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Histopathological and biochemical effects of abamectin on kidney in male albino rats

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Abstract
The present study has been done to evaluate the effect of abamectin on kidney function and histological changes in mal albino rats. The animals were divided into three groups; group1 rats were injected interaperitoneal with a drop of distilled water (0.1 mg/kg) for a period of 15 days and kept under control. And the other two treated groups (1T and 2T) were also daily injected interaperitoneal with abamectin with dose of 0.1 and 0.6 mg/kg body weight respectively for the same period of 15 days. Rats were sacrificed and blood samples were collected immediately from each rat separately in dry clean centrifuge tubes. Blood samples were centrifuged (3000 rpm for 15 min) and plasma was separated to estimate the Creatinine and urea. Tissue sections from the kidney of the control and the treated groups were prepared and stained with haematoxylin and eosin and observed under the microscope. The present results showed that the body weight of rats in the group-1 (control) increased gradually from around 156g to 205.9g in 15 days while in the treated groups 1T and 2T body weight decreased from 268g and 203.6g as initial weight to 259.5g and 196g respectively. The result also showed that the treatment with abamectin caused significant increase in creatinine and urea concentrations compared to control animals Histopathological examination of rat kidneys revealed Congested B.V, Glomerular shrinkage, Glomerular degeneration, Medullary tubular degeneration and Medullary tubular casts in rats treated with abamectin. Data analyses were done by using one way ANOVA at the level of (P<0.05). The results of current study indicated that the abamectin had a great toxic effect on kidney function which causes significant changes in urea and creatinine. Likewise, pathological examination showed abnormalities in the structures of kidney tissues.

Keywords: Abamectin, male albino rats, biochemical parameters, histopathology, Kidney

1. Introduction
Pesticides have played vital role in controlling agricultural, industrial, home and public health pest worldwide [1]. However, their use poses animal and human health concerns because of their toxicity, widespread use and release into the environment [2]. According to the World Health Organization (WHO), approximately 3 million cases of pesticide poisoning occur every year resulting in more than 250,000 deaths [3].
Obviously, exposure to pesticides poses a continuous health hazard especially in the agricultural working environment. By their very nature, most pesticides show a high degree of toxicity because they are designed to kill certain organisms and thus create some risk of harm. Within this context, pesticide use has raised serious concerns not only of potential effects on human health but also about impacts on wildlife and sensitive ecosystems [4-6].
In toxicity studies, a variety of biochemical parameters are measured to evaluate a broad range of physiological and metabolic functions affecting target organ identification and tissue injury assessment [7]. Abamectin was widely employed to control insects and mites of a wide range of agricultural products such as fruit, vegetable and ornamental crops [8]. Moreover, it is widely used as an antiparasitic agent in livestock, animal farms and pets and as the active ingredient of insecticides, acaricide, and anthelmintic for agricultural use [9-10]. Currently, Abamectin has been used extensively all over the world and still one of the most commonly used pesticides in Yemen.
Presently, abamectin used in several countries as a pest control agent in livestock and as an active substance of nematicides and insecticides for agricultural use [11]. This product is a potent insecticide and may be highly toxic to mammals. There are many problems associated with chemical insecticides such as health hazards [12]. Due to its high lipophilic nature, abamectin tends to accumulate in fat tissue which acts as a drug reservoir. The highest levels of abamectin were found in liver and fat while the lowest ones were found in brain tissue [13].
A biomarker may be any measurable biochemical, cellular, physiological or behavioral change

in an organism or population that indicates exposure to chemical pollutants [14]. With focusing on the intermediate sublethal effects of a pollutant, this developing field aims to disclose environmental threats before clear toxic effects such as death of organisms are observed. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals. Also, organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs and in toxicological experiments [15].
Abamectin revealed significant increases in the liver function parameters (i.e. ALT, AST activities, acid phosphatase activity, serum albumin, glucose and total protein levels) [16]. Furthermore, kidney function parameters (uric acid and creatinine concentration) were severely affected [17].

2. Materials and Methods

2.1 Pesticides Used

Abamectin (1.8% E.C; 100mL): a mixture containing a minimum of 80% avermectin B1a (5-O-demethylavermectin) and a maximum of 20% avermectin B1b (5-O-demethyl-25-de-(1-methylpropyl)-25-(1-methylethyl), was supplied by VAPCO Manufacturing CO. Ltd.

2.2 Animals

Wistar male albino rats were obtained from the animal house, faculty of sciences, Sana'a University, Yemen. Animals were housed in stainless still cages under the laboratory conditions of 25 ± 5 ºC and 70 ± 10 % humidity with 12 hours dark: light cycle, provided with diet and water ad libitum. The animals were allowed to acclimatize for two week prior to the start of the study. All the experiments were carried out in compliance with the guide for the care and use of laboratory animals.

2.3 Experimental design

The rats were divided into three groups, each including five animals and daily treated interaperitoneal injection for 15 consecutive days as follows:
Group 1(Control), the rats in this group were daily injected interaperitoneal with a dropped water (0.1 mg/kg) for a period of 15 days without any ingredient of Abamectin. Initial weight of each rat was (156g ±5.10g).
Group 2 (first treated group (1T)) the rats in this group were daily injected interaperitoneal with abamectin as Low dose at a level of 0.1 mg/kg body weight for a period of 15 days. Initial weight of each rat in this group was 268 g ±11.58 g.
Group 3 (second treated group (2T)) the rats in this group were daily interaperitoneal introduced of abamectin as high dose at a level of 0.6 mg/kg body weight for a period of 15 days. Initial weight of each rat in this group was 203.6 g ±9.50 g.
At the end of 15 days, blood samples were individually collected from each rat immediately after slaughtering, the blood transferred into dry clean centrifuge tubes. Plasma was separated after centrifugation (3000 rpm for 15 min) plasma samples were used to estimate the Creatinine and urea.

2.4 Kidney function parameters

Creatinine and urea were spectrophotometrically analyzed using commercial kits from Bio Scope Diagnostics and ERBA Diagnostics Mannheim GmbH (Germany), according to [17-18] respectively.

2.5 Histological Studies

Suitable pieces of kidney were removed from all the animals of each of the experimental groups 1, 2, 3 and fixed for histopathological investigation. The tissues were putted in 10% buffered formalin. The organs were dehydrated in ethanol (70 to 100%), cleared in xylene and embedded in paraffin. Tissue sections were prepared and stained with haematoxylin and eosin (H and E) [19-20]. Stained sections were examined under a light microscope.

2.6 Statistical Analysis

Data were statically evaluated by using one way ANOVA. Wherever the ANOVA values were found to be significant, Duncan's new multiple range test (DMRT) was applied (SPSS computer software). The values were considered significant at the level of $p < 0.05$.

3. Results

3.1 Body weight

The interaperitoneal injection of abamectin showed a great effect on the body weight of wistar male albino rats figure 1. The body weight of rats in the control group-1 significantly increased gradually from around 156 g to 205.9 g over a period of 15 days while in the treated groups 1T and 2T the body weight significantly decrease from 268 g and 203.6 g as initial weight to 259.5 g and 196 g respectively at the period of 15 days figure1.

3.2 Kidney Function Parameters

The present results showed that treatment with abamectin caused significant increase in creatinine and urea concentrations at the level of (p<0.05) compared to control animals (Fig2-3).

3.3 Histopathological Examination

Histological examination of the kidney of control rats revealed normal histological features, illustrated in (Fig. 4). Kidney of abamectin-treated animals at lower dose of abamectin pesticide (0.1 mg/kg bw) treated for 15 days, as presented in Fig. 4. These changes include the presence (B) Congested B.V., (C) Glomerular shrinkage, (D) Glomerular degeneration, (E) Medullary tubular degeneration and (F) Medullary tubular casts.
The kidney tissues of treated animals with a higher dose of abamectin pesticide (0.6mg/kg bw) presented in Fig. 5. These changes include (B) Congested B.V., (C) Glomerular shrinkage, (D) Glomerular degeneration, (E) Medullary tubular degeneration and (F) Medullary tubular casts.

4. Discussion

The present study was conducted to investigate the effects of abamectin on male albino rats and evaluate its impact on body weight and on some biochemical parameters as well as histopathological changes. In the present study, body weight was comparable between all studied groups. The results showed that there was significant (p<0.05) decrease in the body weight of treated animals with abamectin compared with control rats which were increased and gaining body weight significantly as shown in Fig. 1. The study findings were in agreement with some of the previous studies by [21-22], who reported the body weight of abamectin treaded mice was significantly lower than that of the control group at the level of (p< 0.05). Obviously, in toxicological studies, body and organ weights of animals are important criteria for the evaluation of toxicity [23]. Here, the decreasing in the body weight may be attributed to a decreased food intake (anorexia or food avoidance), poor food palatability or increased degradation of lipids and protein due to treatment-related

toxicity [22, 24]. The study results reported that the levels of urea and creatinine were significantly increased in serum of treated rats with abamectin, compared with the control group, as shown in figure 2& 3. These findings are in coincidence with those reported by [25]. The increase in creatinine due to abamectin toxicity was also correlated closely with the histopathological changes in the kidney. Marked congestion and other degenerative changes were observed in the kidneys. Creatinine is a metabolite of creatine and is excreted completely in urine via glomerular filtration. An elevation of creatinine level in the blood is an indication of impaired kidney function [26]. In the present study, the significant increases of plasma urea and creatinine in the treated rats may be due to damage of the kidney cells and/or failure of kidney under the toxic effect of abamectin biocide. Similarly Eissa and Zidan, [27] reported that kidney function (uric acid and creatinine concentration) were severely affected in male albino rats orally treated with LD_{50} of abamectin for 30days. Increase of serum uric acid and creatinine concentrations of treated male albino rats may be due to reduction in glomerular filtration in kidney and reflect dysfunction of the kidney tubules [28].

The results of the histopathological examination showed many histological abnormalities in the kidney of abamectin-treated rats compared to untreated rats in the control group. The kidney showed usual structures in control rats and abnormal structures in the rats which were exposed to the lower and higher dose of abamectin exhibited the presence changes include Congested B.V., Glomerular shrinkage, Glomerular degeneration, Medullary tubular degeneration, Medullary tubular casts, all those presented in Fig. 4 and 5.

Histological changes provide a rapid means for detecting the effects of pesticides in various animal tissues and organs [29]. Hence, the clinical chemistry and histopathological evaluations methods are commonly used for detecting organ specific effects related to chemical exposure [30]. Kidney is an organ which is composed of numerous renal corpuscles connecting with well-developed glomeruli and a system of renal tubules. These histopathological changes of kidney tissues in rats treated with some pesticides have been reported by previous investigators using male rats [31] as well as pregnant rats [32-33]. Abd-Elhady and Abou-Elghar, [34] reported that the change in the biochemical parameters of kidney was also correlated closely with the histopathological. Our current results observed many necrobiotic changes in the kidney tissues in albino rat, similarly, Abd-Elhady and Abou-Elghar, [34] observed that the kidneys showed marked necrobiotic changes in abamectin-treated animals as compared to the normal histological examination of renal tissue in the control rats.

Table 1: Effect of different doses of abamectin on the body weight of male rats after 15 days compared with control group.

Treatment / Parameter	Abamectin treatment for 15 days	
	Initial body weight(g)	**Final body weight(g)**
Control	156 ± 5.10[a]	205.9 ± 18.65[b]
1T. (0.1 mg/kg/bw)	268 ± 11.58[a]	259.5 ± 11.06[b]
2T. (0.6 mg/kg/bw)	203.6 ± 9.50[a]	196 ± 8.72[b]

The mean difference was significant at the p<0.05 level, in comparison with control.

Table 2: Effect of abamectin on blood Urea and Creatinine (mg/dl) in different of experimental groups.

Treatment / Parameter	Abamectin treatment for 15 days	
	Blood urea (mg/dl)	**Creatinine (mg/dl)**
Control	33.2 ± 0.28[a]	0.25 ± 0.05[a]
1T. (0.1 mg/kg/bw)	35± 0.33[b]	0.72 ± 0.09[b]
2T. (0.6 mg/kg/bw)	37.2 ± 0.47[c]	0.86 ± 0.05[c]
The mean difference was significant at the p < 0.05 level.		

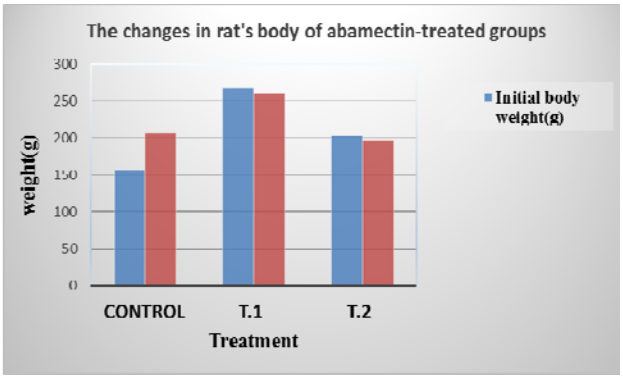

Fig 1: Effect of abamectin on body weight of treated of male rats.

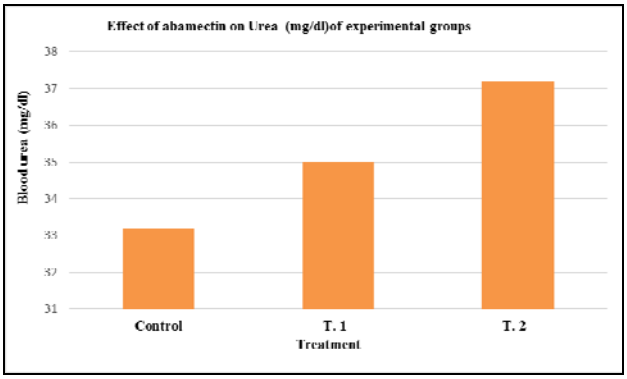

Fig 2: Effect of abamectin on blood urea.

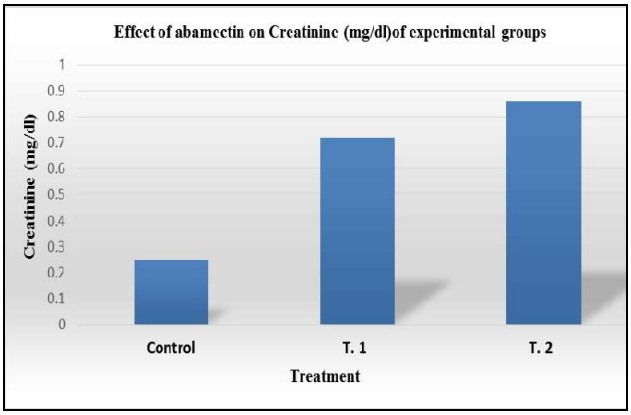

Fig 3: Effect of abamectin on creatinine.

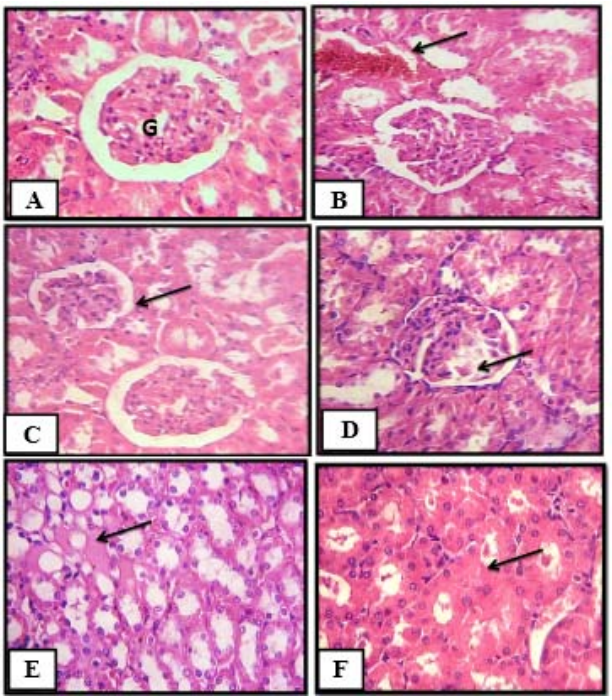

Fig 4: Photomicrograph of the rat kidney sections (0.1mg/kg/bw) shows (A) Normal glomeruli, G indicates normal tubules. (B) Congested B.V. (C) Glomerular shrinkage. (D) Glomerular degeneration. (E) Medullary tubular degeneration. (F) Medullary tubular casts (40X).

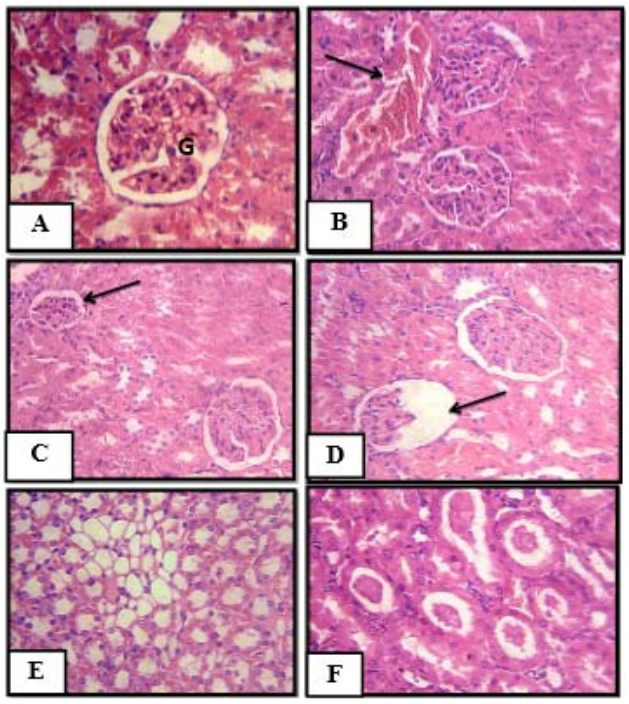

Fig 5: Photomicrograph of the rat kidney sections (0.6mg/kg/bw) shows (A) Normal glomeruli, G indicates normal tubules. (B) Congested B.V. (C) Glomerular shrinkage. (D) Glomerular degeneration. (E) Medullary tubular degeneration. (F) Medullary tubular casts (40X).

5. Conclusion

The results of present study demonstrated that interaperitoneal injection administration of abamectin, at 0.1 mg/kg bw and 0.6 mg/kg bw for 15 days induces toxic effects on kidney function which correlate well with the histopathological changes in the kidney. It causes significant changes in urea and creatinine. Likewise, pathological examination of abnormalities in kidney tissues also observed.

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