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Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbits: Physiological Aspect

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Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbits: Physiological Aspect

Maged M Yassin[1]*, Mohammed S Al-Najjar[2]
[1]Faculty of Medicine, Islamic University of Gaza, Gaza Strip, Palestine.
[2]Faculty of Science, Islamic University of Gaza, Gaza Strip, Palestine.

***Correspondence:** myassin@iugaza.edu.ps

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Abstract
Toxicity studies of methamidophos on the physiological aspects of mammalian organs are limited in the literature. This study aimed to assess hepatic and renal functions in response to a sublethal dose of methamidophos in male domestic rabbits. A daily dose of 1/10 LD_{50} methamidophos was given orally to rabbits for 6 weeks. Control animals were given distilled water. Blood samples were collected and analyzed weekly. Serum glucose and bilirubin were increased upon methamidophos intake versus control, with the significant change commenced from the second and fifth weeks, respectively. In general, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, and alkaline phosphatase showed significant elevation throughout the whole experiment. Conversely, cholinesterase was significantly inhibited all over the experimental intervals studied. Serum urea and creatinine showed a significant increase whereas total protein, albumin, and globulin exhibited a significant decrease during the last five weeks of the experiment. Hypocalcemia and hyperphosphatemia were also observed in methamidophos-treated rabbits. Methamidophos exposure at a sublethal dose had a potential toxic effect on liver and kidney functions as indicated by significant alterations in their biomarkers. Such findings can then be extrapolated to human beings to assess the potential hazards in human populations due to methamidophos exposure.

Keywords: Methamidophos; Toxicity; Liver; Kidney; Male domestic rabbit.

1. INTRODUCTION

Methamidophos is a highly active, systemic, residual organophosphate insecticide/acaricide/avicide with contact and stomach action [1]. The chemical name of methamidophos is O,S-dimethylphosphoramidothiolate, and its common trade names are tamaron, monitor, and nitofol [2]. The acute oral LD_{50} of methamidophos is estimated to be 21 and 16 mg/kg body weight for male and female rats, respectively, 30-50 mg/kg body weight in guinea pigs, and in the range of 10-30 mg/kg body weight in rabbits, cats, and dogs [3]. However, no previous study assessed the exact or specific oral LD_{50} in male domestic rabbits.

The lethal action of methamidophos resides in the fact that this organophosphorus pesticide inhibits irreversibly the activity of the enzyme acetylcholinesterase (AChE), which is essential in the normal transmission of nerve impulses. Inactivation of AChE results in the accumulation of acetylcholine at cholinergic receptor sites, causing a cholinergic crisis that can lead to death [4, 5]. As an organophosphorus pesticide, signs and symptoms of methamidophos poisoning may include weakness, headache, blurred vision, and confusion. Nausea, vomiting, abdominal pain, diarrhea, excessive sweating, and salivating may develop. Difficulty in breathing may be experienced. On severe poisoning, there will be muscle spasms, unconsciousness, and convulsion. Breathing may stop, followed by death [6, 7].

Although the neurotoxic action of methamidophos was extensively investigated, few studies targeted its toxic effect on other mammalian organs including liver and kidney. Methamidophos was reported to alter the physiological and histological aspects related to the liver and kidneys in experimental animals as well as in humans [8, 9]. This study is intended to expand the limited knowledge on hepatic and renal toxicity of methamidophos, which is used in the Gaza Strip as a multipurpose pesticide, particularly in the agriculture sector for the control of insects on a wide range of crops including fruits, vegetables, and flowers [10].

Nowadays large quantities of methamidophos are used in the Gaza Strip (27 metric tons annually), where the protective measures are poorly followed [10]. The use and/or misuse of pesticides including methamidophos caused several cases of death among farmworkers and children in the Gaza Strip [11, 12]. Therefore, the assessment of hepatic and renal toxicity of methamidophos in domestic rabbits could open an avenue toward understanding the extent of its danger, particularly in human populations as a result of methamidophos exposure.

2. METHOD(S)

2.1. Experimental Animals

This research is conducted in the period from April 2014 to March 2015. Healthy adult male domestic rabbits weighting 1,000-1,200 g were used in this study. Animals were maintained under the ambient conditions in the animal house in the Department of Biology, Islamic University of Gaza. They were fed on a commercial balanced diet specially prepared for rabbits (Anber). The diet and tap water were lead free and offered *ad libitum* throughout the experimental period. The Institutional Animal Ethics Committee approved the experimental protocol (No. 03/2014).

2.2. Experimental Design

The study had two phases: the first phase was to determine the oral LD_{50} of methamidophos and the second phase was to assess hepatic and renal toxicity in male domestic rabbits induced by a sublethal dose of methamidophos (1/10 LD_{50}). Analytical-grade methamidophos (97.3% purity) used in this study was purchased from Payer AG Chemical Company, Germany.

2.3. Determination of Methamidophos LD_{50}

A total number of 80 rabbits were used for the determination of LD_{50} of methamidophos. Animals were divided into 10 groups (eight rabbits per group). The first nine groups (1-9) were administered different single doses of methamidophos ranging from 10 to 30 mg kg^{-1} body weight as shown in Table 1. The 10th group was served as a control group. Methamidophos was given orally using a special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury. The rabbit was held between its two ears so that the esophageal opening was clearly and unobstructively opened. The gastric tube was filled with the required dose of methamidophos and then smoothly inserted until it adequately enters the upper part of the esophagus where its contents were emptied. The animals were observed for mortality during the 48-h observation period. The LD_{50} was determined by the graphical method [13].

2.4. Methamidophos Toxicity Experiments

After the determination of methamidophos LD_{50} in the first phase, a sublethal oral dose of methamidophos (1/10 LD_{50}) was given daily in the second phase to assess hepatic and renal toxicity of methamidophos in male domestic rabbits. Animals were divided into two groups: control and experimental groups. The control group comprised 36 rabbits (six rabbits were housed in each cage) and the experimental group also included 36 rabbits (six rabbits were housed in each cage). The experimental groups were orally administrated 1/10 LD_{50} methamidophos daily for overall the experimental duration of 6 weeks. Control animals were given distilled water.

2.5. General Health of Rabbits

Bodyweight of both control and experimental rabbits was measured using a sensitive balance (Model: ONA-15, made in Istanbul 2013) and weights were recorded to the nearest gram. Toxicity symptoms, abnormalities, and mortalities of both control and experimental animals were observed.

2.6. Blood Sampling and Processing

Six animals were taken from each control and experimental groups every week. Blood samples were collected into centrifuge tubes (without any anticoagulant) from rabbit marginal ear vein using the incision method [14]. The samples were left for about 15 min to allow blood coagulation. Then, the samples centrifuged at 3,000 rpm for 20 min. Serum samples were separated into glass tubes, labeled, and kept in the refrigerator for biochemical assay.

Table 1: The first nine groups (1-9) were administered different single doses of methamidophos.

Dose (mg kg^{-1} body weight)	LD_{50} determination group
10.0	1
12.5	2
15.0	3
17.5	4
20.0	5
22.5	6
25.0	7
27.5	8
30.0	9
	10 control group

2.7. Determination of Glucose and Bilirubin

Serum glucose was determined by the glucose oxidase procedure using Dialab reagent kits [15]. Serum bilirubin was measured with the use of surfactants as solubilizing agents [16].

2.8. Determination of Liver Enzymes

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured by an optimized UV test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), using DiaSys reagent kits [17, 18]. Serum γ-glutamyltransferase (γ-GT) and alkaline phosphatase (ALP) activities were determined by the kinetic photometric test according to IFCC, using DiaSys reagent kits [19, 20]. Serum cholinesterase (ChE) activity was measured by a kinetic photometric test according to Ellman's method, using DiaSys reagent kits [21].

2.9. Determination of Kidney Function

Serum urea was determined by using a colorimetric test using DiaSys reagent kits [22]. Serum creatinine was measured by a kinetic test without deproteinization using DiaSys reagent kits [23].

2.10. Determination of Protein Profile

Serum total protein and albumin were measured by photometric test according to the described methods, using DiaSys reagent kits [24, 25]. Serum globulin was calculated according the following formula: Globulin = Total protein – Albumin

2.11. Determination of Calcium and Phosphorus

Serum calcium was assayed following the instructions of the Randox reagent kit manual [26]. Serum phosphorus was determined by phosphomolybdate UV endpoint, using the Ammonium Molybdate Diagnostic kit [27].

2.12. Statistical Analysis

Data were statistically analyzed using SPSS computer program version 21.0 for Windows (Statistical Package for Social Sciences Inc., Chicago, IL). Means were compared by an independent-sample *t*-test. Probability values (*P*) were obtained from the student's table of "*t*" and significance was at $p < 0.05$. The percentage difference was calculated according to the formula: percentage difference equals the absolute value of the change in value, divided by the average of the two numbers, all multiplied by 100. Percent difference = (|(V1 − V2)|/((V1 + V2)/2)) * 100. The graph logarithmic scale of oral LD_{50} of methamidophos was plotted using the Microsoft Excel program 2013.

3. RESULTS

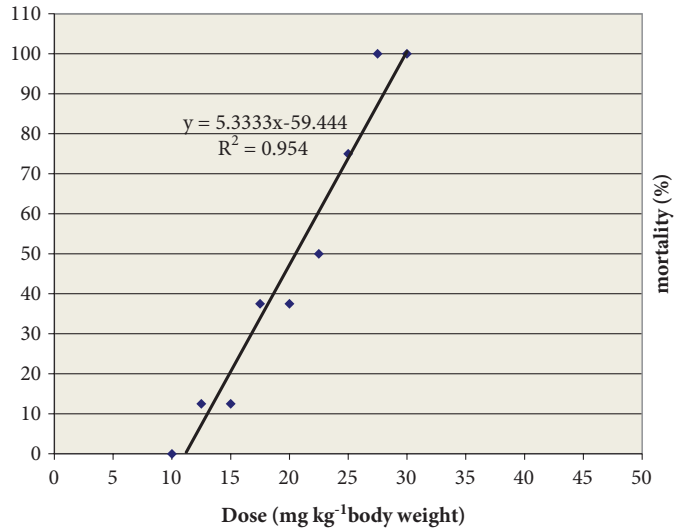
3.1. Oral LD_{50} of Methamidophos

The experimental trials for oral LD_{50} determination of methamidophos after 48 h of administration in male domestic rabbits revealed that the mortality commenced at 12.5 mg kg^{-1} body weight, recording mortality percentage of 12.5% (Table 2). Increasing methamidophos dose to 15.0, 17.5, 20.0, 22.5, and 25.0 resulted in mortality percentages of 12.5%, 37.5%, 37.5%, 50.0%, and 75.0%, respectively. The mortality rate was a function of dose increase. The maximum concentration of methamidophos that kills all animals in the group was found to be 27.5 mg kg^{-1} body weight. The calculated oral LD_{50} of methamidophos in male domestic rabbits from the linear regression was found to be 20.5 mg kg^{-1} body weight (Figure 1).

Table 2: Mortality percentage of male domestic rabbits after 48 h of oral administration of different doses of methamidophos. The number of animals administered methamidophos was 8 in each group (1-9). Control animals were given distilled water and their number was also 8.

Group	Methamidophos dose (mg kg^{-1} body weight)	No. of animals died/total	Mortality (%)
1	10.0	0/8	0.0
2	12.5	1/8	12.5
3	15.0	1/8	12.5
4	17.5	3/8	37.5
5	20.0	3/8	37.5
6	22.5	4/8	50.0
7	25.0	6/8	75.0
8	27.5	8/8	100
9	30.0	8/8	100
10	Control	0/8	0.0

Figure 1: Logarithmic scale of oral LD_{50} of methamidophos in male domestic rabbits. (LD_{50} = 20.5 mg kg^{-1} body weight).



3.2. Body Weight and General Health of Rabbits

The bodyweight of rabbits after 6 weeks of daily oral administration of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight) was significantly decreased compared to controls (915 ± 32.8 *versus* 1170 ± 45.5, $p = 0.003$). Rabbits treated with methamidophos showed varying degrees of toxicity symptoms a few hours after dosing. The symptoms included diarrhea, disorientation, drowsiness, weakness, depression, and mild tremor. However, control rabbits did not display any toxicity symptoms. The mortality records showed that 4 methamidophos-treated rabbits died during the 6-week experimental duration from the total number of 36 rabbits used in the experiment: 1 rabbit in the fourth week, 1 rabbit in the fifth week, and 2 rabbits in the sixth week. However, none of the control rabbits died throughout the experiment.

3.3. Glucose and Bilirubin

As indicated in Table 3, methamidophos administration caused a general increase in the serum glucose of rabbits along the experimental period of 6 weeks compared to controls. This increment was significant in the last five weeks with a maximum % difference of 27.1 during the fourth week ($p = 0.003$). Serum bilirubin was also increased, reaching a maximum % difference of 19.3 during the fifth week ($p = 0.024$).

3.4. Liver Enzymes

Table 4 illustrates that ALT, AST, and γ-GT activities of methamidophos-intoxicated rabbits were, in general, significantly elevated throughout the experimental periods examined with respect to controls. The maximum % differences recorded for ALT, AST, and γ-GT were 31.2, 44.5, and 45.6 in the sixth, fourth, and third weeks, respectively ($p = 0.002$, $p = 0.000$ and $p = 0.000$). The significant increment in ALP activity started from the third week with a maximum % difference of 26.7 during the fourth week ($p = 0.003$). In contrast, ChE activity showed a progressive significant inhibition upon methamidophos administration registering a maximum % difference of 77.8% at the end of the experiment ($p = 0.000$).

3.5. Kidney Function

Serum urea and creatinine were significantly increased in methamidophos-fed rabbits compared to controls (Table 5). The maximum % differences for both urea and creatinine were reached in the fourth week showing values of 45.8% and 31.7% with $p = 0.000$ and $p = 0.003$, respectively.

3.6. Protein Profile

As depicted from Table 6, serum total protein, albumin, and globulin were gradually decreased upon methamidophos supplement compared to controls as the experiment proceeds. Such a decrease was significant during the last five weeks for total protein and albumin and during the last four weeks for globulin. The maximum % differences of 27.0, 25.9, and 24.3 for total protein, albumin, and globulin were recorded at the fifth week, with $p = 0.003$, $p = 0.009$, and $p = 0.016$, respectively.

Table 3: Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum glucose and bilirubin levels in male domestic rabbits. The number of animals was 6 per time interval for each control and methamidophos-treated rabbits. All values are expressed as mean ± SEM. $p < 0.05$: significant.

Parameter	Treatment	Experimental period (weeks)					
		1	2	3	4	5	6
Glucose (mg/dl)	Control	113.7 ± 4.6	114.8 ± 5.3	115.2 ± 4.9	114.0 ± 5.7	115.7 ± 5.1	117.4 ± 4.8
	Methamidophos	115.2 ± 5.4	135.7 ± 6.2	133.4 ± 6.0	149.7 ± 6.9	143.1 ± 6.7	137.0 ± 7.1
	p-value	0.832	0.031	0.043	0.003	0.010	0.042
Bilirubin (mg/dl)	Control	1.62 ± 0.05	1.71 ± 0.06	1.63 ± 0.08	1.60 ± 0.03	1.69 ± 0.04	1.64 ± 0.03
	Methamidophos	1.70 ± 0.06	1.86 ± 0.09	1.81 ± 0.10	1.83 ± 0.12	2.05 ± 0.13	1.92 ± 0.10
	p-value	0.348	0.217	0.192	0.087	0.024	0.039

Table 4: Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum ALT, AST, γ-GT, ALP, and ChE activities in male domestic rabbits. ALT: alanine aminotransferase, AST: aspartate aminotransferase, γ-GT: γ-glutamyltransferase, ALP: alkaline phosphatase, and ChE: cholinesterase. The number of animals was 6 per time interval for each control and methamidophos-treated rabbits. All values are expressed as mean ± SEM. $p < 0.05$: significant.

Enzyme	Treatment	Experimental period (weeks)					
		1	2	3	4	5	6
ALT (U/L)	Control	47.5 ± 1.9	48.0 ± 2.2	47.8 ± 1.7	50.2 ± 2.0	47.4 ± 2.3	48.7 ± 2.1
	Methamidophos	51.8 ± 2.6	55.7 ± 2.4	56.1 ± 3.0	61.4 ± 3.2	57.0 ± 2.9	66.7 ± 3.6
	p-value	0.213	0.042	0.035	0.014	0.027	0.002
AST (U/L)	Control	32.6 ± 1.6	33.7 ± 1.5	35.0 ± 2.0	33.2 ± 1.8	34.5 ± 1.7	33.8 ± 1.4
	Methamidophos	38.9 ± 2.1	49.2 ± 2.8	46.5 ± 2.6	52.3 ± 3.0	45.4 ± 2.8	45.2 ± 2.9
	p-value	0.031	0.001	0.006	0.000	0.007	0.005
γ-GT (U/L)	Control	6.04 ± 0.29	6.15 ± 0.32	5.89 ± 0.25	6.10 ± 0.34	5.82 ± 0.28	6.09 ± 0.31
	Methamidophos	7.34 ± 0.38	8.26 ± 0.45	9.37 ± 0.56	8.58 ± 0.51	9.06 ± 0.54	8.01 ± 0.47
	p-value	0.024	0.004	0.000	0.002	0.000	0.007
ALP (U/L)	Control	96.4 ± 4.1	102.0 ± 4.5	97.1 ± 4.0	98.2 ± 3.8	100.3 ± 4.6	96.5 ± 4.7
	Methamidophos	105.9 ± 5.0	114.8 ± 4.9	114.6 ± 5.7	128.4 ± 6.5	125.5 ± 5.4	117.0 ± 6.1
	p-value	0.172	0.089	0.033	0.003	0.007	0.025
ChE (U/L)	Control	4534 ± 168	4526 ± 159	4663 ± 171	4537 ± 152	4701 ± 163	4542 ± 148
	Methamidophos	2603 ± 117	2442 ± 106	2455 ± 102	2230 ± 98	2228 ± 94	1997 ± 89
	p-value	0.000	0.000	0.000	0.000	0.000	0.000

Table 5: Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum urea and creatinine levels in male domestic rabbits. The number of animals was 6 per time interval for each control and methamidophos-treated rabbits. All values are expressed as mean ± SEM. $p < 0.05$: significant.

Parameter	Treatment	Experimental period (weeks)					
		1	2	3	4	5	6
Urea (mg/dl)	Control	37.1 ± 1.8	37.5 ± 1.6	36.9 ± 1.7	35.7 ± 1.4	34.2 ± 1.5	35.3 ± 2.0
	Methamidophos	45.0 ± 2.4	47.6 ± 2.8	50.6 ± 3.1	56.9 ± 4.0	50.7 ± 3.6	50.0 ± 2.9
	p-value	0.025	0.011	0.003	0.000	0.001	0.002
Creatinine (mg/dl)	Control	0.62 ± 0.02	0.60 ± 0.03	0.57 ± 0.01	0.61 ± 0.02	0.58 ± 0.01	0.61 ± 0.04
	Methamidophos	0.70 ± 0.04	0.72 ± 0.04	0.73 ± 0.05	0.84 ± 0.06	0.75 ± 0.05	0.76 ± 0.03
	p-value	0.086	0.029	0.011	0.003	0.010	0.015

Table 6: Effect of methamidophos (1/10 $LD_{50,}$ 2.1 mg kg^{-1} body weight) on serum total protein, albumin and globulin levels in male domestic rabbits. The number of animals was 6 per time interval for each control and methamidophos-treated rabbits. All values are expressed as mean ± SEM. $p < 0.05$: significant.

Parameter	Treatment	Experimental period (weeks)					
		1	2	3	4	5	6
Total protein (mg/dl)	Control	5.81 ± 0.28	5.76 ± 0.29	6.04 ± 0.31	5.92 ± 0.26	6.10 ± 0.33	5.83 ± 0.32
	Methamidophos	5.27 ± 0.21	4.90 ± 0.18	4.93 ± 0.16	4.71 ± 0.19	4.65 ± 0.14	4.69 ± 0.17
	p-value	0.162	0.035	0.013	0.006	0.003	0.015
Albumin (mg/dl)	Control	3.72 ± 0.18	3.63 ± 0.16	3.91 ± 0.23	3.85 ± 0.21	3.97 ± 0.25	3.75 ± 0.20
	Methamidophos	3.43 ± 0.14	3.15 ± 0.11	3.21 ± 0.10	3.10 ± 0.13	3.06 ± 0.09	3.03 ± 0.13
	p-value	0.232	0.043	0.023	0.016	0.009	0.017
Globulin (mg/dl)	Control	1.99 ± 0.08	2.06 ± 0.10	2.09 ± 0.13	1.96 ± 0.11	2.03 ± 0.14	1.98 ± 0.11
	Methamidophos	1.87 ± 0.09	1.84 ± 0.11	1.73 ± 0.06	1.60 ± 0.07	1.59 ± 0.05	1.61 ± 0.06
	p-value	0.351	0.172	0.028	0.023	0.016	0.020

Table 7: Effect of methamidophos (1/10 $LD_{50,}$ 2.1 mg kg^{-1} body weight) on serum calcium and phosphorus concentrations in male domestic rabbits. The number of animals was 6 per time interval for each control and methamidophos-treated rabbits. All values are expressed as mean ± SEM. $p < 0.05$: significant.

Electrolyte	Treatment	Experimental period (weeks)					
		1	2	3	4	5	6
Calcium (mg/dl)	Control	13.9 ± 0.8	13.7 ± 0.9	14.1 ± 0.6	13.8 ± 0.7	14.0 ± 1.0	13.8 ± 0.7
	Methamidophos	12.5 ± 0.6	12.0 ± 0.4	12.5 ± 0.7	11.9 ± 0.4	12.5 ± 0.3	11.6 ± 0.3
	p-value	0.198	0.096	0.112	0.041	0.162	0.030
Phosphorus (mg/dl)	Control	7.30 ± 0.4	7.46 ± 0.3	6.95 ± 0.2	7.43 ± 0.2	7.44 ± 0.4	7.18 ± 0.3
	Methamidophos	8.18 ± 0.5	8.79 ± 0.4	8.45 ± 0.6	9.44 ± 0.7	8.97 ± 0.4	8.82 ± 0.5
	p-value	0.168	0.034	0.025	0.011	0.029	0.023

3.7. Calcium and Phosphorus

Table 7 revealed that serum calcium was generally decreased in methamidophos-treated rabbits. Such decrease fluctuates throughout the experiment registering significant decrease during the fourth and sixth weeks with maximum % differences of 14.8 and 17.4, respectively ($p = 0.041$ and $p = 0.030$). Conversely, serum phosphorus increased along with the experiment displaying a significant increase commencing from the second week with a maximum % difference of 23.8 in the fourth week ($p = 0.011$).

4. DISCUSSION

The acute oral LD_{50} of methamidophos was estimated to be in the range of 10-30 mg/kg body weight in rabbits, cats, and dogs [3]. However, no published data are available on the exact or specific oral LD_{50} in male domestic rabbits. In this study, the logarithmic scale showed that the oral LD_{50} of methamidophos in male domestic rabbits was 20.5 mg kg^{-1} body weight. This confirms the fact that methamidophos is a highly toxic pesticide and coincides with the idea that the lower the LD_{50} value, the more toxic is the pesticide. Methamidophos was classified as a class I compound, and its use must be restricted [2].

Oral administration of 1/10 LD_{50} methamidophos caused a significant reduction in the bodyweight of rabbits, a finding agreed with that previously reported [9, 28]. Such reduction may be a result of the combined action of cholinergic (reduced food intake and diarrhea) and oxidative stress [29, 30]. The mortalities registered in methamidophos-treated rabbits may be attributed to diarrhea that may be related to the cholinergic crisis, a consistent sign in organophosphate poisoning [31].

Serum glucose was significantly increased in methamidophos-fed rabbits, a finding consistent with that reported earlier [32]. The mechanism by which this organophosphorus insecticide induces hyperglycemia may involve one or more mechanisms: (1) reduction in insulin secretion as a result of the destructive action on the beta cells of Langerhans islets in the pancreas [33], (2) impairment in hepatic function that reduces liver ability to glycogenesis [34], and (3) stimulation of hepatic gluconeogenesis and glycogenolysis [35]. Serum bilirubin was also increased. A similar result was obtained in organophosphorus pesticide-intoxicated rats [36]. Such an increase of bilirubin may be attributed to the impairment of hepatocellular function in acute or subacute hepatic necrosis and may provide further evidence on hepatotoxicity induced by the organophosphorus insecticide methamidophos [8, 9].

Liver enzymes including ALT, AST, γ-GT, and ALP were significantly elevated in methamidophos-treated rabbits. Such elevation was documented by other authors [9, 37]. The liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as methamidophos poisoning [38, 39]. Serum ALT, AST, and γ-GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity [40, 41]. Pesticide exposure causes liver damage and leakage of cytosolic enzymes from hepatocytes into blood [39, 40]. In contrast, ChE was significantly inhibited and such inhibition was previously reported [42]. It is known that organophosphorus pesticides such as methamidophos cause irreversible inhibition of ChE leading to the accumulation of acetylcholine and over activation of acetylcholine receptors at the neuromuscular junction and in the autonomic and central nervous system. This is manifested in cholinergic symptoms including diarrhea, convulsions, and even tremors leading in severe cases to death [31]. This is confirmed by some mortalities and the toxicity symptoms of anticholinesterase action observed in methamidophos-intoxicated rabbits.

Serum urea and creatinine were significantly increased in methamidophos-fed rabbits. In a recent study [9], methamidophos-treated Wistar male rats showed high urea levels. Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function [43]. Thus, the observed increase in urea may be due to (1) impairment in its synthesis as a result of impaired hepatic function, (2) disturbance in protein metabolism, and (3) decrease in its filtration rate in the kidney. As the kidneys become impaired, the creatinine level in the blood will rise due to poor clearance by the kidneys.

Serum total protein, albumin, and globulin were significantly decreased upon methamidophos intake. Similar findings were reported in other studies [44, 45]. The protein level suppression may be due to loss of protein either by reduced protein synthesis or increased proteolytic activity [45, 46]. Low levels of albumin were found in liver disease [47]. Hypocalcemia and hyperphosphatemia were found in methamidophos-fed rabbits. Alterations in serum electrolytes were reported in response to organophosphorus pesticide exposure including methamidophos [48, 49]. This indicates that methamidophos interferes with calcium and phosphorus homeostasis.

5. CONCLUSION

Methamidophos exposure at a sublethal dose had a potentially toxic effect on liver and kidney functions as indicated by significant alterations in their biomarkers. Such findings can expand our understanding of potential hazards in human populations due to methamidophos exposure.

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Authors' Contributions
This work was carried out with the collaboration of all authors. All authors contributed equally to this work.

Conflict of Interest
The authors declare that there is no conflict of interest.

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