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Effect of propachlor toxicity on liver mice enzymes activity

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Abstract

The study was aimed to determined hepatocytes toxicity of propachlor pesticides. The effect was studied in mammalian system (mice) depended on evaluating the enzymatic activity of liver function tests (LFTs) enzymes: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP). The results showed that LFTs enzymes decreased after seven days of gulping mice. Conclusion, propachlor showed acute liver damage that reflected from LFTs activity when comparison with both vitamin C and phosphate buffer solution as a control.

Keywords: Propachlor, Toxicity, Enzymes activity, Liver, Mice.

Introduction

Hepatotoxicity is a general term for liver damage (Mousa, *et al*., 2013; Jaeschk *et al*., 2002). The symptoms of hepatotoxicity can be sign in damage of the liver which reflected in liver enzyme levels in the blood, when liver damaged, enzymes released in to blood stream, the levels can be measured by blood tests, these are called liver function tests enzymes (LFTs) (Keeffe and Friedman, 2004) included alanine transaminase (ALT), an enzyme present in hepatocytes, releases into the blood when hepatocytes damage, rises dramatically in acute liver damage, viral hepatitis or paracetamol overdose aspartate transaminase (AST), associated with liver parenchmal cells. Its raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore, not specific to liver (Nyblom *et al*., 2002; Aggarwal and Shishodia, 2006). Alkaline Phosphatase (ALP) is an enzyme in the cells lining the billary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue (Aggarwal and Shishodia, 2006). Liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents (Mumoli *et al*., 2006). Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ (Lancu *et al.,* 1986).Other chemicals agents ,such as those used in laboratories and industries, natural chemicals and Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins (Pak *et al*., 2004).

Propachlor is 2-chloro-N-isopropylacetanilide ($C_{11}H_{14}ClNO$), trade names: Ramrod, Bexton, and CP 31393 (Morgam, 2003). It is registered for use as a pre-emergence herbicide on corn (all types), soybeans (seed only), grain sorghum (milo), green peas, pumpkins, cotton, and flax. In corn, Propachlor can also be applied as an early postemergence control. Propachlor caused mammalian cell point mutation, cytogenetic damage and chromosomal aberration .In human lymphocytes, or other rodent/human cell line/strains (CDPR, 2003), cytogenetic test of chromosomal aberrations using bone marrow preparations of rats (HSDB, 2003). Due to the wide Propachlor using ranges, the study aimed to understand the residues toxicity of Propachlor on mammalian system, mice.

Material and Methods

Solutions:

- Phosphate buffer solution (PBS) (Hudson and Hay, 1980).
- NaOH (0.4N) prepared according to (Reitman and Frankel, 1957).
- Colchicine solution: Colchicine1mg (one tablet) and sterile distilled water 1ml .The solution was used immediately after preparing 2.5 to 3 hours (Allen *et al*., 1977).

Doses: Vitamin C (180 mg/kg) as comparative groups (Al-Kinani, 2005), propachlor pesticide in (500 mg/kg) as a positive control (CDPR,2003) and the PBS as a negative control (Al-Rubaie, 2005).

Experimental plan: To study the oxidant effect and the antioxidant in laboratory animals Propachlor solution was injected Intraperitonially because it lost after (3-12) hours by urine (CDPR, 2003; HSDB, 2003).

Preparing of liver serum: Weight 1 g from the liver and cut it to very small pieces by sharp knife in 1 ml from PBS and using in the same time the Mechanism pressure of hand to crush the liver tissue till be sticky solution then move the attain to the centrifuge with 9,000 rpm for 20 minutes. Get the upper layer and let the remainder in the bottom of the test tubes, avoid the fatty layer above it, store in freezer (-20)Ċ until evaluate (Lancu *et al.,* 1986; Pak *et al*., 2004).

Enzymatic assay: Enzymes activity of alanine transaminase (ALT), aspartate transaminase (AST) were measured according to Reitman and Frankel (1957), enzyme activity of alkaline phosphatase (ALP) were measured according to (King and King, 1945)

Statistical analysis: The statistical analysis has been used to study the effects of treatments in different trails. The least significant difference (LSD) test was used to signify a comparison between the means (SAS, 2001).

Results and Discussion

Liver function tests (LFTs) changes: The liver carries out numerous synthetic, excretion and detoxification functions, however only a minority of these can be measured by levels of products in the blood figure (1). Liver function testes (LFTs) measure the concentration of various different protein and enzyme in the blood that are either produced by liver cell or released when liver cells are damaged (Longmore *et al*., 2004; Braunweld *et al*., 2001).

Aspartate transaminase enzyme (AST): Table (1) expressed that positive treatment with propachlor related to low value of AST enzyme in the serum (21.52)u/l with significant (p≤0.05) comparing with the negative treatment (35.97)u/l, while comparative group showed high value in enzyme level (44.09)u/l, increasing significantly in comparative with negative and positive treatment. The AST result referred to caused heart attack, infectious mononucleosis, liver disease hepatitis and trauma when the level of this enzyme was increasing (Longmore *et al*., 2004; Braunweld *et al*., 2001)**.**

Alanine transaminase enzyme (ALT): Table (1) showed that the positive treatment as a result by using propachlor was lowing in ALT concentration reached to 44.09u/l and this result indicated to different significant in comparing with negative treatment (62.09u/l) and comparative group (70.04u/l) with p value (p≤0.05). The ALT results which showed caused hepatitis, cirrhosis and infectious mononucleosis(Longmore *et al*., 2004; Braunweld *et al*., 2001).

Alkaline phosphates enzyme (ALP): This enzyme is mainly implicated in the diagnosis of biliary abstraction and was normally found in small bile tracts in the liver, it is also found in the liver, bone, placenta and the evaluated levels may be due to a problem outside the liver such as a malignancy (cancer) (Longmore *et al*., 2004; Braunweld *et al*., 2001). Mice treatment with propachlor was showed significantly (p≤0.05) elevated (250.096u/l), comparison with negative treatment (152.061u/l) but not as Vit. C group (p≤0.05) Table(1).

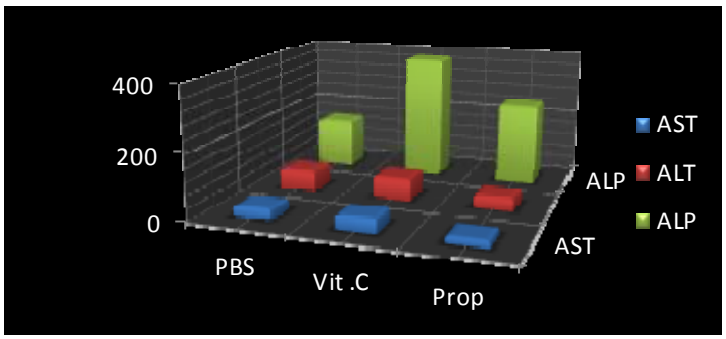

Figure (1): Liver function tests (LFTs) in the serum of white mice.

Table (1): Liver enzymes activity in the serum of white mice (means ±SE, n= 8 mice/group).

Enzymes	Treatments		
	Negative treat (PBS)	Comparative groups, Vit. C (180mg/kg)	Propachlor treatment (500mg/kg)
AST	35.97± 0.02	44.09± 0.25	21.52± 0.96*
ALT	62.09± 0.09	70.04± 0.47	44.09± 0.71*
ALP	152.061± 0.59	382.213± 0.09*	250.096± 0.99

* Probability (p≤ 0.05)

Propachlor is metabolized via the mercapturic acid pathway and the conjugates are excreted in the bile. The second cycle is initiated when the biliary mercapturic acid pathway metabolites are metabolized by microbial/intestinal C-S lyase into reabsorbable metabolites (possibly 2-mercapto- *N*-isopropylacetanilide). The reabsorbable metabolites are further metabolized to glucuronides by glucuronidase enzymes and these are secreted with the bile. These biliary glucuronides subsequently initiate the third cycle in the enterohepatic circulation of propachlor metabolites that cause damage of bile canal and leak in ALP enzyme. No doubt the intestinal microorganisms complicate the metabolic of propachlor (in comparison with the situation in germ-free and antibiotic-treated rats) and create new non-polar compounds from the products of the mercapturic acid pathway, which are reabsorbed into the blood. These new compounds have to be converted again into polar products in order to be excreted (Bakke *et al*., 1980).

Propachlor rate dose a among 25-1000 mg/kg appositive result in DNA repair assay reflect on hepatocytes cells and caused dead cells (Steinmetz and Mirsalis, 1986).

The oxidation which reflected from propachlor damage each of glutathione reductase (Larsen and Bakke, 1983) catalase enzyme, caused pressure on hepatocytes is weak and dead at last (Davison *et al*., 1990).

References

Aggarwal, B.B. and Shishodia, S. 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology, 71: 1397–1421.

Al-Kinani, E.B. 2005. Vitamins roles (E, C and A) in mending immunology and cytogenetic effects of etopside drug in white mice. M.Sc. thesis, Dep. Biology, Ibn-Hautham College, University of Baghdad, Iraq.

Allen, J.W.; Shuler, C.F.; Mendes, R.W. and Latt, S.A. 1977. A simplified technique for *in vivo* of sister chromatid exchange using 5-bromodeoxy-uridine tables. Cytogenetics, 18: 231–237.

Al-Rubaie, E.A. 2005. The antimutagenic effects of *Eruca sativa* and *Daucus carota* in bacteria and mammalian system. M.Sc. thesis, Genetic Engineering and Biotechnology Institute for Post Graduate Studies, University of Baghdad, Iraq.

Bakke, J.E.; Gustafsson, J.A. and Gustafsson, B.E. 1980. Metabolism propachlor by the germ-free rat. Sci., 210: 433-435.

Braunwald, K.; Fauci, H.; Kasper, L.; Hauser, S. and Longo, R. 2001. Jameson. Harrison's Principles of Internal Medicine. 15th ed., McGraw-Hill, New York.

CDPR, 2003. Actively Registered AI's by Common Name. California Department of Pesticide Regulation, California Environmental Protection Agency,

Davison, K.L.; Bakke, J.E. and Larsen, G.L. 1990. Metabolism of the glutathione conjugate of propachlor by in situ perfused kidneys and livers of rats. USDA, ARS, Biosciences Research Laboratory, Fargo, USA, 20(4): 375-83.

HSDB, 2003. Propachlor, Hazardous Substances Data Bank, accessed via TOMES. National Library of Medicine.

Hudson, L. and Hay, F.C. 1980. Practical Immunology. 2nd ed., Black Well Scientific Publ.: London.

Iancu, TC.; Shiloh, H. and Dembo, L. 1986. Hepatomegaly following short-term high –dose steroid therapy. J. Pediatr. Gastroenterol. Nutr., 5(1): 41-6.

Jaeschk, H.; Gores, G.J. Cederbaum, A.I.; Hinson, J.A.; Pessayre, D. and Lemasters, J.J. 2002. Mechanisms of hepatotoxicity. Toxicol. Sci., 65(2): 166-76.

Keeffe, E.B. and Friedman, L.M. 2004. Handbook of liver diseases. Edinburgh: Churchill Livingstone, London. 104-123 pp.

King, P.R. and King, E.G. 1945. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. Clin. Path., 7(4): 322–326.

Larsen, G.L. and Bakke, J.E. 1983. Metabolism of mercapturic acid-pathway metabolites of 2-

chloro-*N*-isopropylacetanilide (propachlor) by gastrointestinal bacteria. Metabolism and radiation research laboratory. Agric. Res. Serv., (13)2: 115-126.

Longmore, P.; Wilkinson, D. and Rajagopalan, L. 2004. Oxford Handbook of Clinical Medicine. 6th ed., Oxford University, Londom.

Morgan, J. 2003. Evidence on the developmental and reproductive toxicity of propachlor. Reproductive and cancer hazard assessment section. Office of environmental health hazard assessment. California Environmental Protection Agency.

Mousa, N.; Eman, A.M.; Shahad, S.S. and Ishrak, A. 2014. Liver histopathological of purification cinnamic acid activity against endoxan in mice. J. Genet. Environ. Resour. Conserve., 2(1): 22-29.

Mumoli, N.; Cei, M. and Cosimi, A. 2006. Drug related hepatotoxicity. North England J. Med., 354(20): 2191-2193.

Nyblom, H.; Berggren, U.; Balldin, J. and OLsson, R. 2002. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol and Alcoholism, 39 (4): 336–9.

Pak, E.; Esrason, K.T. and Wu, V.H. 2004. Hepatotoxicity of herbal remedies: an emerging dilemma. Progress in transplantation. Aliso Viejo. Calif., 14(2): 91-6.

Reitman, S. and Frankel, S. 1957. Biochemical analysis : Amer. J. Clin. Path. 28: 56.

SAS, 2001. SAS/STAT user's guide for personel computers release. 6.12. SAS. Inst. Inc. Cary, NC, USA.

Steinmetz, L. and Mirsalis, C. 1986. Evaluation of the potential of propachlor to induce unscheduled DNA synthesis in the *in vivo- in vitro* rat hepatocyte DNA repair assay. Menlo Park, California, Stanford Research Institute, 37 pp.