected (5, 10, 15). The flies homogenised in buffer solution. The homogenates were centrifuged for 5 minutes. The enzyme activity in the supernatant was determined using p-nitrophenyl phosphate. The result that was produced (fig 5) showed no significant differences on ALP activity with different numbers of flies. However, the graph showed that when the numbers of flies increased the activity of ALP is also increased. However, 10 flies were preferred to 5 and 15 flies in this research to avoid hyposensitivity and inaccurate results with 5 flies, and hypersensitivity and difficulty obtaining the flies with 15 flies.

Effect of alcohol on ALP

In, fig 6 the flies show significant increase preference with ethanol concentration 50% (P=0.0325). It has been found that flies demonstrate increase in activity on exposure to a low to medium alcohol doses, whereas high doses can cause sedation. [5]

Effect of heat stress on ALP activity

Fig 6 shows that there was a significant decrease in ALP activity at 37^0C (P=0.0236) compared to 25^0C and 30^0C, whereas no significant reduction in ALP activity. This outcome indicated that when the flies exposed to heat stress (37^0C), the activity of ALP started to reduce.

Effect of alcohol on ALP with different times and temperatures

Fig 7 shows a significant reduction in ALP activity with ethanol at 25^0C (p=0.0131) with10min and 20min time different, and 30^0C (p=0.0150) with 20min time different. This result showed that the time and temperature differences play a crucial role in determining the activity of ALP.

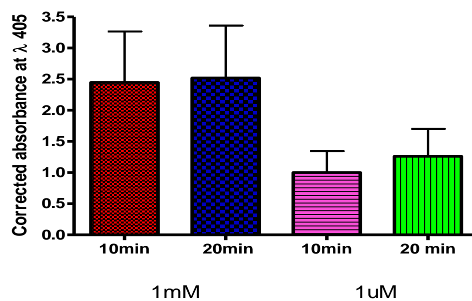

Fig 4: The effect on ALP activity with different concentrations of the pure enzyme and time courses

	10min	20min	10min	20 min
P value (two tailed)	0.0577	0.0577	0.0616	0.0645
Significant (alpha=0.05)?	No	No	No	No

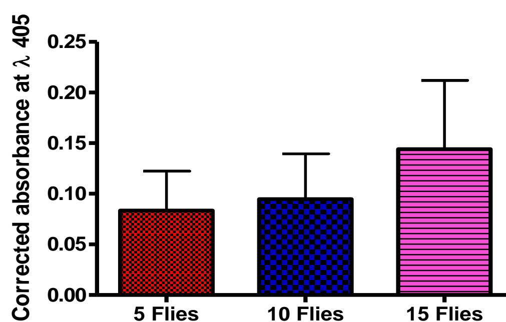

Fig 5: The effect on ALP activity using different numbers of flies

	5 Flies	10 Flies	15 Flies
P value (two tailed)	0.1210	0.1238	0.1245
Significant (alpha=0.05)?	No	No	No

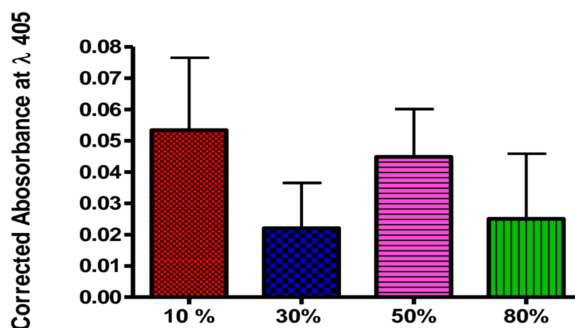

Fig 6: Different alcohol concentrations %

	10 %	30%	50%	80%
P value (two tailed)	0.0695	0.1898	0.0325	0.2839
Significant (alpha=0.05)?	No	No	Yes	No

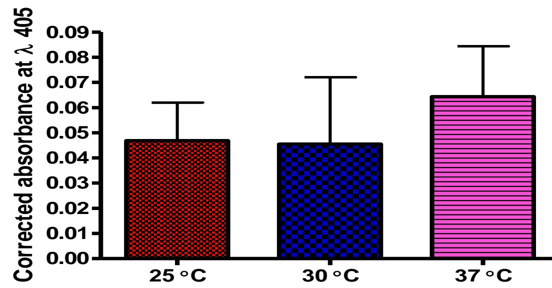

Fig 7: The effect of heat stress on ALP activity

	25 °C	30 °C	37 °C
P value (two tailed)	0.0272	0.1475	0.0236
Significant (alpha=0.05)?	Yes	No	Yes

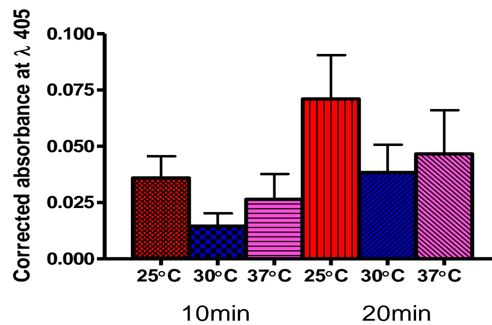

Fig 8: The effect of alcohol on ALP activity at different time and temperature

	25°C	30°C	37°C	25°C	30°C	37°C
P value (two tailed)	0.0131	0.0519	0.0650	0.0150	0.0267	0.0612
Significant (alpha=0.05)?	Yes	No	No	Yes	Yes	No

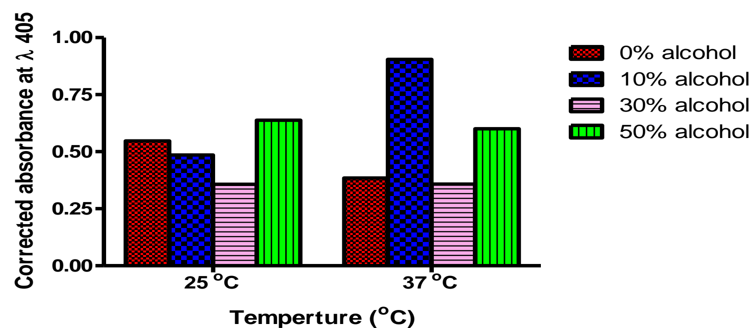

Fig 9: the effect of alcohol on stress response

	0% alcohol	10% alcohol	30% alcohol	50% alcohol
P value (two tailed)	0.1096	0.1868	0.0009	0.0195
Significant (alpha=0.05)?	No	No	Yes	Yes

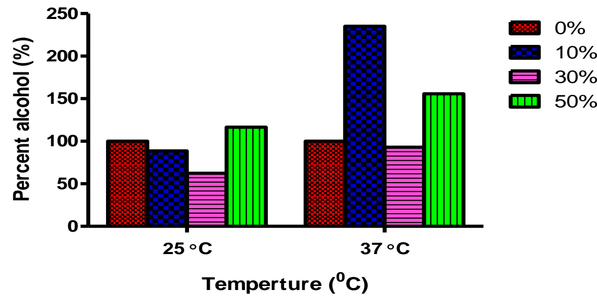

Fig 10: The effect of percent (%) alcohol on stress response

	0%	10%	30%	50%
P value (two tailed)		0.2703	0.1242	0.0910
Significant (alpha=0.05)?		No	No	No

DISCUSSION

It was demonstrated that when the p-nitrophenyl phosphate concentration decreased the activity of ALP is also decreased. And when the p-nitrophenyl phosphate concentrations increased the activity of ALP increased. ALP demonstrated superior substrate specificity to p-nitrophenyl phosphate. [37-39] It was observed that enzyme activity was increased with the increased in the substrate concentration and optimum activity. Additionally, the activity was constant. [37]

Fig 7 showed that the time and temperature differences play a crucial role in determining the activity of ALP. It has been found that when the flies exposed to heat stress (37^{0}C), the activity of ALP started to reduce. By contrast, Mahesh *et al.* [37] investigated the effect of temperature on ALP activity by incubating on different temperatures 10°C - 70°C per 10 minutes. The observation showed that ALP is thermos table, as the temperature increased the activity of ALP also increased and enzyme demonstrated its optimum activity at 65°C and has become inactivated at 70°C. [37] It found that the higher temperature increases the kinetic energy of molecules that break the bond that holding the active amino group and ALP became denatures. Therefore, results in the loss of ALP activity. [37]

Mahesh *et al.* [37] also investigated the effect of time on ALP activity by incubating p-nitrophenyl phosphate at 37°C for different time interval. When the incubation was completed for about 30 minutes, the optimum activity was obtained. After this as the time period increased the activity had become constant. This may be due to the thermal nature of ALP, with the increase in time period the temperature began breaking the bonds between two amino acids. [37]

As it has been mentioned in this research, earlier several research studies shown that ethanol cause a reduction in ALP activity under a particular conditions such as heat stress. [9-11] The results of this research evidence that alcohol ethanol regulates ALP activity in Drosophila by causing a reduction in ALP activity. Certainly, the ALP activity is reduced in flies with increased, as a consequence of ethanol exposure, heat stress, time of exposure, or with the combinations of ethanol exposure and heat stress (fig 6, 7 and 8). It has been hypothesized that ethanol could reduce the activity of ALP in Drosophila under heat stress.

Flies seem to have a stronger attraction to 50% ethanol concentration (fig 6). They showed increasing preference with 50%. So this concentration was used to determine the effect of ethanol on ALP activity (fig 7). The preference of a specific

concentration of ethanol in flies may be due to specific factors such as time of exposure. However, may be over a period of time the flies prefer more ethanol concentrations (e.g. 10% and 30%). Earlier in the result, fig 8 showed that under heat stress (37^0C), the increase in ethanol cause a significant reduction in ALP activity. There was also a considerable reduction in ALP activity with increased alcohol (10% and 30%) at 25^0C as compared to 0% alcohol. This result indicated that ethanol decreases the activity of ALP in Drosophila; particularly under heat stress [29] studies support the research finding that ethanol reduces the activity of ALP under heat stress. [29] found that the ALP activity was reduced under heat stress, but, with the increase of dopamine content not alcohol ethanol, however, and because flies are attracted to ethanol preferred it, [3] and on the basis of the results presented in this research, ethanol could reduce the activity of ALP in Drosophila, specifically under heat stress.

CONCLUSIONS

On the basis of the results presented in this research study, it was concluded that ethanol could reduce the activity of ALP in Drosophila melanogaster, particularly under heat stress.

REFERENCES

1. Morozova, TV. Anholt, RR. and Mackay, TFC. (2006) 'Transcriptional response to alcohol exposure in Drosophila melanogaster', *Genome Biology,* 7, R. 95.
2. Crabbe, JC. Phillips, TI., Harris, RA., Arends, MA. and Koob, GF. (2006) 'Alcohol-related genes: contributions from studies with genetically engineered mice', *Addict Biol,* 11(3-4), pp. 195-269.
3. Devineni, AV. and Heberlein, U. (2009) 'Preferential ethanol consumption in Drosophila models features of addiction', *Curr Biol,* 19(24), pp. 2126-2132.
4. Devineni, AV. and Heberlein, U. (2010) 'Addiction-like behaviour in Drosophila', *Communicative & Integrative Biology,* 3(4), pp. 357-359.
5. Rodan, AR. and Rothenfluh, A. (2010) 'The genetics of behavioural alcohol responses in Drosophila', *Int Rev Neurobiol,* 91, pp. 25-51.
6. Scholz, H., Ramond, J., Singh, CM. and Heberlein, U. (2000) 'Functional ethanol tolerance in Drosophila', *Neuron,* 28, pp. 261-271.
7. Wolf, FW., Rodan, AR, Tsai, LT-Y, Heberlein, U. (2002) 'High-resolution analysis of ethanol-induced locomotor stimulation in Drosophila', *The Journal of Neuroscience,* 22(24), pp. 11035-11044.
8. Guarnieri, DJ. and Heberlein, U. (2003) 'Drosophila melanogaster, a genetic model system for alcohol research', *Int Rev Neurobiol,* 54, pp. 199-228.
9. Zittle, CA. and DellaMonica, ES. (1952) 'Effect of aliphatic alcohols on bovine alkaline phosphatase', *Archives of Biochemistry and Biophysics,* 37(2), pp.419-424.
10. Goz, B., Stowe, AC. and Townsend, AJ. (1983) 'Effect of ethanol on alkaline phosphatase activity in HeLa cells', *Alcohol Clin Exp Res,* 7(2), pp. 176-179.
11. Kaun, KR., Azanchi, R., Maung, Z. et al. (2011) 'A Drosophila model for alcohol reward', *Nature Neuroscience,* 14, pp. 612-619.
12. Eckardt, MJ., File, SE., Gessa, G. (1998) 'Effects of moderate alcohol consumption on the central nervous system', *Alcohol Clin Exp Res,* 22(5), pp. 998-1040.
13. Yazan, A. And Brett, F. (2007) 'Alcohol and the nervous system', *Current Diagnostic pathology,* 13, pp. 203-209.
14. Riley, EP. And McGee, CL. (2005) 'Foetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behaviour', *Exp Biol Med,* 230(6), pp. 357-365.
15. White, AM. (2003) 'What happened? Alcohol, memory blackouts, and the brain', *Alcohol Res Health,* 27(2), pp. 186-196.
16. Nicolas, JM., Fernandez-Sola, J., Anunez, et al. (2000) 'High ethanol intake and malnutrition in alcoholic

cerebellar shrinkage', *QJM,* 93(7), 449-456.
17. Schuchardt, V. (2000) 'Alcohol and the peripheral nervous system', *Ther Umsch,* 57(4), pp. 196-199.
18. Preedy, VR., Ohlendieck, K., Adachi, J. et al. (2003) 'The importance of alcohol-induced muscle disease', Journal of *Muscle Research and Cell Motility,* 24(1), pp. 55-63.
19. Adams, MD., Celniker, SE., Holt, RA. et al (2000) 'The genome sequence of Drosophila melanogaster', *Science,* 287(5461), 2185-2195.
20. Heberlein, U. (2000) 'Genetics of alcohol-induced behaviours in Drosophila', *Alcohol Res Health,* 24(3), pp. 185-188.
21. Heberlein, U., Tsai, L-Y., Kapfhamer, D. et al. (2009) 'Drosophila, a genetic model system to study cocaine-related behaviours: a review with focus on LIM-only proteins', *Neuropharmacology,* 56(Suppl1), pp. 1-24.
22. Roeder, T. (1999) 'Octopamine in invertebrates', *Prog Neurobiol,* 59(5), pp. 533-561.
23. Kitamoto, T. (2001) 'Conditional modification of behaviour in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons', *Journal of Neurobiology,* 47(2), pp. 81-92.
24. Osterwalder, T., Yoon, KS., White, BH. And Keshishian, H. (2001) 'A conditional tissue-specific transgene expression system using inducible GAL4', *PNAS,* 98(22), pp. 12596-12601.
25. Wiwanitkit, V. (2001) 'High serum alkaline phosphatase levels, a study in 181 Thai adult hospitalised patients', *BMC Family practice,* 2:2.
26. Medical Biochemistry. Umea University (Medchem.umu) (2010) *Enzyme kinetics: theory.* Available at: http://www.medchem.umu.se/digitalAssets/35/35898_enzymkinetik_eng_18022010_yk.pdf (Accessed: 02 September 2011).
27. Mirunalini, S., Arulmozhi, V. and Arulmozhi, T. (2003) 'Curative effect of garlic on alcoholic liver diseased patients', *JJBS,* 3(4), pp. 147-152.
28. Ivester, P., Shively, CA., Register, TC. et al. (2003) 'The effects of moderate ethanol consumption on the liver of the monkey, macaca fasciculanies', *Alcohol Clin Exp Res,* 27(11), pp. 1831-1837.
29. Sukhanova, MZ., Grenback, LG., Gruntnko. et al. (1996) 'Alkaline phosphatase in Drosophila under heat stress', *Journal of Insect Physiology,* 42(2), pp. 161-165.
30. Geer, BW., Heinstra, PWH. and Mckechnie, S.W. (1993) 'The biological basis of ethanol tolerance in Drosophila', *Comp Biochem Physiol B,* 105(2), pp. 203-229.
31. Kamping, A. and Van Delden, W. (1978) 'Alcohol dehydrogenise polymorphism in populations of Drosophila melanogaster.lI. Relation between ADH activity and adult mortality', *Biochemical Genetics,* 16, pp. 541-551.
32. David, JR. and van Herrewege, J. (1993) 'Adaptation to alcoholic fermentation in Drosophila species: Relationship between alcohol tolerance and larval habitat', *Comparative Biochemistry and Physiology*, 74A, pp. 283-288.
33. Chambers, GK. (1991) 'Gene expression, adaptation and evolution in higher organisms. Evidence from studies of Drosophila alcohol dehydrogenases. *Comparative Biochemistry and Physiology',* 99B, pp. 723-730.
34. Phillips, TJ. And Shen, EH. (1996) 'Neurochemical bases of locomotion and ethanol stimulant effects', *Int Rev Neurobiol,* 39, pp. 243-282.
35. Bainton, RJ., Tsai, LT., Sing, CM. et al. (2000) 'Dopamine modulates acute responses to cocaine, nicotine, and ethanol in Drosophila', *Curr Biol,* 10(4), pp. 187-194.
36. Bogomolova, EV., Rauschenbusch, IYu. et al. (2010) 'Dopamine down-regulates activity of alkaline Phosphatase in Drosophila: the role of D2-like receptors', *Journal of Insect Physiology,* 56, pp. 1155-1159.
37. Mahesh, M., Guleria, N., Rajesh, TS. et al. (2010) 'Isolation and characterisation of extracellular thermostable alkaline

phosphatase enzyme from Bacillus SPP', *IJABPT,* 1(1), pp. 21-33.
38. Robert, RB. and Evan, RK. (2003) 'Characterization of a monomeric E.coli alkaline phosphatase formed upon a single amino acid substitution', *J Biol Chem*, 278, pp. 23497-23501.
39. Sebastian, Z., Jean, LR., Didier, H. et al. (2001) 'Characterization of highly thermostable alkaline phosphatase from the Euryarchaeon pyrococus abyssi', J. Appl Env Microbiol, 67, pp. 4504-4511.

How to cite this article: Obied EAM. Effect of ethanol on alkaline phosphatase (ALP) activity in drosophila melanogaster (fruit fly). Int J Health Sci Res. 2016; 6(4):254-265.

****

International Journal of Health Sciences & Research (IJHSR)

Publish your work in this journal

The International Journal of Health Sciences & Research is a multidisciplinary indexed open access double-blind peer-reviewed international journal that publishes original research articles from all areas of health sciences and allied branches. This monthly journal is characterised by rapid publication of reviews, original research and case reports across all the fields of health sciences. The details of journal are available on its official website (www.ijhsr.org).

Submit your manuscript by email: editor.ijhsr@gmail.com OR editor.ijhsr@yahoo.com