Niger. J. Physiol. Sci. 37 (June 2022): 119 – 126
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full-Length Research Article

Hepatoprotective and Renoprotective effect of *Moringa oleifera* Seed Oil on Dichlorvos-induced Toxicity in Male Wistar rats

Saka W. A., Adeshina O. A., Yusuf M. G., Omole A. I.

Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

Summary: The liver and the kidney are vital organs of the body. Drug-induced toxicity is one of the most common problems encountered by these organs. The search for an effective medicine to treat this toxicity without any side effects has led to the use of traditional-based medicine. This study evaluated the effect of ethanolic extract of *Moringa oleifera* seed oil on hepatic and renal markers in dimethyl 2, 2-dichlorovinyl phosphate (DDVP, known as dichlorvos)-exposed Wistar rats. Twenty-one male Wistar rats were randomly divided into three groups of seven animals each. Group A served as the negative control and was not exposed to dichlorvos. Group B served as the positive control and was exposed to dichlorvos for 2 minutes but received no extract. Group C was exposed to the dichlorvos and received 300mg/kg of extract (*Moringa oleifera* seed oil) for 7days before and 21days after exposure. Exposure to DDVP led to a significant increase in hepatic & renal markers, inflammatory markers, decrease in plasma protein and alteration of plasma electrolyte. *Moringa oleifera* seed oil regulated and significantly enhanced plasma protein, reduced elevated level of hepatic & renal markers, inflammatory markers in the study sample. In addition, histopathology observation showed that Moringa seed oil was able to regenerate the hepatorenal damage on exposure to dichlorvos. *Moringa oleifera* seed oil exhibited hepatoprotective, nephro-protective properties and could be explored in nutrition and health.

Keywords*: Hepatic, Renal, Histopathology, Moringa oleifera, Dichlorvos.*

**Address for correspondence: E-mail: mshehu84@gmail.com, wsaka@lautech.edu.ng; Tel: +2348063101718*

Manuscript received- December- 2021; Accepted- April, 2022

DOI: https://doi.org/10.54548/njps.v37i1.15

INTRODUCTION

The liver and kidney are crucial organs required by the body to maintain gastrointestinal homeostasis and bodily function. Other functions of these organs include regulation of internal milieu such as detoxification and excretion of toxic exogenous and endogenous metabolites and drugs (Ferguson *et al*., 2008; Najafian *et al*., 2014). Thus, its optimal function is essential for health and diseases (Saleem *et al*., 2008). Various drugs and chemicals are used experimentally to induce liver and renal damage, which are assessed in the laboratory by physiological, biochemical, and pathological biomarkers. Hepatotoxin and Nephrotoxin, such as dichlorvos, malathion, gentamicin, acetaminophen, and Nickel, have been used for ages to cause hepatic and renal dysfunction in rats (Sohn *et al*., 2009; Adeyemi and Elebiyo, 2014; Sief *et al*., 2014; Dungca, 2016; Ajao *et al*., 2017; Nwaichi *et al*., 2019). Potential hepatoprotective and nephroprotective agents are screened for efficacy and safety by observing their effects on these preclinical models, and their utility in clinical conditions is predicted (Sharma and Paliwal, 2012).

Dichlorvos, an organophosphate pesticide,is reported to cause toxicity tothe reproductive system (Joshi *et al*., 2003), pancreas (Hagar and Fahmy, 2002), kidney and spleen (Verma and Srivastva, 2003), liver and lung (Owoeye *et al*., 2012), brain (Mogda *et al*., 2009; Diwivedi *et al*., 2010) and immune system (Neishabouri *et al*., 2004).The blood and other body fluids are the central transport system of the body, and any alteration caused by chemical intoxicant, pathogen, injury, and stress is reflected by changes in the blood picture and vital internal organs such as the liver and kidneys (Ihedioha *et al*., 2004; Poiout-Belissent and McCartney, 2010). Like another organophosphate, the mechanism of toxicity of dichlorvos is the inhibition of acetylcholinesterase (AchE), which is attributed to the accumulation of acetylcholine, thus leading to excess stimulation of parasympathetic nerves (Wang *et al*., 2004). This stimulation resulted in perspiration, nausea, lacrimation, vomit¬ing, diarrhea, excessive bronchial secretion and death (ATSDR, 1997). Other effects on skeletal muscles and the nervous system include muscle cramps, muscle fasciculation, muscle weakness, flaccidity, drowsiness, fatigue, mental confusion, headache, convulsions, coma, and even death (ATSDR, 1997). However, new methods and drug investigations are needed for protective clinical treatment against hepatorenal damage caused by organophosphorus pesticide toxication (Ihedioha *et al*., 2004). Several antidotes have been assessed for the regular treatment of organophosphorus pesticides poisoning (O.P.s), and the currently proposed drugs are atropine and pralidoxime chloride (Yadav *et al*., 2012). Atropine has been used as an antidote against O.P.s for ages, as it effectively blocks the muscarinic receptors, but not nicotinic

receptors, against the toxic effects of AchE (Gunay *et al*., 2010). Side effects of these drugs include headache, salivation, blurred vision, vomiting, etc. (CEPA, 1996). The need for effective medicine with minimal side effects has led to traditional-based treatment.

Medicinal plants have been essential in protecting the body system against harmful chemicals or drugs that may cause oxidative stress and alteration in antioxidant enzyme and non-enzymatic systems (Alabi *et al*., 2005). Among many plants, Moringa oleifera is one of the best known and most widespread tree species crop of the Moringaceae family. Moringa, commonly known as 'drumstick tree' and horseradish tree, is a multipurpose tree used for human food, medicine, and oil production (Anwer *et al*., 2007). It is cultivated in Africa, Central and South America, and South Asia (Ricardo, 2012). The leaves, flowers, roots, gums, fruits, and seeds are widely used in folk medicine for treating inflammation (Mahajan and Mehta, 2008), cardiovascular dysfunction, liver disease (Rao and Misra, 1998), hematological, hepatic, and renal dysfunction (Mazumder *et al*., 1999). It is rich in phytochemicals such as kaempferol, rhamnetin, quercetin, chlorogenic acid, rutin, apigenin, ascorbic acid, and carotenoids (Anwar *et al*., 2007; Karthivashan *et al*., 2013). Leaves of this plant have been traditionally reported to possess a wide variety of biological activities, including antimicrobial, anti-inflammatory, anti-cancer, anti-diabetic, antispasmodic, stimulant, cough expectorant, and diuretic agent (Anwar *et al*., 2007; Mbikay, 2012).

Several works of literature exist on the protective effects of the leaf, seed, and pod extract of Moringa oleifera against tramadol-induced nephrotoxicity,7,12-dimethyl benzylanthracene (DMBA) induced renal cancer, and the GENT-induced nephrotoxicity, (Sharma and Paliwal, 2012; Ouédraogo*et al*., 2013; Nafiu *et al*., 2019). However, no scientific report is available on the protective effects of Moringa oleifera seed oil on liver and kidney toxicity. Hence, the present study was designed to investigate the protective effect of ethanolic extract of Moringa oleifera seed oil on dichlorvos-induced toxicity inthe liver and kidney of male Wistar rats.

MATERIALS AND METHODS

Animals: For this experiment, healthy male adult Wistar rats with weights ranging from 160-190g were bought from a private animal breeder around Taki in Ogbomoso North L.G.A, Oyo state. They were kept in well-ventilated plastic cages and housed in the animal house of the Department of Physiology, Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso, Oyo State.

Treatment: The 21 rats were randomly divided into three groups as follows:
Group I: Negative control; consists of 7 rats that received only feed and clean water for 28 days.
Group II: Positive control (DDVP treated group); consists of 7 rats exposed to dichlorvos via inhalation at a dose of1ml per rat daily and feed and water for 28 days.
Group III: DDVP and *Moringa oleifera* treated group; consists of 7 rats pretreated with *Moringa* seed oil at a dose of 300mg/kg body weight for 7days before and 21days after exposure to the dichlorvos.
They were exposed to dichlorvos via inhalation at a dose of 1ml per rat daily and *Moringa* seed oil at a dose of 300mg/kg body weight for 21 days in addition to feed and water.

Plant Materials and Preparation of Ethanolic Extract: The oil of *M.oleifera* seed was extracted as Ogushina *et al*. (2014) previously reported with some modifications. Briefly, *Moringa oleifera* seeds used in the study were purchased at the New Waso market in Ogbomoso, identified and approved in the Department of Pharmacology and Therapeutics, Bowen University Teaching Hospital (BUTH), Ogbomoso, Oyo state. The husks of the seeds were crushed on a hard surface to obtain the sources. The seeds obtained were pounded into smaller particles using mortar and pestle. The particles obtained after hitting were poured inside a cone-shaped filter paper and then placed inside the extractor. 97% ethanol was run on the *Moringa oleifera* seed particles, and with the extractor being operational, at 78°C temperature, the oil in the seed was extracted and collected inside a beaker. The extract's cup is then placed inside an incubator at 37°C for preservation.

Drugs and Chemicals: The trade name of the local pesticide used for this study is **sniper™.** DDVP, containing 1000g/liter of 2,3-dichlorovinyl dimethyl phosphate (DDVP); manufactured by (Forward (Beinaj) Hepu Pesticide Co. Limited, China, for Saro Agrosciences Limited, Oyo State, Nigeria) was used. The pesticide, which contains dichlorvos as the active ingredient, was purchased from New Waso market, Ogbomoso, Oyo State.

Exposure to DDVP: Group B and C animals were exposed to 98.54g/m^3 of DDVP via inhalation, as Saka *et al*. (2020) reported. In addition, 1ml of the pesticide was soaked in cotton wool and placed in a desiccator. The rat was also placed inside the desiccator and allowed to inhale the dichlorvos for 2 minutes daily for 28 days to induce liver and kidney toxicity.

Phytochemical analysis of *Moringa oleifera* seed oil: Qualitative phytochemical analysis of *M.oleifera* seed oil was performed and reported as previously documented by Chitravadivu *et al*. (2009).

Saponins: 5ml of the seed oil was boiled in 20ml of distilled water in a water bath and filtered. Approximately 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously to obtain a stable, persistent foam. The resulting foam was then mixed with three drops of olive oil and shaken vigorously. The formation of emulsion indicated the presence of saponin

Alkaloids: 1ml of the seed oil was stirred with 5ml of 1% (v/v) aqueous HCl on a steam bath and filtered when hot. Distilled water was added to the residue, and then 1ml of the filtrate was treated with a few drops of Mayer's reagent, Wagner's reagent, and Dragendoff's reagent. Alkaloids were confirmed by forming a yellow color with Mayer's reagent, red precipitate with Dragendoff's reagent, and reddish-brown precipitate with Wagner's reagent

Terpenoids: 5ml of the seed oil was added to 2ml of chloroform. 3ml of concentrated H_2SO_4 was then carefully added to form a layer. Reddish-brown discoloration of the interfaced was observed, indicating the presence of terpenoid.

Steroids: 2ml of acetic anhydride was mixed with 2ml of the seed oil, followed by the addition of 2ml of concentrated H_2SO_4. A color change confirmed the presence of steroids from violet to blue or green.

Glycosides: 5ml of the seed oil was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underplayed with 1ml of concentrated sulphuric acid. The presence of glycoside was confirmed by forming a violet-green ring below the brown circle.

Flavonoids: One milliliter (1ml) of 10% (w/v) NaOH was added to 3ml of the seed oil. The formation of yellow color confirmed the presence of flavonoids.

Tannins: One millimeter (1ml) of the seed oil was boiled with 20ml of distilled water in a test tube and filtered. Three drops of 0.1% ferric chloride were added to the filtrate. The formation of green color confirmed the presence of tannin.

Anthraquinones: Five millimeters (5ml) of the seed oil was mixed with 10ml of benzene and filtered. Five millimeters (5ml) of 10% NH_3 solution was added to the filtrate. The presence of anthraquinones was established by developing red color in the ammoniacal (lower) phase.

Sample collection and Preparation of Tissue Homogenate: Animal sacrifice was performed using cervical dislocation, and the blood sample was obtained from the animal by cardiac puncture and collected into plain bottles (Saka *et al.*, 2011). Serum was obtained by centrifuging at 3000r.p.m for 15 minutes to obtain supernatant for biochemical assay.

Biochemical Assay

Estimation of hepatic markers: Alkaline phosphatase (A.L.P.), alanine aminotransferase (A.L.T.), aspartate aminotransferase (A.S.T.), albumin, globulin, and total protein were measured using standard laboratory kit according to manufacturer's guide.

Estimation of renal markers: Serum sodium, potassium, and calcium levels were determined using the Flame photometry method (410 flame photometer, Chiron Diagnostics) following the manufacturer's guidelines (Akhigbe *et al.*, 2008). Serum bicarbonate, chloride ion, urea, and creatinine were determined using the standard assay kit following back titration, electrometric method, diacetylmonoxime, and alkaline picrate methods.

Estimation of Inflammatory markers: Tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) markers of inflammation were assayed using ELISA kits (Elabscience Biotechnology Co, Ltd, U.S.A.) following the manufacturer protocols. At the same time, Myeloperoxidase (M.P.O.) was determined using colorimetric methods (Akhigbe and Ajayi, 2000).

Histopathological examination: The organs (i.e., kidney and liver) were also excised, and the excised organs were washed using standard saline solution and later fixed in 10% formalin solution. A paraffin embedding technique was carried out, and sections were taken at 5mM thickness. Hematoxylin and eosin solutions were used to stain the cut-out tissue sections and then examined microscopically for histopathological changes (Preece, 1972).

Ethical considerations: Animals used for the research were treated humanely according to the institution's guidelines and criteria for humane care by the National Institute of Health Guidelines for the care and use of Laboratory Animals.

Statistical Analysis

Results obtained were expressed as mean ± standard error of the mean (n=7). In addition, the data were subjected to a one-way analysis of variance (ANOVA), and differences between samples were determined by Tukey multiple comparison test using the Graph Pad Prism 5 (GraphPad Software Inc., San Diego, CA). While the level of significance used for the analysis was set at $p<0.05$nt.

RESULTS

Table 1 shows the findings of the qualitative phytochemical studies. *Moringa oleifera* seed oil extract contained saponins, terpenoids, steroids, tannin, and antiquinones in varying concentrations.

Table 1:
Phytochemical analysis of Moringa seed oil

Phytochemicals	Presence
Saponins	+
Alkaloids	-
Terpenoid	++
Steroids	++
Glycoside	-
Flavonoids	+++
Tannins	+
Anthraquinones	++

-not detected; + presence in low concentration;++ present in moderate concentration; +++ present in high concentrations.

Body weight changes: The effects of dichlorvos and ethanolic extract of Moringa oleifera seed oil on body weight changes is depicted in Figure 1. Administration of dichlorvos decreased the weight of rats when compared to control. However, treatment with *Moringa oleifera* seed oil increased weight of the rats as compared to dichlorvos-induced rats.

Estimation of Hepatic markers: The effects of dichlorvos and ethanolic extract of *Moringa oleifera* seed oil on hepatic markers are summarized in Table 2. Administration of Dichlorvos induced extensive damage to the liver tissues, evident by the significant increase in alanine aminotransferase (A.L.T.), aspartate aminotransferase (A.S.T.), alkaline phosphatase (A.L.P.) and a corresponding decrease in albumin, globulin and total protein in the

pathogenic rats (Group B) when compared with control rats. On the other hand, treatment with ethanolic extract of *Moringa oleifera* seed oil (Group C) significantly decreases A.L.T., A.S.T.& A.L.P. In addition, it elevates the plasma protein level compared with the dichlorvos-induced rats.

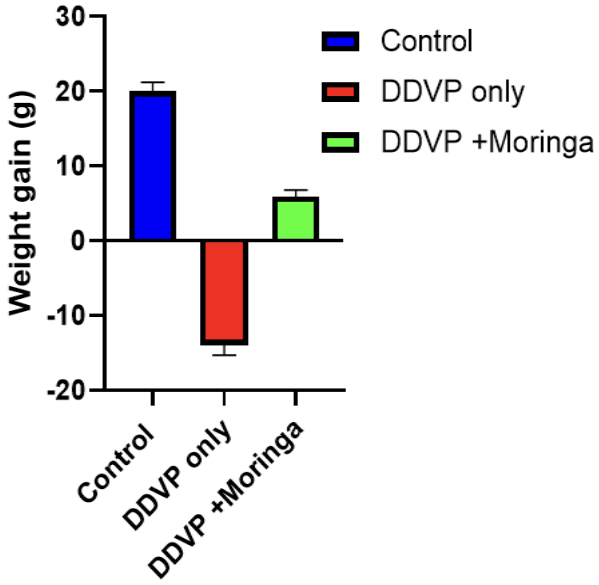

Figure 1
Effect of Ethanol Extract of Moringa oleifera on weight gain male Wistar rats.

Table 2:
Effects of ethanolic extract of *Moringa oleifera* seed oil on hepatic markers in experimental rats.

GROUPS	I	II	III
AST (U/L)	82.03 ± 7.91	97.29 ± 2.64[a]	82.71 ± 7.02[b]
ALT (U/L)	27.33 ± 1.59	54.44 ± 3.50[a]	32.16 ± 3.36[b]
ALP (U/L)	31.31 ± 1.68	55.53 ± 0.49[a]	32.23 ± 0.57[b]
Albumin (g/dl)	1.74 ± 0.09	1.08 ± 0.04[a]	1.68 ± 0.05[b]
Globulin (g/dl)	1.32 ± 0.16	0.57 ± 0.07[a]	1.14 ± 0.14[b]
Total protein (g/dl)	2.70 ± 0.18	1.95 ± 0.11[a]	2.50 ± 0.18[b]

Values are expressed in Mean ± S.E.M. (n=7)
Groups with different superscript(s) are significantly different from other at p≤0.05. [a]Represent significant difference when compared with negative control. [b]Represent statistical significance when compared with positive control.
Group I = Negative control; Group II = Positive control; Group III = DDVP +300mg/kg of *Moringa oleifera* seed oil.

Estimation of serum urea and creatinine: The effects of dichlorvos and ethanolic extract of *Moringa oleifera* seed oil on serum urea and creatinine are summarized in Table 3. Dichlorvos administration induced extensive damage to kidney tissue. As a result, serum creatinine and urea significantly increased in the pathogenic rats (Group B) when compared with control rats (P<0.05). Similarly, there was a significant decrease in the elevated levels of serum creatinine after treatment with ethanolic extract of Moringa seed oil (Group C) (P=0.017) and urea (P=0.02) when compared with dichlorvos-induced rats.

Table 3:
Effects of ethanolic extract of *Moringa oleifera* seed oil on renal markers in experimental rats.

Group	I	II	III
Creatinine (mg/dl)	0.44 ± 0.07	0.96 ± 0.06[a]	0.62 ± 0.10[b]
Urea (mmol/L)	9.08 ± 1.08	16.65 ± 1.27[a]	11.02 ± 1.63[b]
Sodium (ppm)	214.76 ±27.91	494.12 ±19.42[a]	340.98 ±57.50[b]
Potassium (ppm)	2.62 ± 0.10	0.65 ± 0.06[a]	2.10 ± 0.17[b]
Calcium (ppm)	14.63 ± 0.46	10.84 ± 0.75[a]	14.16 ± 0.26[b]
Bicarbonate (mEq/L)	1.22 ± 0.09	0.88 ± 0.07[a]	1.02 ± 0.05
Chloride (mEq/L)	142.67 ± 7.18	170.67 ± 4.52[a]	150.00 ± 3.33[b]

Values are expressed in Mean ± S.E.M. (n=7)
Groups with different superscript(s) are significantly different from other at p≤0.05; [a]Represent significant difference when compared with negative control. [b]Represent statistical significance when compared with positive control.
Group I = Negative control; Group II = Positive control; Group III = DDVP +300mg/kg of Moringa oleifera seed oil.

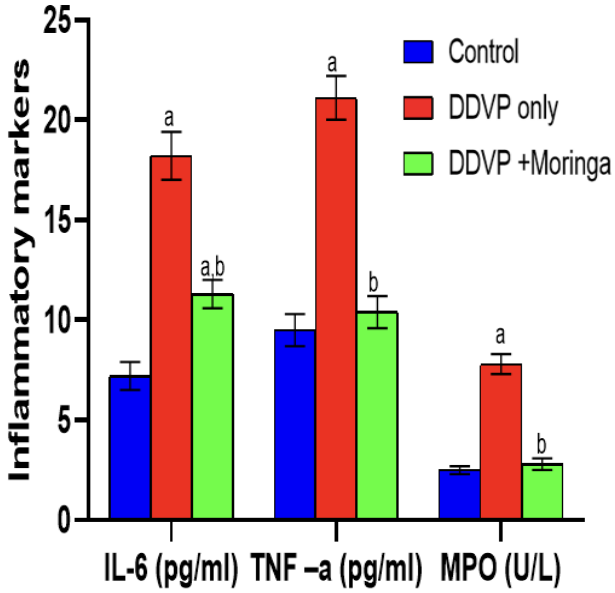

Figure 2
Effect of Ethanol Extract of Moringa oleifera on inflammatory markers in male Wistar rats.

Estimation of inflammatory markers: Exposure of male rats to DDVP led to a significant increase in M.P.O., TNF-α, and IL-6 compared to the control. However, treatment with *Moringa oleifera* seed oil significantly reduced elevated levels of these markers compared to DDVP-induced rats.

Estimation of serum electrolytes: The result (Table 3) showed a significant decrease in potassium, calcium, and bicarbonate ions (P<0.05) in the test group after

administration of dichlorvos as compared to the control rats. However, treatment with Moringa seed oil shows a significant increase in potassium ion, calcium ion ($P<0.05$), and a non-significant increase in bicarbonate ion ($P>0.05$) when compared with the dichlorvos-induced group. Also, there was a significant increase in sodium and chloride ions ($P<0.05$) in the test rats compared to control rats. On the other hand, treatment with Moringa seed oil significantly decreases sodium and chloride ions compared to dichlorvos-induced rats ($P<0.05$).

Histology: Photomicrograph of the liver section (Plate 1) of the standard control group (group A) showed normal central venules without congestion (white arrow), and the morphology of the hepatocytes appeared normal (blue arrow). In addition, the sinusoids seemed to be normal and not infiltrated (slender arrow); no pathological lesion was seen. Still, a photomicrograph of the liver section of group B induced with dichlorvos revealed a non-congested central venule with degenerated cells and macrophage within its lumen (white arrow), portal tracts with dilated portal venules seen (black arrow), the hepatocytes show hypochromic and severe necrosis (blue arrow),the focal area with degeneration and destroyed liver plates seen within the parenchyma (green arrow). Finally, a photomicrograph of the liver section of group C pre-treated with 300mg/kg of *Moringa oleifera* seed oil before administration of dichlorvos showed mildly congested central venules (white arrow), the morphology of the hepatocytes appeared normal (blue arrow), the sinusoids appear mildly dilated but not infiltrated (slender arrow).

A photomicrograph of kidney sections of the standard control group (group A) showed typical kidney architecture, renal cortex with normal glomeruli, mesangial cells and capsular spaces (white arrows), normal renal tubules (blue arrow), and common interstitial spaces (blue arrow) but a photomicrograph of kidney section of group B induced with dichlorvos showed poor architecture, renal cortex with sclerotic glomeruli and widened capsular spaces (white arrow), collapsed renal tubules (blue arrow) and severe atrophic mesangial cells (black arrow).

A photomicrograph of the kidney section of group C pre-treated with 300mg/kg of *Moringa oleifera* seed oil before administration of dichlorvos shows the moderate architecture, renal cortex with normal glomeruli, mesangial cells and capsular spaces (white arrow), normal interstitial spaces (slender arrow) and attenuated renal tubules (blue arrow) when compared with DDVP-induced group

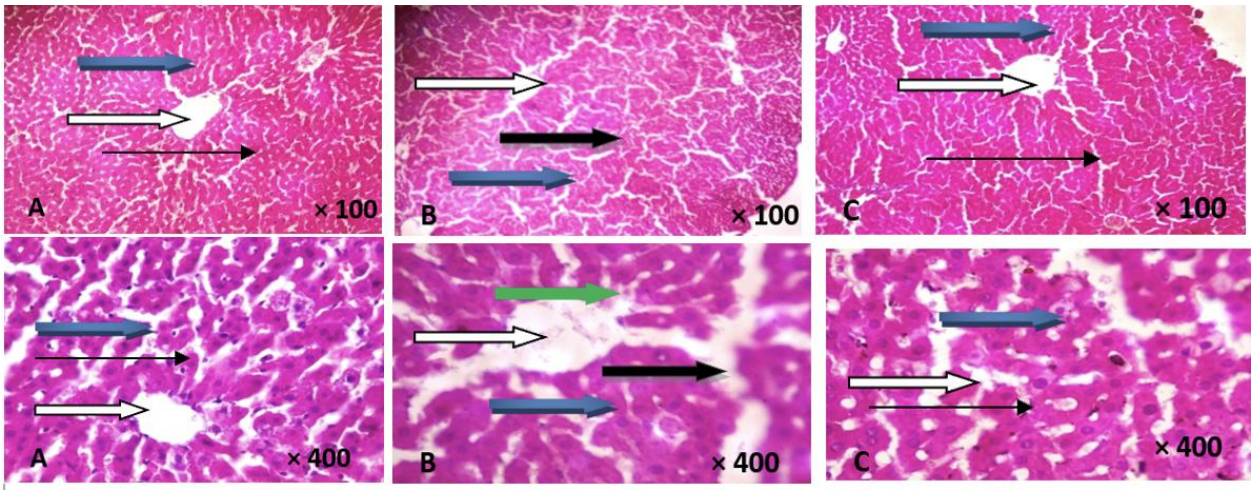

Plate 1:
Histopathological examination of the liver
A: Control, **B** Dichlorvos-induced only, **C:** *Moringa oleifera* seed oil (300mg/kg)

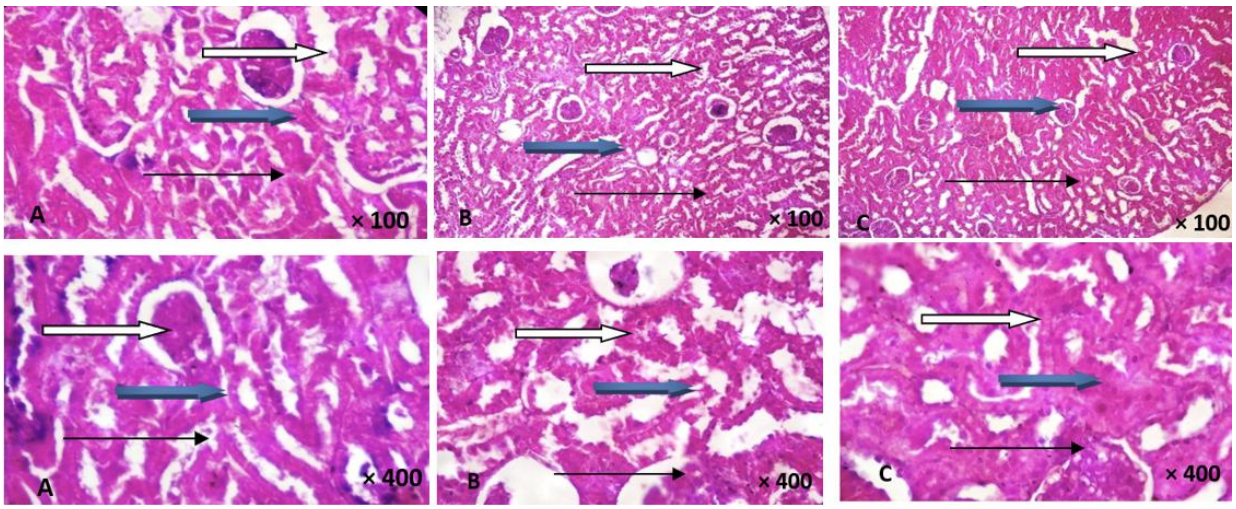

Plate 2:
Histopathological examination of the kidney
A: Control **B**: Dichlorvos-induced only C: *Moringa oleifera* seed oil (300mg/kg).

DISCUSSION

The continuous use of pesticides has increased human exposure to environmental pollutants that may have acute and chronic adverse effects on health. In addition, synthetic chemicals in pesticides may contribute to long-term impacts on the environment. Humans are concomitantly exposed to insecticides whose toxicity profiles have not been delineated in homes. Dichlorvos, a highly hazardous chemical classified by the WHO as (Class I) (WHO, 1992), accumulate in humans and cause toxic effects in the various organ of the body (Tsitsimpikoua *et al.,* 2013). These organs are either affected by direct or indirect exposure to dichlorvos (Sheiner *et al.,* 2003). The effects of Moringa oleifera seed oil on dichlorvos-induced toxicity in liver and kidney organs of male Wistar rats were investigated in the study. This is done by assessing the liver enzyme activities, renal function tests, and inflammatory markers, which serve as the biomarkers for liver and kidney function, respectively.

The results of the acute exposure clearly show that dichlorvos has the potential to promote hepatic and renal damage. This was evident in the study by significant increases in hepatic and renal markers (Table 2;4). Hepatic impairment is characterized by an elevation in serum liver enzymes viz A.L.T., A.L.P., and A.S.T. A.S.T. and A.L.T. are the most common because they are readily released into the extracellular space by the hepatocytes (Ozer *et al.,* 2008). A.S.T. is a highly sensitive hepatocellular marker than A.L.T. (Al-Mammary *et al.,* 2002). However, the concentration of A.L.T. in the body is more than A.S.T. as the body generates more A.L.T. than A.S.T. (Mayne, 1996). Thus, the high levels of A.S.T., A.L.T., and A.L.P. observed in this study (Table 2) indicate the hepatoxic effect of dichlorvos. This was in tandem with the previous report by Celik *et al*. (2009) and Garba*et al*. (2013), who suggested that dichlorvos causes liver damage in rats. Creatinine and urea are known biochemical markers of acute and chronic nephrotoxicity (Naggayi *et al.,* 2015). Thus, serum creatinine and urea levels are often considered reliable predictors of renal function (Yaman & Balikci, 2010; Naggayi *et al.,* 2015). The significant elevation is seen in the plasma level of urea, creatinine, and other renal markers in the untreated rats could be attributed to the nephrotoxic effect of dichlorvos, which further suggest impairment of the glomerular function and tubular damage of the kidneys (Gross *et al.,* 2005; Adeyemi and Akanji, 2012). This is consistent with the findings of Edelstein (2008), who posited elevated levels of the renal marker in dichlorvos exposed population. The degeneration and destruction observed in the hepatic and renal tissues can be linked to the generation of reactive oxygen species (R.O.S.) initiated by dichlorvos (Sharma and Singh, 2012).

It is a fact that excessive R.O.S. generation and oxidative stress may trigger an inflammatory response and vice versa. Oxidative stress usually enhances a variety of cell signaling pathways involved in inflammation (Khalil *et al.,* 2019). Exposure to DDVP led to marked increased inflammatory markers such as TNF-α, IL-6, and M.P.O., which play a central role in the development and progression of liver and kidney dysfunction. This aligns with previous reports, which confirm that although DDVP acts via permanent binding and inhibition of acetylcholinesterase activity, most of its harmful biological effects are oxidative-stress mediated (Burke *et al.,* 2016; Pearson *et al.,* 2016; Wang *et al.,* 2016; Imam *et al.,* 2018).

The toxicity of dichlorvos did not spare the tissues (kidney and liver) under examination, as revealed by the histopathological results. Dichlorvos induced histological alterations in the liver of rats ranging from degenerated venules to necrotic hepatocytes. This might indicate hepatic damage, as Abdelhalim and Jarrar (2012) reported. Also, the histopathological examination of the renal tissue attacked by dichlorvos revealed many alterations ranging from tubular degeneration (distorted renal tubules) and atrophy of the glomeruli (glomeruli with obliterated capsular spaces) to congestion of renal blood vessels. This was in tandem with the report of Elhalwagy *et al.* (2008), Kalender *et al.* (2007), Sulak *et al.* (2005), and Mohnsen (2001) that pesticides cause various histopathological changes in the liver and kidney tissues of experimental animals.

From this study, *Moringa oleifera* seed oil has shown its potential in reducing and controlling stress response and played an enormous role in protecting the organs affected by dichlorvos exposure, as demonstrated by the photomicrograph histopathology results. However, it may require more time for complete recovery. This ability of Moringa seed oil to regulate the activities of these biomarkers can be attributed to the presence of bioactive compositions such as phenols, flavonoids, terpenoids, and saponins (Bharali *et al*., 2003; Anwar *et al.,* 2007; Karthivashan *et al.,* 2013). These compounds scavenge R.O.S., chelate metal ions, and regenerate membrane-bound antioxidants. This finding agrees with Kumar and Pari, (2003) and Arabshahi *et al*. (2007). which demonstrated the antioxidant activity of *Moringa* extract. Furthermore, reports have shown that saponins possess tumor-inhibiting activity *in vivo* (Akindahunsi & Salawu, 2005). Thus, its presence can control human cardiovascular disease and reduce blood cholesterol. Other bioactive compounds found in Moringa seed oil are high pro-vitamin A, vitamin C, tocopherols, carotenoids, and anthocyanins, which explain their mode of action in the induction of antioxidant profiles in the present investigation. However, the underlying process is yet to be elucidated.

Moringa seed oil also contains the bioactive agent β-carotene, which supports healthy liver and kidney functioning, as well as flavonoids, shown to have anti-inflammatory, anti-cancer, anti-diabetic, antispasmodic, stimulant, cough expectorant, and diuretic agent (Bharali *et al*., 2003; Anwar *et al.,* 2007; Kumar *et al.,* 2010; Mbikay, 2012; Minaiyan *et al.,* 2014). These features might have resulted in the observed hepato-protection against homeostatic imbalance imposed by dichlorvos exposure. Moreover, the maintenance of normal levels of serum electrolytes by *Moringa oleifera* seed oil is consistent with reported medicinal potential, including the improvement of the health of renal tissues and general well-being (Anwar *et al.,*2007; Ndong *et al.,* 2007). The efficacy of Moringa seed oil in improving plasma protein, lowering elevated liver enzymes (A.S.T., A.L.T., and A.L.P.), and renal biomarkers (electrolyte, urea, and creatinine) were observed. All this may be attributed to phytochemicals present in the plants. The consumption of this plant oil may provide health benefits in terms of hepatoprotective, nephroprotective, and

anti-inflammatory effects (due to the presence of β-carotene, flavonoid, tannins, alkaloids, etc.) and scavenging of free radicals in the body (due to the presence of tocopherols, phenolics, and carotenoids) (Karthivashan *et al.*, 2013;Bhatnagar and Krisna, 2013)

In conclusion, this study revealed the harmful effect of dichlorvos exposure on liver and kidney organs. This wasconfirmed by the assay of liver and renal biomarkers, inflammatory markers, and histopathological examination of the organs. The Moringa seed oil also showed its regenerative potential in recuperating the damaged organs. Thus, Moringa seed oil possesses both hepatoprotective and nephroprotective potential, which depends on its bioactive compounds. This study has shown that Moringa seed oil can regulate and reduce impaired cellular integrity expressed in hepatic and renal markers in individuals that are constantly exposed to household pesticides (i.e.,dichlorvos) and could be explored in the treatment of hepatic and renal conditions and also, in the protection of the hepatorenal integrity from some oxidative stress. Future studies are required to fully evaluate the contents of *Moringa oleifera* responsible for the protection against hepatotoxic and nephrotoxic agents.

REFERENCES

Abdelhalim, M.A., Jarrar, B.M. (2012). Histological alterations in the liver of rats induced by different gold nanoparticle sizes, doses and exposure duration. J Nanobiotechnology. 10: 5. PubMed Abstract | Publisher Full Text | Free Full Text.

Adeyemi, O. S. and Elebiyo, T.C. (2014). Moringa oleifera Supplemented Diets Prevented Nickel-Induced Nephrotoxicity in Wistar Rats. Journal of Nutrition and Metabolism Volume 2014, Article ID 958621, 8 pages http://dx.doi.org/10.1155/2014/958621

Adeyemi, O.S. and Akanji, M.A. (2012). "Psidium guajava leaf extract: effects on rat serum homeostasis and tissue morphology," Comparative Clinical Pathology, vol. 21, no. 4, pp. 401–407.

Ajao, M.S., Sansa, A.B., Imam, A., Ibrahim, A., Adana, M.Y., Oluwafuyi, A.A., *et al*. (2017). Protective Effect of Nigella Sativa (Black Caraway) Oil on Oral Dichlorvos Induced Hematological, Renal and Nonspecific Immune System Toxicity in Wistar Rats. IJT. 11(6):1-5.

Akhigbe RE, Ige SF, Afolabi AO, Oyeyipo PI, Ajao FO, Ajayi FA (2008). Water balance and serum levels of some electrolytes in oral contraceptive-treated female wistar rats. J Med Sci 8:591-4.

Akindahunsi, A.A., Salawu, S.O. (2005). Phytochemical screening and nutrient antinutrient composition of selected tropical green leafy vegetables. Afr J Biotechnol. 4(6): 497–501.

Alabi, D.A., Onibudol, M.Z. and Amusa N.A (2005). Chemicals and nutritional composition of four botanicals with fungitoxic properties. World Journal of Agricultural Science 1 (1):84-88

Al-Mamary M., Al –Habori, Al-Aghbari, A.M. and Basker, M.M. (2002). Investigations into the toxicological effects of Catha edulis leaves: A short term study in animals. Phytother. Res. 16: 127-132.

Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H. (2007). "Moringa oleifera: a food plant with multiple medicinal uses," Phytotherapy Research, vol. 21, no. 1, pp. 17–25.

Arabshahi, D.S., Devi, V., Urooj, A. (2007). Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chemistry, 100: 1100–1105.

ATSDR: Agency for Toxic Substance and Disease Registry (1997). Toxicologi¬cal profiles of Dichlorvos. U.S. Department of Health and Human Services. Atlanta, GA. Pp 1–178.

Bharali, R., Tabassum, J., Azad, M.R.H., (2003). Chemomodulatory effect of Moringa oleifera, Lam. on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pacific Journal of Cancer Prevention, 4: 131-139.

California Environmental Protection Agency (CEPA) (1996). Dichlorvos (DDVP) Risk Characterization Document, Medical Toxicology and Worker Health and Safety Branches Department of Pesticide Regulation California. Environmental Protection Agency. California, CEPA USA. pp.1-119.

Celik, I., Yilmaz, Z., & Turkoglu, V., (2009). Hematotoxic and hepatotoxic effects of dichlorvos at sublethal dosages in rats. Environ Toxicol, 24 (2), 128-132.

Chitravadivu, C., Manian, S., Kalachelvi, K. (2009). Qualitative analysis of selected medicinal plants, Tamil nadu, India. Mid. East J. Sci. Res; 4:144-6

Diwivedi, N., Bhutia, Y.D., Kumar, V., Yadav, P., Kushwaha, P., Swarnkar, H., *et al*. (2010). Effects of combined exposure to dichlorvos and monocrotophos on blood and brain biochemical variables in rats. Hum Exp Toxicol. 29:121–9.

Dungca, N.T.P. (2016). Protective effect of the methanolic leaf extract of Eclipta alba (L.) Hassk. (Asteraceae) against gentamicin-induced nephrotoxicity in Sprague Dawley rats. J Ethnopharmacol. 184:18-21.

Eddleston, M., Buckley, N.A., Eyer, P., Dawson, A.H. (2008) Management of acute organophosphorus pesticide poisoning. Lancet. 96(12):597-607.

Elhalwagy, M.E.A., Darwish, N.S., Zaher, E.M. (2008) Prophylactic effect of green teapolyphenols against liver and kidney injury induced by fenitrothioninsecticide. Pestic Biochem Physiol. 91(2): 81–89.

Ferguson, M.A., Vaidya V.S., Bonventre, J.V., (2008). Biomarker of nephrotoxic acute kidney injury. Toxicology 245, 182-193.

Garba, U.S., Nasiru, A.A., Haruna, M.A., *et al*. (2013). Biochemical and histopathologic changes in liver of albino rats exposed to 1% dichlorvos pesticide at subacute period liver toxicity of a nigerian dichlorvors pesticide. J Pharm Biomed Sci. 3(2): 1–6.

Gross, J.L., de Azevedo, M.J., Silveiro, S.P., Canani, L.H., Caramori, M.L., and Zelmanovitz, T. (2005). "Diabetic nephropathy: diagnosis, prevention, and treatment," Diabetes Care, vol. 28,no. 1, pp. 164–176.

Gunay, N., Kose, B., Demiryurek, S., Ceylan, N.O., Sari, I. and Demiryurek, A.T. (2010) "Protective effects of Y-27632 on acute dichlorvos poisoning in rats," The American Journal of Emergency Medicine, vol. 28, no. 3, pp. 268–274, 2010.

Hagar, H.H., Fahmy, A.H. (2002). A biochemical, histochemical, and ultrastructural evaluation of the effect of dimethoate intoxication on rat pancreas. Toxicol Lett. 133:161–70.

Ihedioha, J.I., Okafor, C. and Ihedioha, T.E. (2004). The hematological profile of Sprague-Dawley outbred albino rats in Nsukka, Nigeria. Animal Research International, 1(2):125-132

Joshi, S.C., Mathur, R., Gajraj, A., Sharma, T. (2003). Influence of methyl parathion on reproductive parameters in male rats. Environ Toxicol Pharmacol. 14:91–8.

Kalender, S., Kalender, Y., Durak, D., *et al*. (2007) Methyl parathion induced nephrotoxicity in male rats and protective role of vitamins C and E. Pestic Biochem Physiol. 88(2): 213–218.

Karthivashan, G., Tangestani, F.M., Arulselvan, P., Abas, F., Fakurazi, S. (2013). Identification of bioactive candidate compounds responsible for oxidative challenge from hydro-ethanolic extract of Moringa oleifera leaves. Journal of Food Science 78:C1368-C1375.

Kumar, A., Pari, L. (2003). Antioxidant action of Moringa oleifera Lam (drumstick) against antitubercular drugs induced lipid peroxidation in rats. Journal of Medicinal Food, 6: 255–259.

Kumar, P.S., Mishra, D., Ghosh, G. and Panda, G.S. (2010). "Medicinal uses and pharmacological properties of Moringa

oleifera," International Journal of Phytomedicine, vol. 2, no. 3, pp. 210–216.
Mahajan, S.G., Mehta A. A. (2008). Effect of Moringa oleifera Lam seed extract on ovalbumin-induced airway inflammation in guinea pigs. Inhalation Toxicology, 20: 897–909.
Mayne P.D. (1996). Clinical Chemistry in Dignosis and Treatment 6th Edn, Arnold London/Oxford University Press Inc., New York.
Mazumder, U.K., Gupta M., Chakrabarti, S., Pal, D. (1999). Evaluation of haematological and hepatorenal functions of methanolic extract of Moringa oleifera Lam root treated mice. Indian Journal of Experimental Biology, 37: 612–614.
Mbikay, M. (2012) "Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review," Frontiers in Pharmacology, vol. 3, article 24.
Mogda, K., Mansour Afaf, A.I., El-Kashoury, M.A., Rashed, K.M., Koretem, K.M. (2009). Oxidative and biochemical alterations induced by profenofos insecticide in rats. Nat Sci. 7:1–15.
Mohnsen, M. (2001). Biochemical and histopathological changes in serum creatinine and kidney induced by inhalation of Thimet (Phorate) in male Swiss albinomouse, Mus musculus. Environ Res. 87(1): 31–36.
Nafiu A. O, Akomolafe R. O, Alabi Q. K, Idowu C. O (2019). Effect of fatty acids from ethanol extract of Moringa oleifera seeds on kidney function impairment and oxidative stress induced by gentamicin in rats; Journal of Biomedicine and Pharmacotherapy 117 ; 109154
Naggayi, M., Mukiibi, N. & Iliya, E. (2015). The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. Afr. Health. Sci., 15(2): 598-605.
Najafian, M., Mokaber, H., Pourahmad, M., Farzam, M., Jahromi, K.H. (2014). Pathological changes of Gentamicin in liver tissue and antioxidant property of cinnamon extract on wistar rats. Biomed & Pharmacol j., Vol 7 (1), 341-347
Ndong, M., Uehara, M., Katsumata, S.I. and Suzuki, K. (2007). "Effects of oral administration of Moringa oleifera Lam on glucose tolerance in Goto-Kakizaki and wistar rats," Journal of Clinical Biochemistry and Nutrition, vol. 40, no. 3, pp. 229–233.
Neishabouri, E.Z., Hassan, Z.M., Azizi, E., Ostad, S.N. (2004). Evaluation of immunotoxicity induced by diazinon in C57bl/6 mice. Toxicology. 196:173–9.
Nwaichi, E.O., Essien, E.B and Ibeh, U.C. (2019) "Influence of Daucus carota on the Hepatic and Renal Biomarkers of Dichlorvos-Exposed Albino Rats". EC Pharmacology and Toxicology 7.10: 1067-1075.
Ogunsina, B.S., Indira, TN., Bhartnagar, AS., Radha, C., Debnath, S., Krishna, A.G. (2014). Quality characteristics and stability of Moringa oleifera seed oil of Indian origin. J Food Sci Technol; 51: 503-10.
Ouédraogo, M., Lamien-Sanou, A., Ramdé, N., Ouédraogo, A.S., Ouédraogo, M., Zongo, S.P., *et al*. Protective effect of Moringa oleifera leaves against gentamicin-induced nephrotoxicity in rabbits. Exp Toxicol Pathol. 2013;65(3): 335-9.
Owoeye, O., Edem, F. V., Akinyoola, B. S., Rahaman, S., Akang, E. E. & Arinola, G. O. (2012). Histological changes in liver and lungs of rats exposed to dichlorvos before and after vitamin supplementation. Eur. J. Anat., 16(3):190-8.
Ozer, J., Ratner, M., Shaw, M., Bailey, W., Schomaker, S. (2008). The current state of serum
Poiout-Belissen, F.M. and McCartney, J.E. (2010). Interpretation of hematology data in preclinical toxicology studies. Pages 78-84. In: Weiss, D.J. and Wardrop, K.J. (Eds.) Schalm's Veterinary Hematology, 6th edition, Wiley-Blackwell, Iowa.
Preece, A. (1972). A Manual for Histologic Technicians. 3rd Edn., Little, Brown and Company, Boston, MA., USA., ISBN-13: 9780316717656, Pages: 428.
Rao, K.S., Misra, S. H. (1998). Anti-inflammatory and antihepatotoxic activities of the rats of Morringa pterygosperma Geaertn. Indian Journal of Pharmaceutical Science, 60: 12–16.
Ricardo, A. (2012); Seed and oil yields of Moringa oleifera variety Periyakalum-1 introduced for oil production in four ecosystems of South America, Ind. Crops Prod. 36 (1):70–73.
Saka W.A. Akhigbe R.E, Popoola O.T and Oyekunle O.S (2012). Changes in serum electrolytes, urea and creatinine in aloevera-treated rats. J. Young Pharmacist. 4(2):78-81. DOI:10.4103/0975-1483.96620
Saka, W.A., Ayoade, T.E., Akhigbe, T.M. and Akhigbe, R.E. (2020). Moringa oleifera seed oil partially abrogates 2,3-dichlorovinyl dimethyl phosphate (Dichlorvos)-induced cardiac injury in rats: evidence of role of oxidative stress. J Basic Clin Physiol Pharmacol:20190313
Saleem, M.T.S., Christina, A.J.M., Chidambaranthan, N., Ravi, V., Gauthaman, K. (2008). Hepatoprotective activity of Annona squamosa (Linn) on an experimental animal model. Int. J. Applied Res. Nat Pro, 1(3):1-7
Sharma, P. & Singh, R. (2012). Dichlorvos and lindane induced oxidative stress in rat brain: Protective effects of ginger. Pharmacognosy Res., 4(1):27-32.
Sharma, V. and Paliwal, R. (2012) "Chemo protective role of Moringa oleifera and its isolated saponin against DMBA induced tissue damage in male mice: a histopathological analysis," International Journal of Drug Development & Research, vol. 4, no. 4, pp. 215–228.
Sheiner EK, Sheiner E, Hammel RD, *et al*. (2003) Effect of occupational exposures on male fertility: literature review. Ind Health. 41(2): 55–62. PubMed Abstract | Publisher Full Text
Sief, M.M., Khalil, F.A., Abou-Arab, A.A., *et al* (2015). Ameliorative role of melissa officinalis against hepatorenal toxicities of organophosphorus malathion in male rats. MOJ Toxicol. 1(3):103–109.
Sohn, S.H., Lee, E.Y., Lee, J.H., Kim, Y., Shin, M., Hong, M., *et al* (2009). Screening of herbal medicines for recovery of acetaminophen-induced nephrotoxicity. Environ Toxicol Pharmacol. 27(2):225-30.
Sulak, O., Altuntas, I., Karahan, N., *et al*. (2005). Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamins E and C. Pestic Biochem Physiol. 83(1): 21–28.
Tsitsimpikou, C., Tzatzarakis, M., Fragkiadaki, P., Kovatsi, L., Stivaktakis, P., Kalogeraki, A., Kouretas, D. & Tsatsakis, A.M (2013). Histopathological lesions, oxidative stress and genotoxic effects in liver and kidneys following long term exposure of rabbits to diazinon and propoxur. Toxicology., 10; 307:109-14.
Verma, R.S., Srivastva, N. (2003). Effect of chlorpyrifos on thiobarbituricacid reactive substances scavenging enzyme and glutathione in Rat tissues. Indian J Biochem Biophysics. 40:423–8.
Wang, H.H., Chou, Y.C., Liao, J.F., Chen, C.F. (2004). The effect of the insecticide di¬chlorvos on esterase activity extracted from the psocids, Liposcelis bostry¬chophila and L. entomophilia. J Insect Sci 4: 1–5.
WHO: World Health Organization. (1992). International Programme on chemical safety. WHO recommended classification of pesticide by hazards and guidelines to classification 1994–1995, UNEP/ILO/WHO.
Yadav, P., Jadhav, S.E., Kumar, V., Kaul, K.K., Pant, S.C. and Flora, S.J.S. (2012) "Protective efficacy of 2-PAMCl, atropine and curcumin against dichlorvos induced toxicity in rats," Interdisciplinary Toxicology, vol. 5, no. 1, pp. 1–8.
Yaman, I. & Balikci, E. (2010) Protective effects of nigella sativa against gentamicin-induced nephrotoxicity in rats. Exp. Toxicol. Pathol., 62(2):183-90.