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Comparative Study on Toxicity of Synthetic Pyrethroids And Azadirachtin Insecticides in Experimental Rats

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Abstract

The current work was planned to compare the toxic effects of a commercially available insecticide (Butox® 50%) that was widely used in the Egyptian veterinary field with Achook® insecticide (a product of agricultural application only) derived from neem (*Azadirachta indica*) seed oil extract on the experimental animals in a trail to predict their side effects if used topically upon livestock and accidently ingested or licked by animal. The insecticides toxicity was evaluated on basis of signs of toxicity, changes of biochemical parameters of experimental animals in addition to histopathological findings observed in examined internal organs. Statistical analysis clarified that the total body weight of Butox® 50% treated groups had a significant increase than the weight of control group. On contrary, Achook 0.15% insecticide did not have any significant effect on the body weight as weight did not changed when compared with control group. Butox® 50% and Achook 0.15% insecticides caused no significant signs of toxicity or mortalities indicating high safety margin of azadirachtin on non-target organisms. Butox® 50% toxicity on overdose led to significant decrease of ALT, AST, ALP and LDH which may be a result of severe liver damage. Hypercholesterolemia was observed that may be due to the ability of pyrethroids to disrupt lipid metabolism in liver. Finally, there was a reverse relation between AChE and testosterone hormone was already observed in the results of the tested insecticides and it is worth mentioning that Achook insecticide caused the highest increase in AChE and the lowest level of testosterone hormone when compared with the control group and other insecticide groups. The histopathological examination of selected tissues showed that the safest insecticide was Achook 0.15% as it led to the least alterations in liver and kidney tissues; in addition, it did not produce any changes in histology of other organs as brain and testes unlike Butox® 50% insecticide.

Keywords: insecticide, toxicity, Butox, Azadirachtin

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Introduction:

The world has suffered greatly from the serious effects of synthetic pesticides which use has increased five folds in the last 30 years **(Nawab et al., 2003).** They were made by the hands of man and has been used for many years and now human is reaping the harvest of what presented with his hands in the form of poor health and a steady increase in non-curable diseases, and let us do not forget the case of severe pollution affected the surrounding environment including water, soil and air. Therefore, the researchers always compete to minimize these problems by resorting to natural alternatives to synthetic insecticides own the same power of currently used pesticides but safer to the environment, animals and humans as the same. Presently, ectoparasites control is focused on the repeated use of organophosphates (OPS) and pyrethroid group of acaricides **(Magadum et al., 2009)**. However, recent studies considered the organophosphorus compounds the most toxic class of pesticides regarding to vertebrate animals. So, now synthetic pyrethroids are becoming predominant insecticides for agricultural and urban applications. They are synthetic compounds structurally derived from pyrethrin I, one of the six active components of pyrethrum, which is an extract from the dried flower heads of *Chrysanthemum cinerariaefolium* **(Holmes et al., 2008**). Plant extracts were widely used against phytophagus pests and mosquitoes including neem (*Azadirachta indica*). The principle insecticidal component of neem extract is Azadirachtin which has a diverse range of bioactivities, such as insecticidal, anthelmintic, antifeedancy and insect growth regulation effects **(Yan et al., 2012)**. Several studies approved the efficacy of neem extract on hard ticks **(Al-Rajhy et al., (2003), Magadum et al., (2009) and Garcia, et al. (2012)).** Due to the residual contamination of chemical pesticides to agricultural crops and food commodities, the use of azadirachtin as an alternative to synthetic pesticides has increased significantly **(Hanhong and Li, 2001)**. However, little is known about its toxicity, mutagenicity and clastogenicity in mammalian species. The continued usage of the same compound for controlling certain insect for long period resulted in developing resistance against the used compound so the present and future challenge is to get safe, cheap and environmentally friendly alternative pesticide so the current work was planned to compare the toxic effects of a commercially available insecticide (Butox® 50%) widely used in the Egyptian veterinary field and Achook® insecticide (a product of agricultural application only) derived from neem (*Azadirachta indica*) seed oil extract on the experimental animals in a trail to predict their side effects of application on live stock.

Materials and Methods

Tested insecticides:

Butox® 50%: It was one of the synthetic Pyrethroids pesticides. It was produced by the Arab Company for Chemical Industry, Egypt. It contained 5% deltamethrin active principle.

Achook 0.15%: Achook was an insecticide product for agricultural use only and had no recommended dose for veterinary application. It contained 0.15% azadirachtin that was the main component of neem seed oil extracted from seeds of neem plant (*Azadirachta indica*) and it was classified as a bio-insecticide. The tested product was produced by Egyptian Agricultural Development Company, Egypt.

Albino rats used in assessment of toxicity of the selected insecticide: A total of 30 apparently healthy, male albino rats (wistar) initially weighing 120 ±10 g was used in this study. Animals were purchased from laboratory animal house in Tanta city, Egypt and housed in the Department of Animal Hygiene and Zoonoses of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University. Rats were left for two weeks for acclimatization and kept in cages in a rate of 5 rats per cage at room temperature and supplied with standard diet and water ad libitum during the whole experiment period (four weeks). All procedures and investigation were reviewed and approved by the animal ethics committee and were performed in accordance with the guiding principles of the care of the laboratory animals. They were divided into 3 groups; each group consisted of 10 animals. Group I (control group) received saline only while groups II and III received Butox® and Achook, respectively.

Assessment of insecticides toxicity in albino rats:

Experimental albino rats intoxication: The experimental sub-acute intoxication of male albino rats with the tested insecticides was conducted over 4 weeks; rats received treatments orally on daily basis (6 days/week) and they were noticed twice daily for appearance of toxicity signs and their body weights were recorded on weekly basis. The experimental groups were designed as follow:

Group I (control group): Rats received only saline.

Group II: Rats were intoxicated with Butox® 50% at dose of 0.6 mg deltamethrin /kg Bwt according to **Oda and El Maadawy, (2011**).

Group III: Rats received Achook at dose of 9 mg azadirachtin /kg Bwt according to **Abou- Tarboush et al.** (**2009**).

Blood sampling: Blood samples were collected from retro-orbital sinus by the aid of capillary tubes in weatherman tubes without adding anticoagulant then the samples were centrifuged at 3500 rpm for 5 minutes to obtain the serum then the obtained

serum was removed to clean, dry and well-marked Eppendorf tubes, and kept in refrigerator until tested.

Biochemical analysis: biochemical blood parameters for liver function were done according to IFCC, (2011)., parameters for renal function according to Kaplan et al., (1984c) and Other parameters estimated in serum like Cholinesterase enzyme (ChE), according to Tietz and Saunders (1999) and Testosterone hormone, according to Wheeler (1995).

Histopathological examination:

Vital organs including, liver, kidneys, testes and brain were collected as fast as possible from sacrificed rats then washed in saline solution before, they preserved in formalin 10% for fixation. The fixed organ samples were in turn subjected to the paraffin embedding technique to produce paraffin blocks. The produced paraffin blocks were used to prepare 5 microns thick sections which were stained with Hematoxylin and Eosin (H&E) according to the method described by **Toman et al., (2012)**.

Statistical analysis:

Statistical analysis was carried out using one-way Analysis of Variance **(ANOVA)** for study the effect of different treatment groups (Control, Butox-50 and Achook) on different studied variables of either body weight, body weight gains as well as the different hematological and biochemical parameters according to **(SAS, 2004)**.

Results

Effects of tested insecticides on body weight of the experimental rats: there was effect of tested insecticides on the total body weight of experimental rats (Table-1).

Effects of tested insecticides on clinical signs and mortality of rats: the effects of toxic doses of the tested insecticides on the experimental rats (Table-2).

Effect tested insecticides on blood biochemical parameters of rats: there Butox® 50% toxicity led to significant decrease of liver enzymes including ALT, AST, ALP and LDH. This reduction may be a result of severe liver damage and this prospect was in accordance with the pathological changes noticed in the examined hepatic tissue (Table-3, Photo-1/2).

Effect of insecticides on biochemical serum parameters related to kidney functions of experimental rats: there was reduced urea and creatinine levels in rat's serum as a result of Butox® 50% administration indicating dysfunction of hepatic and renal tissues (Table-4, Photo-3/4).

Effect of the tested insecticides on Acetylcholinesterase (AChE) and testosterone levels in the experimental animals' serum: Testosterone hormone did not change significantly due to Butox® 50% intoxication (Table-5) however the histological examination of testes showed pathological changes (Photo-5/6).

Table (1): Effect of insecticides on body weight of experimental rats

Insecticide	1st Week	2nd Week	3rd Week	4th Week	Total B. Wt. (g)
Butox® 50%	150.45±3.47[a]	166.82±4.54[a]	183.00±7.79[a]	211.50±11.08[a]	221.67±13.10[a]
Achook 0.15%	131.00±1.80[bc]	152.22±3.13[b]	159.44±4.89[b]	182.22±6.24[bc]	189.38±6.91[bc]
Control	127.50±2.11[c]	144.38±2.58[b]	167.14±4.21[ab]	170.00±5.12[c]	181.43±5.08[c]

Means within the same column of different litters are significantly different at (P < 0.01).

Table (2): Effect of insecticides on clinical signs and mortality rate in experimental rats

Insecticide	Experimental toxic dose	Signs of toxicity	
		Nervous manifestation	Mortality rate (%)
Butox® 50%	0.6 mg/kg Bwt	Absent	0.0
Achook 0.15%	9 mg/kg Bwt	Absent	0.0

Table (3): Effect of insecticides on biochemical parameters related to liver functions of experimental rats

Parameters	Unit	Control	Butox® 50%	Achook 0.15%
ALT	u/l	50.67±6.36[b]	38.67±1.86[c]	35.00±1.73[d]
AST	u/l	149.00±7.81[a]	122.00±7.81[b]	119.00±29.02[b]
ALP	u/l	190.67±46.92[c]	163.67±17.61[d]	290.33±57.92[a]
LDH	u/l	2835.00±480.82[c]	1934.67±152.60[d]	3245.67±596.93[a]
Glucose	mg/dl	144.00±12.34[a]	114.33±8.76[c]	130.67±4.67[b]
Cholesterol	mg/dl	70.67±2.85[b]	90.00±1.73[a]	72.67±1.76[b]
Albumin	g/dl	3.27±0.03[c]	3.63±0.09[a]	3.43±0.09[b]
Protein	g/dl	5.90±0.23[b]	6.77±0.63[a]	5.77±0.33[b]
Bilirubin	mg/dl	0.16±0.01[a]	0.14±0.01[b]	0.14±0.01[b]

Means within the same raw of different litters are significantly different at (P < 0.05).

Table (4): Effect of insecticides on biochemical serum parameters related to kidney functions of experimental rats

Parameters	Unit	Control	Butox® 50%	Achook 0.15%
Urea	mg/dl	33.33±2.33[a]	25.33±2.33[b]	33.67±0.88[a]
Creatinine	mg/dl	0.57±0.03[a]	0.47±0.03[b]	0.60±0.00[a]
Uric acid	mg/dl	3.70±0.15[a]	3.30±0.15[a]	3.57±0.09[a]

Means within the same raw of different litters are significantly different at (P < 0.05).

Table (5): Effect of the tested insecticides on Acetylcholinesterase (AChE) and testosterone levels in the experimental animals' serum

Parameters	Unit	Control	Butox® 50%	Achook 0.15%
AChE	u/l	1981.33±53.65[b]	1553.00±59.14[c]	2251.33±161.66[a]
Testosterone	ng/ml	2.52±0.91[b]	2.34±0.91[b]	2.07±0.41[c]

Means within the same raw of different litters are significantly different at (P < 0.05).

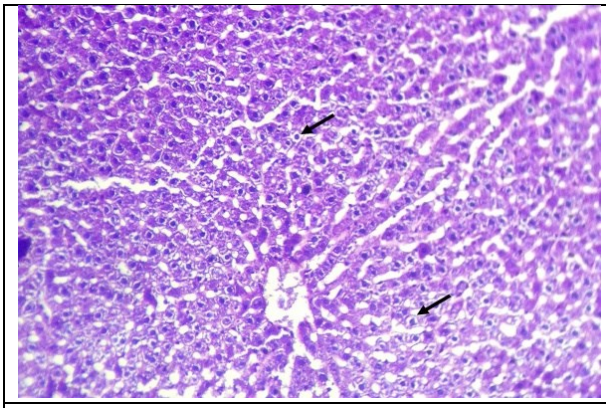

Photo (1): Liver of a rat showing moderate hydropic degeneration of some hepatocytes (arrows) due to Butox toxicity

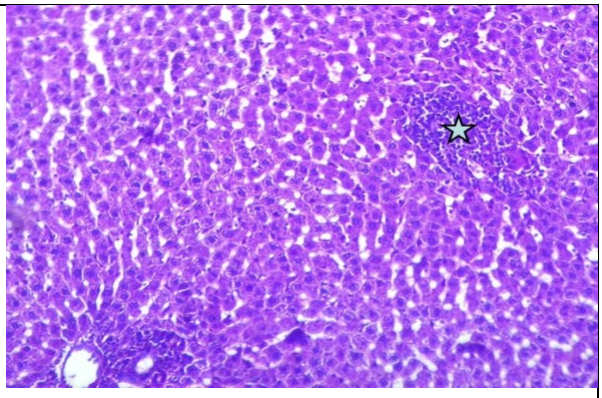

Photo (2): Liver of a rat showing hepatic necrosis with inflammatory cell infiltration (star) due to Butox toxicity

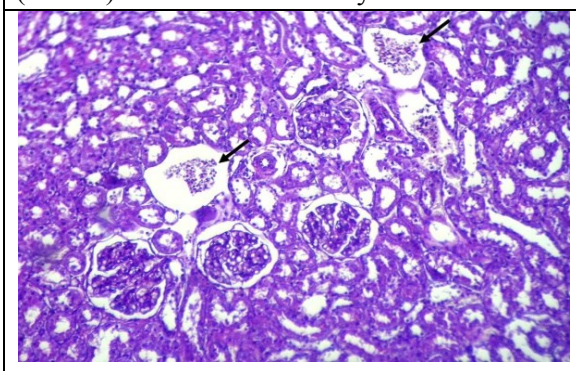

Photo (3): Kidney of a rat showing congestion of blood vessels (arrows) due to Butox toxicity.

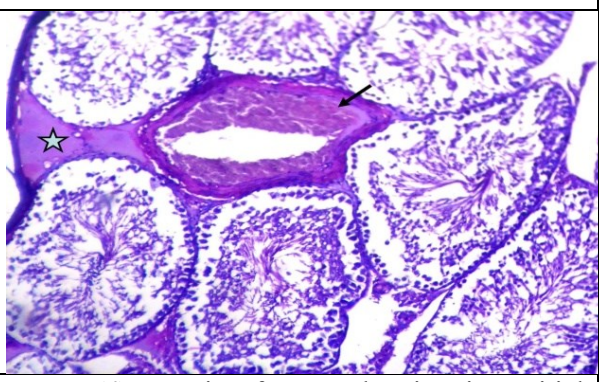

Photo (4): Testis of a rat showing interstitial edema which is characterized by faint esinophilic albuminous fluid (star) with congestion of blood vessel (arrow) due to Butox toxicity.

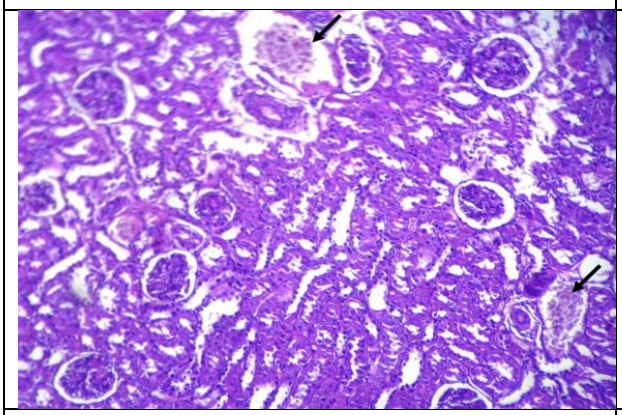

Photo (5): Kidney of a rat showing congestion of blood vessels (arrows) due to Achook toxicity.

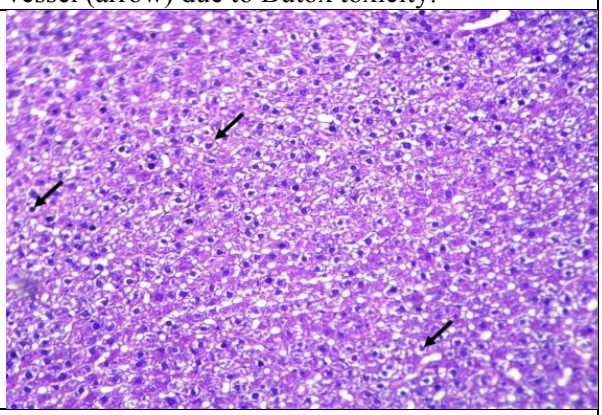

Photo (6): Liver of a rat showing moderate hydropic degeneration of some hepatocytes (arrows) due to Achook toxicity.

Discussion

Regarding the Effects of tested insecticides on body weight of the experimental rats; The illustrated data in **Table (1)** showed the effect of tested insecticides on the total body weight of experimental rats. Statistical analysis clarified that the total body weight of Butox® 50% treated group had a significant increase than the weight of control group that may be a result of

deltamethrin effect on lipid metabolism that led to formation of fat masses inside abdominal cavity. On contrary, it was found that Achook 0.15% insecticide did not have any significant effect on the treated animal's body weight as the group weight did not changed when compared with control group weight. The obtained results disagreed with **Suwanchaichinda et al., (2005)** who mentioned that administration of deltamethrin for 14 days did not affect body weight of Swiss albino mice, but the short duration of administration and the type of experimental animal should be considered, as when the duration prolonged for 45 days **Moid et al., (2014)** recorded a significant decrease in body and tissue weights of treated Swiss albino male mice that may indicate the severe deterioration state of these animals. The recorded result of Achook insecticide was compatible with **Chang et al., (2007)**, **Kupradinun et al., (2010), Aladakatti et al., (2011)** and **Hyunjoo et al., (2014)** who found that administration of either *A. indica* extract or azadirachtin showed non-significant changes in the body weight of experimental rats. On contrary, it disagreed with **Panda and Kar, (2000)** and **Rahman et al., (2001)** who recorded significantly decreased body weight of rats after administration of neem leaf extract and Vepacide (azadirachtin containing insecticide), respectively. This variation in results may be returned to usage of different experimental animal of different susceptibility and the different components of leaf extract or commercial insecticide that should be considered.

Regarding to the Effects of tested insecticides on clinical signs and mortality of rats; The recorded results in **Table (2)** showed the effects of toxic doses of the tested insecticides on the experimental rats. It was illustrated that oral administration of Butox® 50% at a dose of 0.6 mg/kg Bwt to albino male rats did not produce any nervous manifestations characterizing pyrethroids toxicity reflecting the wider safety margin of the administrated dose. This result disagreed with the finding of **Suwanchaichinda et al., (2005)** who administrated 5-10 mg/kg Bwt of deltamethrin to Swiss albino mice for 14 days and observed nervous signs of toxicity. However, **Manna, et al. (2005)** did not notice any gross effect in rats when he tried higher dose of deltamethrin (up to 100 mg/kg Bwt). On the other side, no mortalities were recorded within experimental animals during the current work that agreed with **Yavuz et al., (2012)** who did not report any mortality after daily deltamethrin administration for 28 days. As Butox® 50%, oral administration of azadirachtin at a dose of 9 mg/kg Bwt caused no significant signs of toxicity or mortalities indicating high safety margin of

azadirachtin on non-target organisms that agreed with the results of **Raizada et al., (2001)** who used technical azadirachtin at single oral dose of 5000 mg/kg Bwt, **Chang et al., (2007)** who orally administered azadirachtin at doses of 0.04%, 0.20% and 1.00% in the diet of male and female rats for 90 days and **Sirvastava and Raizada, (2007)** who fed rats 5, 25 and 50 mg/kg Bwt of technical azadirachtin in diet and they did not notice any signs of toxicity appeared on rats.

Regarding to the Effect of tested insecticides on blood biochemical parameters of rats; Butox® 50%: The recorded data in **Table (3)** showed that Butox® 50% toxicity led to significant decrease of liver enzymes including ALT, AST, ALP and LDH. This reduction may be a result of severe liver damage and this prospect was in accordance with the pathological changes noticed in the examined hepatic tissue **(Photo-1/2)**. This result supported by the finding of **Manna et al., (2005)** who mentioned that AST and ALT activities in serum changed due to damage of liver intoxicated with deltamethrin, **Eraslan et al., (2007)** who decided that the reduction of ALP may be a result of severe liver damage and **Kumar et al., (1999)** who recorded a reduction in LDH activity in fish due to impaired function of liver induced by deltamethrin. On contrary, the recorded result disagreed with **Abdel-Daim et al., (2013)** and **Tewari and Gill, (2014)** who reported significant increases in liver enzymes in serum due to deltamethrin toxicity. This variation may be due to different doses of deltamethrin, administration period and susceptibility of tested animals to the toxicant. Referring to serum glucose level, it was observed that administration of Butox® 50% led to significant decrease in serum glucose level that was similar to that recorded by **Kumar et al., (1999)** who noticed reduced glucose level in fish intoxicated with deltamethrin. Occurrence of hypoglycemia may be attributed to the direct effect of deltamethrin on the pancreatic tissue that responsible for insulin hormone synthesis **(Eraslan et al., 2007).** In contrast many authors documented a significant increase in serum glucose level due to deltamethrin toxicity as **Manna et al., (2005)** and **Yavuz et al., (2010)**. Also, Butox® 50 % caused hypercholesterolemia that may be due to the ability of pyrethroids to disrupt lipid metabolism in liver **(Yousef et al., 2003)**. This result was synchronized with that of **Abdel-Daim et al., (2013)**. Also, the data in **Table (3)** showed an increase in serum protein and albumin levels in the current work. This result indicated liver dysfunction as liver seemed unable to metabolize proteins, which were reflected by the pathological changes detected in hepatic tissue. This finding was

supported by **Charlton, (1996)** who mentioned that liver controlled the metabolism of proteins and amino acids and liver diseases disturbed the regulation of protein metabolism, while this result disagreed with results obtained by **El-Sayed et al., (2007)** and **El Zayat et al., (2008)** who reported decreased total protein level due to deltamethrin toxicity. The reduced bilirubin level occurred due to Butox® 50% may be resulted from the rapid renal clearance or excessive execration of bilirubin in bile as a trial from the body to remove its excess level resulted from hemolysis of RBCs or due to defect in the enzyme responsible for its formation "biliverdin reductase" that present in all body tissues especially in reticulo-macrophages of the liver and spleen. The obtained result agreed with **Tewari and Gill, (2014)** who found a statistically significant decrease in activity of total bilirubin of mice exposed orally to deltamethrin at dose of 0.5 mg/kg Bwt for a period of 30 days. On contrary, it disagreed with **Yousef et al., (2006)** and **Abdel-Daim et al., (2013)** who recorded a significant increase in total bilirubin level due to deltamethrin toxicity in rats. The recorded data in **Table (4)** showed reduced urea and creatinine levels in rat's serum as a result of Butox® 50% administration indicating dysfunction of hepatic and renal tissues **(Photo-3)**. Low creatinine level may be emerged from either a defect in creatine synthesis in liver that enter in formation of creatinine or due to high execration rate from affected renal tissue. **Khan et al., (2012)** mentioned that urea is created in liver by deamination of amino acids, so reduced urea level may arise from a defect in synthesis of enzymes responsible for this process which was confirmed in this work by increased level of total protein in serum. Another probable cause of low serum urea may be the increased renal glomerular filtration rate and execration of urea in urine. This result agreed with **Tewari and Gill, (2014)** who noticed a significant reduction in serum urea level in mice intoxicated with deltamethrin. On contrary, it disagreed with **Amin and Hashem, (2012)** and **Yavuz et al., (2012)** who observed a reverse effect where deltamethrin led to significant increase of creatinine and urea levels. The recorded data in **Table (5)** showed a significant reduction in AchE activity in serum due to deltamethrin intoxication that also was recorded by **Vani et al., (2011)**. According to **Ells et al., (1992)** pyrethroids caused multiple nerve impulses that stimulated release of acetylcholine by nerves that might lead to exhaustion of AchE and its subsequent reduction in serum. Testosterone hormone did not change significantly due to Butox® 50% intoxication **(Tabl-5)** however the

histological examination of testes showed pathological changes **(Photo, 4)**. This finding disagreed with that of **Oda and El-Maddawy, (2012)** and **Issam, et al. (2012)** who noticed reduction in testosterone level. **Achook 0.15%:** The tabulated results in **Table (3)** revealed that Achook 0.15% caused some changes in liver related biochemical parameters including liver enzymes, glucose, albumen and bilirubin. Liver enzymes showed increase in ALP and LDH serum activities while ALT and AST showed reduced activities. The reduced serum activities of AST and ALT may be indicators for the liver disorder which was noticed also in microscopic examination of liver **(Photo-2).** This result agreed with the result of **Gupta et al., (2001)** while it differed from results of **Park et al., (2014)**. The increased levels of ALP, which was also reported by **Kupradinun et al., (2010)**, and LDH indicated liver dysfunction or defect in other organ like heart. Also, the increased level of LDH was noticed by **Ashafa et al., (2012).** It was found that the serum glucose level was reduced significantly than the control group. The hypoglycemic effect of *A. indica* in normal and experimentally induced diabetic animals was studied by **Osadebe et al., (2004), Akhtar, et al., (2011)** and **Shailey and Basir, (2012)**. **Jelodar et al., (2005)** suggested that such glucose lowering properties may return to the stimulatory effect of the extract on pancreas to release insulin. They also supposed that the extract may has the ability to regenerate the β-cells. In contrast to that result, **Radwan et al., (2001)** and **Park et al., (2014)** reported significant increase in serum glucose level of rats exposed to azadirachtin while **Gupta et al., (2001)** found no significant change in blood glucose level of rats exposed to semi-chronic intoxication with petroleum ether extract of neem. The increased albumin level in serum of Achook intoxicated rats may be related to the pathological changes found in their kidneys **(Photo-5)**. The increased serum albumin also noticed by **Radwan et al., (2001)**. Also, Achook insecticide led to reduction of bilirubin level in serum which may be related also to defected kidneys or affected liver that disagreed with **Abdel Megeed et al., (2001)** and **Ashafa et al., (2012)** who reported increased level of bilirubin when they treated rats with azadirachtin. The recorded data in **Table (5)** showed an increase in serum AChE activity compared to control group that was compatible with the result obtained by **Tahir et al., (1992)** who noticed stimulation of AChE of blister beetle due to azadirachtin application at concentration of 0.05% and 0.025%. Also, **Mordue et al., (2010)** suggested that azadirachtin has the ability to occupy various sites at the surface of AChE

molecule rather than binding with the active site. Also, it was mentioned that small volumes of azadirachtin had no considerable inhibitory activity but in larger amounts it inhibited the enzyme by blocking the access of substrate to it **(Sami et al., 2016)**. On the other hand, **Gupta et al., (2001)** and **Parveen et al., (2004)** noticed reduction of AChE activity due to application of neem extracts that may be also related to the suggestion of **Sami et al., (2016)** that azadirachtin affect the level of mRNA of AChE genes which lower the expression of AChE protein. The antifertility effect of neem extracts was reported as it has spermicidal activity (**Kaushic and Upadhyay, 1995)**. Also, several studies reported the reduction in testosterone hormone as **Raji et al. (2003)** who used neem stem bark ethanol extract intraperitoneally and recorded a dose dependent decrease in the level of testosterone and **Shaikh et al., (2009)** who noticed lower levels of all male reproductive hormones including FSH, LH and testosterone. The obtained result in the present work was compatible with previously mentioned studies as Achook 0.15% insecticide led to significant reduction in testosterone hormone **(Tab-5).** This reduction was explained due to the insecticide effect on pituitary gonadotropins through its alteration of AChE. Finally, it is worth mentioning that Achook insecticide caused the highest increase in AChE and the lowest level of testosterone hormone when compared with the control group and other insecticide groups.

Conclusion

From the obtained results of the current study, it was concluded that intoxication of experimental albino rats with Butox® 50% insecticides caused significant increase in rat's body weight, while Achook insecticide did not change the weight comparing to control group. Liver related biochemical blood parameters were extremely altered due to rat's intoxication with Butox® 50%, while Achook insecticide also caused alteration in liver enzymes and its related serum chemicals with the exception of serum cholesterol and total protein levels. Administration of Butox® 50% led to the most severe alterations of serum parameters related to renal function, while Achook insecticide did not cause any changes in renal function tests when compared with control group. Unlike Butox® 50%, Achook insecticide did not produce any significant alterations in brain or testes histology. Based on the obtained results in the current study, further studies should be carried out to detect the toxic effects of Achook insecticide on the other body organs and their functions.

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