Investigation of erythrocyte cholinesterase inhibition and liver toxicity by artificial fruit ripener Ethereal *in vivo*

T.T. D. Chathuranga[1]

L. Dinithi C. Peiris[1] (Corresponding author)

[1]Department of Zoology (Centre for Biotechnology), University of Sri Jayewardenepura. Nugegoda, Sri Lanka

Tel: +94-12804515 E-mail: dinithi@sci.sjp.ac.lk

The research is financed by: University of Sri Jayewardenepura

Abstract

A novel liquid plant growth regulator dubbed ethereal (ET) employed primarily for artificial fruit ripening has been deemed a systemic health concern with documented reports of severe hepatotoxicity. The present study evaluated the viability of the use of Wistar rats (*Rattus norvegicus*) as valid test model for human acetylcholinesterase (AChE) through computational phylogenetics followed by investigation of the ET toxicity using male Wistar rats. The adverse effects were studied using a population of 36 male rats (n=9) who were orally treated either with 100, 250, 500 mg/kg of ET or Distilled Water (DW: Control) for 90 days. The reversible effects and delayed toxicity from ET exposure was studied using two separate sets of animals (n=6) who were gavaged with 500 mg/kg of ET and DW for 90 days (main group) and kept for another 28 days without treatment (recovery group). On days 91 and 119 erythrocyte cholinesterase (EChE), which is homologous to AChE and liver toxicity (ALT and AST levels) were determined. Treatment with ET induced a significant inhibition of EChE at both 250, 500 mg/kg groups and also in the recovery group. exploratory behaviour parameters, muscle strength and coordination, However, ET had Similar results were observed with ALT, AST levels both main and recovery groups. These effects were also evident by liver histopathology. It can be concluded that continuous exposure to ethrel could induce liver toxicity and alteration in EChE activity even after withdrawal from exposure.

Keywords: Fruit ripener, ethrel, ethephon, ALT, AST, erythrocyte cholinesterase acetylcholine esterase

1. Introduction

Fruits are an indispensable commodity with high nutritional value and commercial importance. They are vital components of a balanced diet providing vitamins and growth factors that are essential for maintaining a healthy wellbeing. In fact, a plethora of literature indicate that fruit consumption decreases risks for several health diseases including cancer, heart disorders, brain disorders and atherosclerosis (Rodriguez-Casado, 2016). However, as a consumable with global demand, the fruit horticulture industry is faced with pressing concerns in contriving a profitable and sustainable schema in fruit crop production. Namely, reduced post-harvest life and short ripening period are both major economic setbacks faced by the fruit farming industry (Asif, 2012). Solutions to these concerns entails employing a variety of chemicals such as ethylene, ethane, calcium carbide and ethereal (ET), as pre-treatments to improve consumer acceptance and for better marketing. These compounds, particularly ET, the trade name of the generic ethephon, essentially serve as artificial fruit ripening agents, which exploits and expedites the biochemistry of the natural ripening process constituting starch to sugar conversion, increased respiration rates and production of ethylene gas; the natural fruit ripening agent (Kulkarni, Kudachikar, & Keshava Prakash, 2011).

Ethrel, was first registered as an organophosphorus pesticide in the United States in 1995 and the active constituent of ET is ethephon (States & Substances, 1998) . It is now being widely used as an artificial ripening agent in Sri Lanka, and thus is the focus of the present study. This factoid is noteworthy, since suspending the breakdown of ET in to ethylene (the bioactive ripening agent) via pH regulation, ensures a uniform ripening time (Mohamed & Abu-Goukh, 2003). While improving the products market value, ET is also notorious for its organ wide damage to an organism's tissues. Ethephon being an organophosphorus compound, found to inhibit the plasma cholinesterase activity in mammals resulting in cholinergic effects specially affecting intestinal muscle. (Bhadoria, Bhadoria, Nagar, & Bahrioke, 2015; Haux, Quistad, & Casida, 2000). Cholinesterase's including acetylcholinesterase (AChE) and erythrocyte cholinesterase (EChE) are affected by ethephon. Ethylene, had shown to increase tumours, kidney weight, cause necrosis of the stomach and fibrosis in the heart (Bhadoria et al., 2015). Exposure can also result in liver damages (Çetinkaya & Baydan, 2010) and infertility ((Dissanayake, Keerthirathna, & Peiris, 2019; Peiris, Chathu, Perera, & Moore, 2019; L. D. Peiris & Moore, 2001).

Ethrel is used by Sri Lankan vendors to keep up with the increasing consumer demand. However, despite enforcing regulations on recommended doses and providing instructions on mode of application, failure to comply is a common problem among Sri Lankan fruit vendors. Recently, local newspapers reported that the Consumer Authority Agency (CAA) destroyed 75,000 kg of fruit from vendors who had directly sprayed ET unripe fruits in order to speed up the ripening process. The increasing trend of ET misuse warrants an extensive data mining effort to determine potential effects of long-term exposure. According to the literature, the toxic effects of the parental compound is greater than that of the active ingredient (El Raouf & Girgis, 2011).

It is important to study the mechanism of action of neurotoxicity exert by the active compound of ET through a proper model organism. Hence, here we investigated the neurotoxic effects of ET in Wistar rats exposed to chronic doses. Further, we also measured the liver toxicity.

2. Materials and methods

2.1 Test material

Commercially available ET (IUPAC name: 2 chloroethyl phosphonic acid; active ingredient: 480g/l ethephon; class- plant growth regulator; Purity: 98% pure) purchased from Harrisons chemicals (Pvt) Ltd Sri Lanka and is available in liquid formulation.

2.2 Dose regime

Male Wistar male (*Rattus norvegicus*), aged between 8-12 weeks, and weighing 250 -330g were obtained from the Medical Research Institute (MRI), Sri Lanka. Animals were kept in well ventilated plastic cages under ideal conditions (temperature 25 ±2°C; photoperiod approximately 12 h dark and 12 h light; relative humidity 55%) at the Faculty of Medical Sciences, University of Sri Jayewardenepura. Nutrition was in the form of pelleted food and tap water provided *ad libitum*. Ethical approval for the present study was obtained from the institutional Ethics Review Committee (No:24/14) and the research was carried out in agreement with the institutionally accepted principles for care and use of laboratory animals and guidelines. Upon acclimatization for one week, 36 male rats were divided randomly and three groups were treated orally with ET and the control group received DW. Ethrel was given at doses of 100, 250, 500 mg/kg/day for 90 days. The control group was administered with equal volumes of DW.

To study the recovery of animals following ET treatment, another 12 animals were divided randomly into two groups (recovery test) and treated with 500 mg/kg of ET or DW for 90 consecutive days. The animals were maintained for an additional 28 days without any treatment to determine the reversibility, persistence or delayed toxicity (Wills, McGlasson, Graham, & Joyce, 2007). Throughout the study period, animals were monitored for mortality and any clinical sign of toxicity following ET treatment. Animals were scarified and blood was collected from cardiac punctures the same procedure was followed for the animals in the recovery test groups at the end of 28 days.

2.3 Inhibition of erythrocyte cholinesterase activity

Erythrocyte Cholinesterase (EChE) activity was evaluated by the method describe by Mohammad, et al., (Mohammad, Alias, & Ahmed, 2007). In presence of EChE, hydrolysis of acetylcholine and production of acetic acid that subsequently decreases the pH of the reaction mixture. Cholinesterase activity was expressed as (Δ pH/incubation time) = (pH1 – pH2) - Δ pH of blank. pH was measured using a pH meter (Model: pH3110.WTW Co., Weilheim, Germany).

2.4 Behavioural test analysis

Behavioural tests were conducted after the treatment to measure motor function. From the 13th week onwards, the behaviour of animals was determined using a rat hole-board technique (13). Rat hole-board was an open field with a total area of 68×68 cm^2 and with four equidistant holes (3 cm diameter). When the rats were placed in the middle of the board, they moved about crossing the demarcated lines and dipped their heads in to the holes. Prodding their nose into any of the holes in the board is considered as a normal behaviour indicating inquisitiveness and utilized to determine the exploratory behaviour. Only one rat was tested each time. The head dip count and the dipping time (in seconds) was recorded for a period of five minutes each (time allowed for

curiosity behaviour). Head dip was scored only if both eyes disappeared into the hole. The hole-board was carefully cleaned with 5% ethanol before each animal was introduced. Same procedure was followed for the animals in the recovery test groups at the end of 28 days.

Muscle strength and co-ordination was evaluated using pole and bridge test. The elevated bridge test evaluates motor coordination and balance, while the pole test evaluates the locomotor action (Glat, Ben-Zur, Barhum, & Offen, 2016; Kudavidanage, Dissanayake, Keerthirathna, Nishshanke, & Peiris, 2020). On day 91 the rats were individually allowed to stand with their fore limbs on a horizontal bar (diameter: 0.8 cm and length: 36 cm) to determine their muscle stamina. The time taken for the rat to fall from the bar was determined. Subsequently, the bridge test was carried out to determine the motor co-ordination and the time elapsed to slide off was recorded. Same procedure was followed for the animals in the recovery test groups at the end of 28 days.

2.5 *In vivo* liver toxicity

Blood collected from the heart puncture was allowed to clot for 25-30 mins at room temperature. Subsequently, centrifuged at 3000 rpm for 10 mins to separate serum. Serum samples were investigated for ALT (alanine transferase) and AST (aspartate transferase) activities using enzyme test kit (Biolab Reagent, France). Absorption was measured using a spectrophotometer ((Labomed, INC. Los Angeles, USA) at 340 nm wavelength.

2.6 Histopathological study

Portions of livers from both treated, recovery and control animals were fixed in Bouin's fluid. Subsequent routine processing, paraffin sections of 6 µm thick were cut using a microtome (Philip Harris Limited, Shenstone, England). The sections were double stained using haematoxylin – eosin stains and mounted for microscopic examination (Olympus Corporation, Japan) for any signs of pathological changes.

2.7 Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and two sample *t*- test. Data were expressed as the mean± standard error mean (SEM). Significance level was set at $P< 0.05$.

3. Results

3.1 In vivo inhibition of AChE

The result indicated that activities of AChE in 100, 250 and 500 mg/kg BW of ET treated animals were significantly ($P<0.05$) lowered by 5.8%, 9.9% and15.8% respectively when compared to control group. Similarly, the animals from 500 mg/kg -recovery also exhibited a significantly ($P< 0.05$) lower AChE activity (by 14.6%) when compared to the respective control (Table 1),

3.2 Behavior testing

The results of all behaviour testes are summarized in Table 1. Animals of the 100, 250, 500 mg/kg BW of ET had no significant difference in the number of head-dips, crossing, rearing and blouses. Further, there was no difference between head-dipping duration between treated and control animals. Similarly, no significant

differences between ET treated and control animals were observed for the latency to fall off from the Bar and Bridge tests (Table 1).

3.3 Liver toxicity

As shown in Table 2, the serum ALT and AST enzymes of animals treated with 250 and 500 mg/kg of ET exhibited a statistically significant ($P< 0.05$) dose dependent relationship. Significantly ($p<0.05$) high levels of plasma ALT and AST levels was evident even in the recovery groups.

The liver cells of the animals treated with ET showed abnormal histological appearance compared to their respective control (Figure 2). The hepato-lobular architecture of the control animals exhibited a normal histological appearance. The vein in the center of the hepatic lobule is filled with blood and hepatocytes, arranged in form of cords are rounded to polyhedral shapes and radiate peripherally. Hepatocytes also showed the nuclei with clear nuclear membranes (Figure 8a). On the contrary, the animals treated with both doses of ET exhibited tissue damage with dilated portal triad accompanied with widespread cytoplasmic vacuolations (Figure 2b).

4. Discussion

The use of artificial ripening agents to expedite and scale-up production is a convention prompting much debate owing to the potential toxic effects to consumers. Ethereal (ET), widely purveyed by Sri Lankan vendors especially local fruit cultivators are suffering a severe paucity in data with regards to its potential chronic toxicity, both holistic, multi-faceted approach to toxicity is dearth. To meet the growing demand, vendors have resorted to the use of artificially ripened fruits. Though ET is widely used in Sri Lanka, its effects on public health are unknown.

A separate recovery group was maintained to study the post-treatment effects after the withdrawal of the treatment. Upon the longitudinal 90-day follow-up to demonstrate general clinical toxicity of ET treated male rats, no overt signs indicative of toxicity or mortality was observed. In fact, the treated rats indicated a body weight gain displaying indifference to the ET treatment. The recovery model (both the control and retreated samples at a maximum dosage of 500 mg/kg/day) followed a similar trend, indicative of no overall growth inhibition or hypothalamic growth-inhibiting effects induced by ET intake. These findings are in align with myriad of findings with combined inferences harmonizing with a value of greater than 2000 mg/kg of bodyweight, to produce significant body weight dips.

Cholinesterase belonging to serine hydrolase group show high affinity towards choline esters (Assis et al., 2018). Blood and tissue cholinesterase are diagnostic of nervous system impairment, whereby the predominant neurotransmitter acetylcholine is not degraded following post-synaptic depolarization, resulting in a protracted action potential, thus preventing repolarization, which can have exhaustively deleterious effects on nervous function. Thus, AChE is a highly pragmatic tool in diagnosing OP (organophosphorus pesticide) and carbamate-based insecticide poisoning (Kudavidanage, Enoka P; Peiris Dinithi, 2016; Mason, 2000). The EChE presents high degree of homology with AChE from neuromuscular junctions and constitutes the predominant form found in human blood (Assis et al., 2018). Erythrocyte ChE is often used as a biomarker of acute OP effects and it is comparable to the enzyme found in cholinergic synapses. Erythrocyte ChE, can be used as a biomarker for organophosphorus and carbamate insecticides is evident since it is the first specific target/barrier of the action

of these pesticides, besides plasma butyryl cholinesterase (Assis et al., 2018). Erythrocyte ChE can be considered as the best proxy to extrapolate OP inhibitory effects at the nervous system synapses (Chen, Sheets, Nolan, & Mattsson, 1999).Therefore, erythrocyte cholinesterase activity can be considered as the better biomarker than the plasma cholinesterase activity (Kwong, 2002), which was used in this study.

The activity of the EChE decreases individually with time accompanying the life-span of the cell and decreases as whole activity following the increasing oxidative stress (Lionetto, Caricato, Calisi, Giordano, & Schettino, 2013). AChE undergo slow spontaneous regeneration against anti-cholinesterase agents after 1-3 months compared to EChE (a few hours to days), thus increasing the chances of detecting such compounds after a longer exposure interval and indicating the prognostic importance of EChE (Thiermann, Szinicz, Eyer, Zilker, & Worek, 2005). The erythrocyte AChE specific activity presents higher correlation with the central nervous system AChE than other peripheral ChEs such as lymphocyte, platelet AChE and plasma beutyl-cholinesterase.

The EChE activity in the treated groups including the recovery group were inhibited in a dose dependent trend and had not recovered after 29 days. A 20-30% reduction in serum cholinesterase activity indicates exposure to cholinesterase inhibitor. More than 50% inhibition of cholinesterase activity is important in the diagnosis of chemical poisoning and indicates a hazardous status (Strelitz, Engel, & Keifer, 2014). The lowest enzyme inhibition percentage (5.82%) observed at dose level of 100 mg/kg and enzyme inhibition was increased by 9.93% and 15.75% respectively at 250 and 500 mg/kg dose levels in main test. Hence, the present study suggests that ET could be detrimental as reduction in erythrocyte cholinesterase activities are evident even at lower dose levels.

On the other hand, the highest percentage of enzyme inhibition (15.75%) is lower than the threshold reduction value (of < 50%) to substantiate poisoning. These results indicate that ET oral treatment at dose level of 500 mg/kg for 90 days did not behave like typical organophosphate. Chlorpyrifos, an organophosphate pesticide, displayed a 50% of enzyme inhibition for a dose level as low as 10 mg/kg when orally administered to adult rats for 30 days (Dhanushka & Peiris, 2017). Ethrel being a dibasic phosphonic acid does not act like a typical organophosphorus compound towards cholinesterase enzymes. However, ET phosphorylate serine residues in the active site of cholinesterase via phosphonic acid dianion. In the present study, percentage of enzyme inhibition at 500 mg/kg recovery test (14.62%) has not change significantly at the end of recovery period. This could be due to short time to restore depressed EChE levels to normal level. Furthermore, EChE is synthesized in the liver (Assis et al., 2018). Decrease in EChE is evident in liver diseases (Hor et al., 2018). Both ALT, AST levels and histopathology in the ET treated rats indicated liver damages which may be the cause for reduction of blood cholinesterase levels observed in the present study.

The lack of changes in the passive avoidance paradigm indicates that none of the treatments induced alterations in the acquisition or retention of the learned response. Exploration in an alien or novel environment is an essential part of normal behaviour (File & Wardill, 1975). However, sedative effects or muscle weakness as a result of ingestion of certain chemicals may reduce the scores of behaviour parameters in the standard rat hole-board test (Merino et al., 2018). Nonetheless, exploratory behaviour parameters such as rearing, head dipping, crossings, time per head dip and passed faecal boluses remained unaltered with respect to the control in the present study, indicative that ET at the applied maximum dosage of 500 mg/kg of body weight failed to influence rat exploratory behaviour. However, examination of muscle contractility and co-ordination with standard bar & bridge tests revealed a palpable effect of ET at the highest dose level. In fact, the latency to fall off bar and latency to slide off bridge employed as measures of muscle relaxation and co-ordination and a reduction of both parameters suggestive of skeletal muscle defect (Ratnasoonya, Peiris, & Amarasekera, 1994),

which indicated a significant difference amongst the control and maximum dosage treatment groups in the original group. However, the recovery test showed a reversing of the effect.

Hepatocyte analysis is the main diagnostic marker of toxicity and hence it is highly important in studying effects of various chemicals. Further, plasma enzymes produced by liver is a valid candidate to measure the toxicity of xenobiotics. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are valuable indices to detect inflammation and necrosis of the liver or hepatocellular damage (Klaunig, Li, & Wang, 2018). These indices can also be used as a quantitative assessment of the degree of liver damage (Limdi & Hyde, 2003). In the current study, both ALT and AST activities of 250 and 500 mg/kg of ET treated main test and recovery test (500 mg/kg of ET) were significantly different from respective control groups. This suggests, chronic administration of ET at higher doses may have hepatotoxic effects in rats. Histopathology refers to the microscopic examination of tissue sections to study the manifestation of disease (Hinton et al., 2018) and histopathology assessment in the liver was performed for all groups to determine liver damage which is characterized by the presence of fatty change, enlargement of hepatocytes, macro vesicular fatty change and obliteration of sinusoids of the liver and disorganized cords of hepatocytes (Klaunig et al., 2018). This was evident by the histopathological studies conducted in the present study. There was an increase in liver to body weight ratio at 500 mg/kg group. However, in the recovery group, though an increase of liver to body weight ratio was observed, it was not enough to substantiate damage. Increase of an organ weight is an indication of inflammation while a reduction in the same parameter can be adduced to cell shape changes and tissue movements thus becoming an indication of a possible toxic effect by a certain drug or chemical (Bhatia, Underhill, Zaret, & Fox, 2014). However, the liver weights were decreased indicating signs of non-alcoholic fatty liver disease or hepatic fibrosis, which is depicted by accretion of excess liver triacylglycerol leading to liver damage (Dixon, Bhathal, Hughes, & O'Brien, 2004) and this is evident by fat globules observed in the treated animals.

5. Conclusions

In summary, ET, the commercial variant of ethephon is a widely used artificial fruit ripener. It was analysed for toxicity using male Wistar rats. These organisms were proved to be a viable test candidate due to the genetic similarity of its Ache with that of humans as revealed through phylogenetic analysis. A range of parameters including biochemistry, morphology and behaviour was considered to provide a holistic approach to deducing ET's toxicity. Consequently, the experiment revealed a rise in liver ALT and AST and hepatocyte inflammation, alongside, a blood and tissue Cholinesterase reduction resulting in muscle relaxation. Upon physical and behavioural examination of body weights and exploratory behaviour via a bar and bridge test no overt signs of toxicity were observed. However, since these latter results are in disparity with most citable literature, a revised examination is warranted.

References

Asif, M. (2012). Physico-chemical properties and toxic effect of fruit-ripening agent calcium carbide. *Annals of Tropical Medicine and Public Health*, *5*(3), 150. https://doi.org/10.4103/1755-6783.98602

Assis, C. R. D., Linhares, A. G., Cabrera, M. P., Oliveira, V. M., Silva, K. C. C., Marcuschi, M., … Carvalho, L. B. (2018). Erythrocyte acetylcholinesterase as biomarker of pesticide exposure: new and forgotten insights. *Environmental Science and Pollution Research*, *25*(19), 18364–18376. https://doi.org/10.1007/s11356-018-2303-9

Bhadoria, A., Bhadoria, P., Nagar, M., & Bahrioke, V. (2015). Effect of ethephon on the liver in albino rats: A histomorphometric study. *Biomedical Journal*, *38*(5), 421. https://doi.org/10.4103/2319-4170.155589

Bhatia, S. N., Underhill, G. H., Zaret, K. S., & Fox, I. J. (2014). Cell and tissue engineering for liver disease. *Science Translational Medicine*, *6*(245), 245sr2. https://doi.org/10.1126/scitranslmed.3005975

Çetinkaya, M., & Baydan, E. (2010). Investigation of in vitro effects of ethephon and chlorpyrifos, either alone or in combination, on rat intestinal muscle contraction. *Interdisciplinary Toxicology*, *3*(1), 35–39. https://doi.org/10.2478/v10102-010-0002-6

Chen, W. L., Sheets, J. J., Nolan, R. J., & Mattsson, J. L. (1999). Human Red Blood Cell Acetylcholinesterase Inhibition as the Appropriate and Conservative Surrogate Endpoint for Establishing Chlorpyrifos Reference Dose. *Regulatory Toxicology and Pharmacology*, *29*(1), 15–22. https://doi.org/10.1006/rtph.1998.1256

Dhanushka, M. A. T., & Peiris, L. D. C. (2017). Cytotoxic and Genotoxic Effects of Acephate on Human Sperm. *Journal of Toxicology*, *2017*, 1–6. https://doi.org/10.1155/2017/3874817

Dissanayake, D. M. I. H., Keerthirathna, W. L. R., & Peiris, L. D. C. (2019). Male Infertility Problem: A Contemporary Review on Present Status and Future Perspective. *Gender and the Genome*, *3*, 247028971986824. https://doi.org/10.1177/2470289719868240

Dixon, J. B., Bhathal, P. S., Hughes, N. R., & O'Brien, P. E. (2004). Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss. *Hepatology (Baltimore, Md.)*, *39*(6), 1647–1654. https://doi.org/10.1002/hep.20251

El Raouf, A. A., & Girgis, S. M. (2011). Mutagenic, teratogenic and biochemical effects of ethephon on pregnant mice and their fetuses. *Global Veterinaria*, *6*(3), 251–257.

File, S. E., & Wardill, A. G. (1975). Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia*, *44*(1), 53–59. https://doi.org/10.1007/BF00421184

Glat, M. J., Ben-Zur, T., Barhum, Y., & Offen, D. (2016). Neuroprotective Effect of a DJ-1 Based Peptide in a Toxin Induced Mouse Model of Multiple System Atrophy. *PloS One*, *11*(2), e0148170. https://doi.org/10.1371/journal.pone.0148170

Haux, J. E., Quistad, G. B., & Casida, J. E. (2000). Phosphobutyrylcholinesterase: phosphorylation of the esteratic site of butyrylcholinesterase by ethephon [(2-chloroethyl)phosphonic acid] dianion. *Chemical Research in Toxicology*, *13*(7), 646–651.

Hinton, D. E., Baumann, P. C., Gardner, G. R., Hawkins, W. E., Hendricks, J. D., Murchelano, R. A., & Oklhiro, M. S. (2018). Histopathologic biomarkers. In *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress* (pp. 155–209). https://doi.org/10.1201/9781351070270

Hor, Y.-Y., Lew, L.-C., Lau, A. S.-Y., Ong, J.-S., Chuah, L.-O., Lee, Y.-Y., … Liong, M.-T. (2018). Probiotic Lactobacillus casei Zhang (LCZ) alleviates respiratory, gastrointestinal & RBC abnormality via immuno-modulatory, anti-inflammatory & anti-oxidative actions. *Journal of Functional Foods*, *44*, 235–245. https://doi.org/10.1016/J.JFF.2018.03.017

Klaunig, J. E., Li, X., & Wang, Z. (2018). Role of xenobiotics in the induction and progression of fatty liver disease. *Toxicology Research*, *7*(4), 664–680. https://doi.org/10.1039/C7TX00326A

Kudavidanage, Enoka P; Peiris Dinithi, D. (2016). EXPOSURE OF JUDO 40 ALTERS DNA INTEGRITY AND SPERM FUNCTION OF RAT Multidisciplinary. *EPRA Internat Onal Journal of Multidisciplinary Research*,

9(2), 11–19. Retrieved from https://www.academia.edu/29299680/EPRA_International_Journal_of

Kudavidanage, E. P., Dissanayake, D. M. I., Keerthirathna, W. L. R., Nishshanke, N. L. W., & Peiris, L. D. C. (2020). Commercial Formulation of Chlorpyrifos Alters Neurological Behaviors and Fertility. *Biology*, *9*(3), 49. https://doi.org/10.3390/biology9030049

Kulkarni, S. G., Kudachikar, V. B., & Keshava Prakash, M. N. (2011). Studies on physico-chemical changes during artificial ripening of banana (Musa sp) variety "Robusta." *Journal of Food Science and Technology*, *48*(6), 730–734. https://doi.org/10.1007/s13197-010-0133-y

Kwong, T. C. (2002). Organophosphate pesticides: biochemistry and clinical toxicology. *Therapeutic Drug Monitoring*, *24*(1), 144–149.

Limdi, J. K., & Hyde, G. M. (2003). Evaluation of abnormal liver function tests. *Postgraduate Medical Journal*, *79*(932), 307–312.

Lionetto, M. G., Caricato, R., Calisi, A., Giordano, M. E., & Schettino, T. (2013). Acetylcholinesterase as a Biomarker in Environmental and Occupational Medicine: New Insights and Future Perspectives. *BioMed Research International*, *2013*, 1–8. https://doi.org/10.1155/2013/321213

Mason, H. J. (2000). The Recovery of Plasma Cholinesterase and Erythrocyte Acetylcholinesterase Activity in Workers after Over-exposure to Dichlorvos. *Occupational Medicine*, *50*(5), 343–347. https://doi.org/10.1093/occmed/50.5.343

Merino, P., Santos-López, J. A., Mateos, C. J., Meseguer, I., Garcimartín, A., Bastida, S., … González-Muñoz, M. J. (2018). Can nonalcoholic beer, silicon and hops reduce the brain damage and behavioral changes induced by aluminum nitrate in young male Wistar rats? *Food and Chemical Toxicology*, *118*, 784–794. https://doi.org/10.1016/j.fct.2018.06.004

Mohamed, H. E., & Abu-Goukh, A. B. A. (2003). Effect of ethrel in aqueous solution and ethylene released from ethrel on mango fruit ripening. *Journal of Horticultural Science and Biotechnology*, *78*(4), 568–573. https://doi.org/10.1080/14620316.2003.11511665

Mohammad, F. K., Alias, A. S., & Ahmed, O. A. H. (2007). Electrometric measurement of plasma, erythrocyte, and whole blood cholinesterase activities in healthy human volunteers. *Journal of Medical Toxicology*, *3*(1), 25–30. https://doi.org/10.1007/BF03161035

Peiris, L. D. C., Chathu, P., Perera, D. D. B. D., & Moore, H. D. (2019). 1,3-Dinitrobenze-Induced Genotoxicity Through Altering Nuclear Integrity of Diploid and Polyploidy Germ Cells. *Dose-Response*, *17*(3), 155932581987676. https://doi.org/10.1177/1559325819876760

Peiris, L. D., & Moore, H. D. (2001). Effects of acute and chronic doses of methoxy acetic acid on hamster sperm fertilising ability. *Asian Journal of Andrology*, *3*(3), 209–216. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11561192

Ratnasoonya, W. D., Peiris, L. D. C., & Amarasekera, A. S. (1994). Analgesic activities of Murraya koengnigii leaf extract. *Medicinal Science Research*, *22*, 837–840.

Ratnasooriya, W. D., Peiris, L. D. C., & Jayathunga, Y. N. A. (1995). Analgesic and sedative action of monchrotophos following oral administration in rats. *Medicinal Science Research*, *23*, 401–403.

Rodriguez-Casado, A. (2016). The Health Potential of Fruits and Vegetables Phytochemicals: Notable Examples. *Critical Reviews in Food Science and Nutrition*, *56*(7), 1097–1107. https://doi.org/10.1080/10408398.2012.755149

States, U., & Substances, T. (1998). *Reregistration Eligibility Decision (RED)*. (September).

Strelitz, J., Engel, L. S., & Keifer, M. C. (2014). Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers. *Occupational and Environmental Medicine*, *71*(12),

842–847. https://doi.org/10.1136/oemed-2014-102315

Thiermann, H., Szinicz, L., Eyer, P., Zilker, T., & Worek, F. (2005). Correlation between red blood cell acetylcholinesterase activity and neuromuscular transmission in organophosphate poisoning. *Chemico-Biological Interactions*, *157–158*, 345–347. https://doi.org/10.1016/j.cbi.2005.10.102

Wills, R. B. H., McGlasson, W. B., Graham, D., & Joyce, D. C. (2007). Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and ornamentals. In *Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and ornamentals* (pp. 28–167). https://doi.org/10.1017/CBO9781107415324.004

Tables

Table 1: Exploratory behaviour parameters (no of rears, no of head dips, crossings and time per head dip), muscle strength and co-ordination of animals treated with ethereal for 90 days.

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	Recovery Test	
					Control	500 mg/kg
Exploratory behaviour tests						
No of rears	29.89±2.10	28.90±2.40	28.89±4.50	28.40±3.40	29.92±2.30	28.20±1.29
No of head dips	6.50±1.09	6.11±2.00	6.05±1.22	5.84±2.00	6.00±0.80	5.92±1.88
Crossings	31.00±3.22	29.98±2.22	29.00±2.34	28.77±2.89	30.00±3.34	28.88±2.36
Time/ head dip (S)	1.00±0.22	1.10±0.20	1.08±0.15	1.09±0.25	1.05±0.11	1.10±0.22
No of faecal boluses	1.42±0.14	1.32±0.25	1.35±0.22	1.56±0.23	1.44±0.33	1.66±0.35
Bar & bridge tests						
Latency to fall in the bar holding test(s)	20.50±1.87	20.98±1.44	20.85±1.12	20.67±1.22	20.00±1.35	20.83±1.45
Latency to slide off in the bridge test(s)	40.02±1.66	40.22±2.82	39.23±2.22	39.25±2.81	40.78±2.50	41.83±2.01

Data are mean ± SEM; *p <0.05 compared to control (n=6)

Table 2 Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and erythrocyte cholinesterase activity (EChE) activity of male rats exposed to ethereal and distilled water (control) for 90 days.

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	Recovery	
					Control	500 mg/kg
EChE activity (ΔpH/30 min)	0.292 ± 0.002	0.275±0.001*	0.263±0.001*	0.246 ± 0.001**	0.294±0.003	0.251±0.002**
Inhibition of EchE (%)	0	5.82	9.93	15.75	0	14.62
ALT activity (iu/l)	58.32±2.32	60.56±2.21	62.12± 3.24	68.58± 2.32**	62.32±3.32	70.62±4.22**
AST activity (iu/l)	56.63±6.23	57.41±2.07	59.91±1.43	67.69±3.71**	60.86±5.13	69.41±5.22**

Data are expressed as mean ± SEM; p <0.05 and **p < 0.01 compared to control (n=6)

Figures

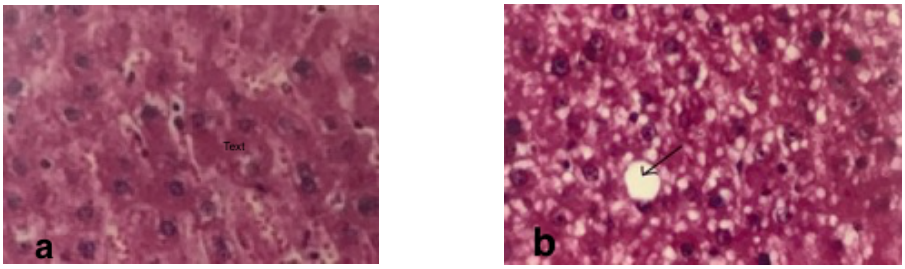

Figure 1. Photomicrographs of transverse section of the livers obtained from rats treated either with distilled water, control (a) or 500 mg/kg of ethereal (b) for 30 days (mag. x 200). Note numerous fat globules exhibited by the treated animals (b) indicating liver damage (indicated by arrows).