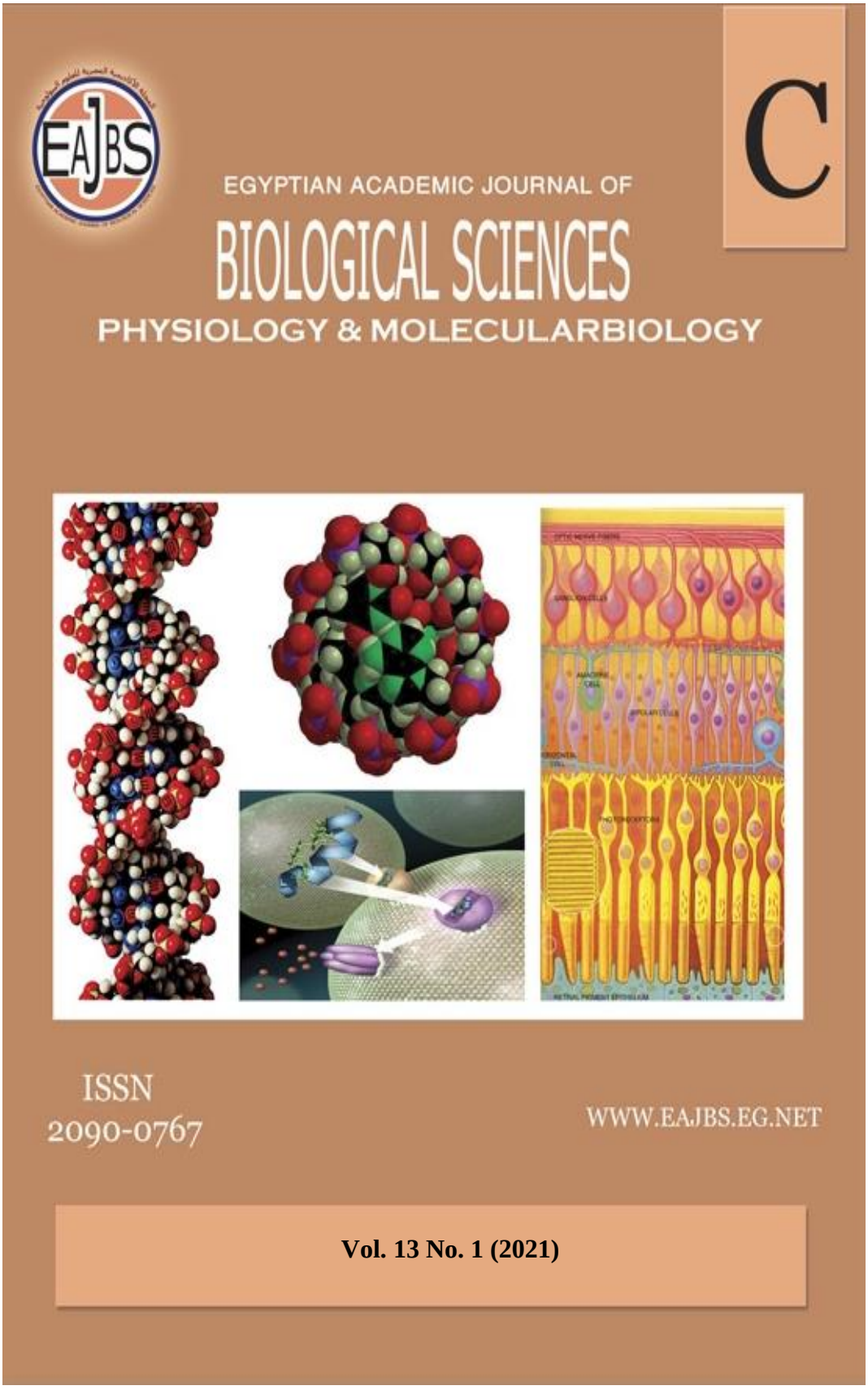

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Sustainable Hypoglycemic Action of Neem Oil on Male Rabbits and Its Impact on Some Glucose Regulating Hormones

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ABSTRACT

Neem tree (*Azadirachta indica*) is one of the crucial medical plants found in tropical and sub-tropical regions around the world especially in dry forests in India, Pakistan, Sri Lanka and Burma. The present study deals with the assessment of the sustainable hypoglycemic action of *Azadirachta indica* in rabbits after using two sub-lethal doses of neem oil extract, low dose (1/10 LD_{50}) and high dose (1/4 LD_{50}). Blood glucose level, insulin, cortisol, adrenaline and noradrenaline were measured through the treatment period for 60 days followed by 30 days withdrawal period. The results showed that neem oil has an obvious hypoglycemic effect and has a noticeable rule in raising the serum levels of the investigated hormones. Nonetheless, the observed changes were still potent during the withdrawal period. In conclusion, it can be deduced that the high dose of neem oil is more efficient than the low dose of *Azadirachta indica* which may serve as an important alternative source in the remedy of *diabetes mellitus* involved in dropping increased blood glucose during diabetes, after taking all precautionary measures to avoid the toxicity of the extract.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder which affects over 170 million people worldwide and, possibly, over 365 million in the year 2030 (Wild *et al.,* 2004 and Saeedi *et al*., 2019). Almost half of all deaths attributable to high blood glucose occur before the age of 70 years (Sarwar *et al.,* 2010). According to WHO, will be the 7th leading cause of death in 2030 (Bourne *et al.*, 2013). Type 2 DM is rapidly evolving as one of the greatest global health challenges of the 21st century (Ginsberg *et al., 2000*).

Insulin is considered the main hormone in the regulation of blood sugar; it is secreted from β cells in the islets of Langerhans in the pancreas (USRDS annual data report, 2014). However, there are others hormones related to the blood glucose level in addition to insulin such as cortisol. Cortisol is a steroid hormone that is produced by the adrenal glands (which are located on the top of the kidneys) and it is regulated by the hypothalamus.

Also, cortisol is known as an anti-inflammatory agent and can suppress the immune system in times of stress thus the HPA axis will release more or less cortisol in the body to maintain homeostasis which is controlled by glucocorticoids receptors (GRs) (Caballero *et al.,* 2005).

Other hormones of great importance affect glucose level, adrenaline and noradrenalin, they are called the emergency hormones in response to negative or positive stress and their work is opposite to the action of insulin, where they convert glycogen to glucose, so that, their concentration in the blood increase in the emergency situation and also increase the heart rate above the normal rate, expand the arterioles in the skeletal muscles, expand the respiratory passages, reduce the stress of these muscles, and increase the respiratory rate above the normal rate. All this happens to meet the emergency situation that the organism may be exposed to while exercising its daily vital activity (Kannan *et al.,* 2006). This serious metabolic disorder directly affects the metabolism of carbohydrates, proteins, fats, water and electrolytes (Saxena *et al.,* 2004).

The traditional alternative and sustainable medicinal systems that use herbs and medicinal plants on a large scale appeared in India called (Ayurveda and Unani), used as a treatment for illnesses and for health maintenance in general. More than 800 plants are used as traditional treatments in one way or another for the treatment of diabetes and have been scientifically evaluated, of which 10 plants are mentioned in Ayurveda for the treatment of diabetes. From this standpoint, research has tended towards the necessity to discover safe and sustainable medicines from natural plant sources such as *Acorus calamus* AC. It has been used in the Indian and Chinese medicinal system for hundreds of years for its useful role in treating several types of diseases, especially nervous system disorder, and its roots have been widely used as traditional medicine in the treatment of diabetes among Americans and has spread in Indonesia (Hao *et al.,* 2009).

Numerous studies have proven that nature is the source of treatment or therapeutic drugs for thousands of years, as most medicines are the result of distillation, amalgamation, or reproduction of various materials found in nature. Certain types of plants are widely used in cooking and have been proven to have anti-diabetic properties such as *Murraya konenigii* and *Mentha piperite*. These plants grow wild in India and are widely cultivated in home gardens and temples and have a long history in medicine except for their use in cooking and religious purposes (Sharma *et al.,* 2009).

Azadirachta indica is known as neem in many countries of the world. It is a large evergreen tree that belongs to the family Meliaceae. It is believed to have originated from Burma in south Asia (National Research Council (1992)) and grow well in tropical and sub-tropical regions around the world with the ability to resist many adverse environmental conditions such as drought, infertile soil, stony, shallow, or acidic soil (Jacobson. 1990). However, the chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, lavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. The biologically most active compound is azadirachtin, it is actually a mixture of seven isomeric compounds labelled as azadirachtin A-G and azadirachtin E is more effective (Verkerk *et al.,* 1993).

Through the study of some of what was researched about this tree and its medicinal benefits, that made the interests of scientists and specialists focused on it in order to decipher its therapeutic uses for many diseases and its medical effects, especially its hypoglycemic effect, the

direction of our research was focused on the extent of the effect of the neem oil extracted From the neem tree, on some hormones related to sugar metabolism in order to interpret the secret of its hypoglycemic effect.

MATERIALS AND METHODS

Animals:

Ninety male rabbits weighted 900–1100 g were used in these experiments. Rabbits were kept at constant environmental conditions throughout the experimental period, and nutritional conditions were 16.5% fibers, 14.5% proteins, 3.5 % fats, Vit. A 10000 IU, Vit. D 950 IU and Vit. E 25 mg, the total calories were 2400 kcal/kg.

Animal Grouping:

Animals were divided randomly into 3 groups in separate cages (30 rabbits in each group):

Group I: Normal Control that is symbolized by the symbol C

Group II: The low dose group that is symbolized by the symbol L.

Group III: The high dose group is symbolized by the symbol H.

Neem Oil:

It was prepared with two doses, high dose (H) 1/4 LD50 and low dose (L) 1/10 LD50, so the value of the low dose (L) 1/10 LD50 = 450 kg/mg and the high dose (H) 1/4 LD50 = 1125kg/mg. These doses were calculated at this value in this paper according to the LD50 value that was previously estimated for this oil and on the same experimental animal (Ghariani, 2009).

Experimental Protocol:

The rabbits were given the required dose orally (the dose is calculated based on the weight of the animal immediately before administration) every 5 days for 60 days, i.e., at a rate of 12 times for each group (H - L) each according to its prescribed dose and then the neem was stopped. Then the recovery period for 30 days, 5 rabbits were slaughtered from each group for each trial period according to the following 15, 30, 45, 60 days (treatment period) and 15, 30 days (withdrawal period).

Sampling and Biochemical Parameters:

Blood samples were collected from all animals after the slaughter process, blood was collected from each animal directly. Blood samples were centrifuged at 4000 r.p.m for serum separation which was collected in clean tubes and directed for estimation. The biochemical parameters that were measured in this study included blood glucose level, insulin and cortisol for all groups during the treatment period for groups C, L and H. Also, the level of the same previous hormones and the blood glucose level after stopping the administration of the neem oil (withdrawal period) for the three groups.

Blood glucose level was measured by the colorimetric method. Insulin concentrations were measured in serum by the Enzyme-Linked Fluorescent Assay (ELFA) principles according to kit instructions, cortisol was measured by the electrochemiluminescence immunoassay (ECLIA) is intended for use on immunoassay analyzers. HPLC was used to determine the level of Adrenaline and Noradrenaline hormones.

Statistical Analysis:

Statistics were performed using the statistical graph pad prism 5. One way analysis of variables (ANOVA) was used posted by Newman-keuls test. All results are expressed as mean ± SE and the level of significance between groups were *$p<0.05$, ** $p<0.01$, *** $p<0.0001$.

RESULTS

The study was conducted on 90 rabbits which classified into three groups (control group, group for low dose and the third group for high dose), to study the effect of the neem oil on the blood sugar level of male rabbits (the experimental animal) and to study the extent of the changes and effects that oil may have on the level of hormones related to sugar metabolism insulin hormone and Cortisol, so the results will be in two stages for each

hormone and two stages for the level of blood glucose level, the first dose is low (L) and the second high dose (H).

I-Low Dose Effect (1/10 LD50):

The first dose is low (L) with a value of (450 mg / kg body weight), which is equivalent to 1/10 LD50. After the experimental animal received this dose, then followed by two withdrawal periods (15 days, 30 days) of this dose according to the established plan, the results were recorded at each period of slaughtering the experimental animal to take a blood sample.

1- Low Dose Effect (1/10 LD50) on Blood Glucose Level:

As in Figure 1, The results of analyzing blood glucose levels to find out the effect of the low dose (L) 1/10 LD50 of neem oil showed that there was a decrease in the level of blood glucose. Statistically, the results were highly significant (P <0.01). As for the withdrawal phase from this dose, with periods, the percentage of change decreased from 12.4% after 15 days to 8% after 30 days of withdrawal compared to the control group for the same period under the same conditions and statistically, the results were Significant (P <0.01).

Table 1: Level of glucose (mg/dL) in Blood of male rabbits after treatment with (1/10 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	103±0.81	90±2.71 **	-12.6
	30	97±2.16	85±2.16 **	-12.4
	45	96±5.35	82±4.08 **	-14.6
	60	95±1.41	74±4.32 ***	-22.1
Withdrawal Period	75	97±1.41	85±2.58 **	-12.4
	90	100±1.41	92±1.63 **	-8

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at p<0.05, 0.01, 0.0001.

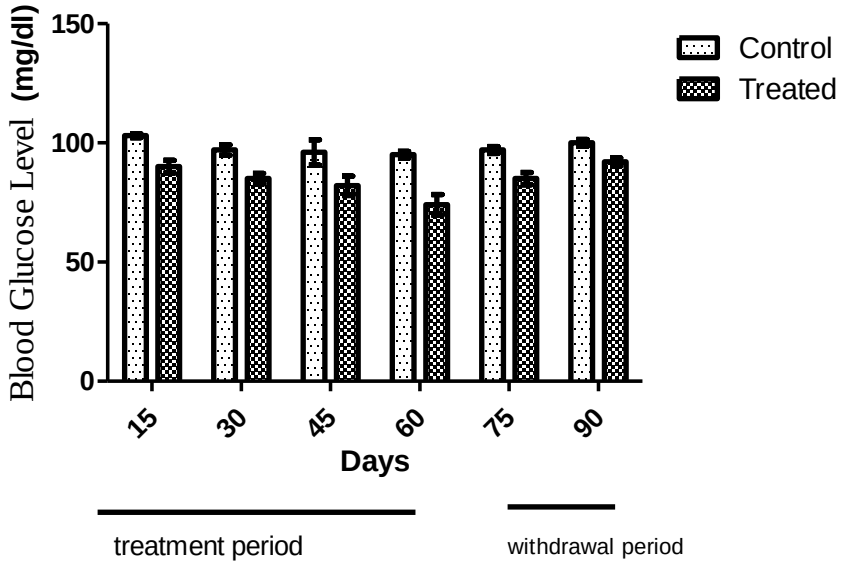

Fig 1: Blood glucose level (mg/dl) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

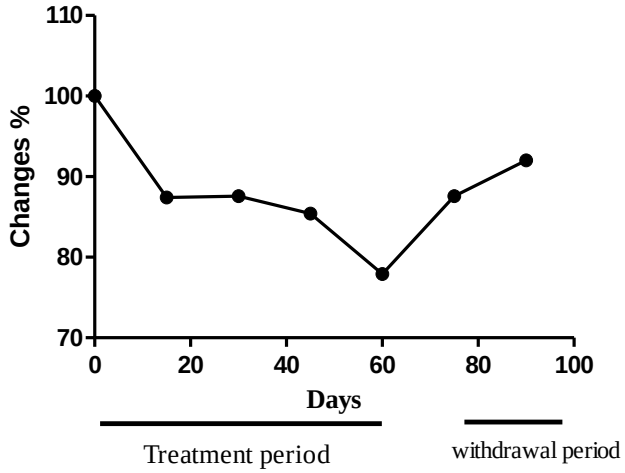

Fig 2: Percentage change of blood glucose level of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

2- Low Dose Effect (1/10 LD50) on Insulin:

The results showed that the effect of the low dose (L) 1/10 LD50 on this hormone during the treatment period showed a significant increase in the level of the insulin hormone (Fig 3), where the percentage of this increase ranged between (18.1%) after 15 days and (56.5%) after 60 days of treatment with this oil (Fig. 4) with significant values. As for the withdrawal period, the increased hormone level during the treatment period began to gradually decline, reaching 57 % and 48.7% after 15 and 30 days of withdrawal, respectively. The results were statistically significant ($P < 0.05$, $P < 0.01$), as shown in (Table 2).

Table 2: Level of insulin (IU/ml) in Blood of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean ± SD	% Change
Treatment Period	15	8.3±1.41	9.8±1.98 *	+18.1
	30	8.2±2.18	11.3±1.47 *	+37.8
	45	7.8±1.44	11.6±1.44 *	+48.7
	60	8.5±1.41	13.3±1.64 **	+56.5
Withdrawal Period	75	7.9±1.36	12.4±1.31 **	+57
	90	7.8±1.33	11.6±1.75 *	+48.7

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001

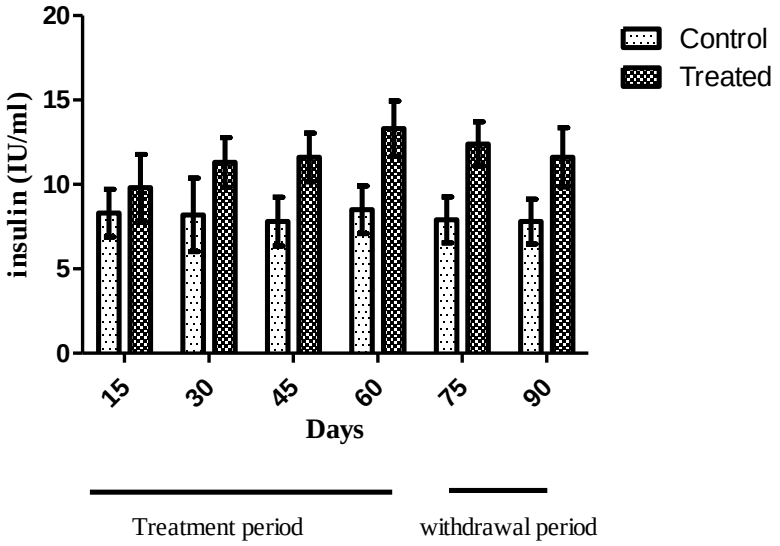

Fig. 3: Level of insulin (IU/ml) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

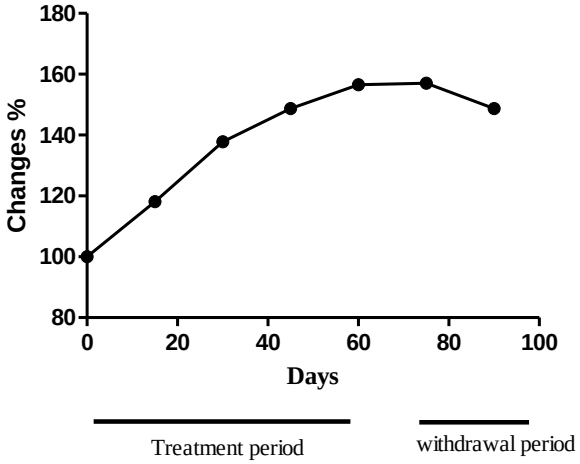

Fig. 4: Percentage change in level of insulin (IU/ml) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

3- Low Dose Effect (1/10 LD50) On Cortisol:

The effect of the low dose (L) 1/10 LD50 of neem oil on cortisol was the emergence of an increase in the level of this hormone in the blood serum of the experimental animal during the specified treatment period. The percentage of increase in the level of this hormone was gradual from 65.5% to 193.8% and statistically, the results were Significant. As for the withdrawal, which is after 15 days and 30 days, a significant decrease in the hormone level was observed, with a decrease of 147% after 15 days and to 38%. After 30 days. (Table 3, Fig. 5 and Fig. 6).

Table 3: Level of Cortisol (n mol/L) in Blood of male rabbits after treatment with (1/10 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	5.8±2.12	9.6±2.17 *	+65.5
	30	7±2.60	13.3±0.79 **	+90
	45	6.7±1.62	19.8±1.43 **	+195.5
	60	8±2.16	23.5±1.78 ***	+193.8
Withdrawal Period	75	6.6±1.01	16.3±0.93 **	+147
	90	6.8±1.47	9.4±1.16 *	+38

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001.

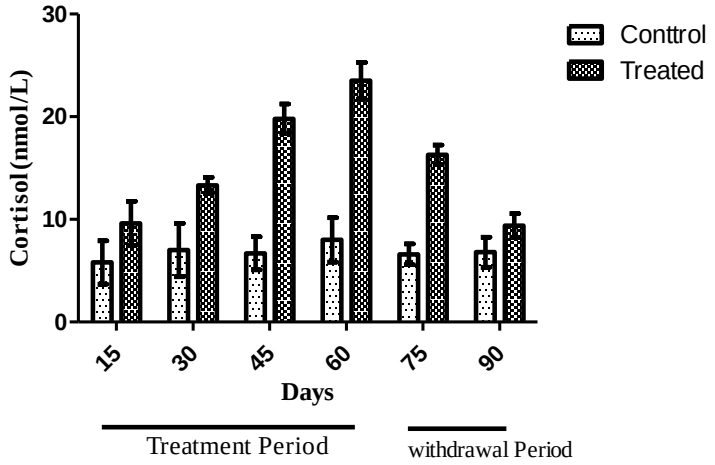

Fig. 5: level of cortisol (n mol/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

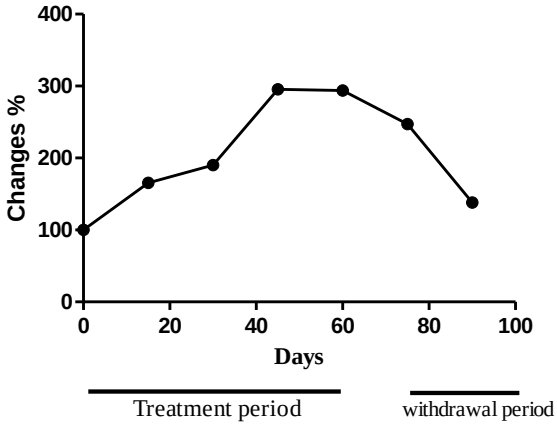

Fig. 6: Percentage change in level of cortisol hormone (n mol/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

4- Low Dose Effect (1/10 LD50) on Adrenaline:

The results of this research showed that the low dose of neem oil caused an increase in the level of adrenaline compared to the control group (C) after giving it to the experimental animal for 60 days as shown in Table (4) and Figure (7). This increased from 13.8% after 15 days of treatment with this dose to 58% after 60 days of treatment (Fig. 8), and statistically, the increase was from Significant ($P<0.05$ to $P<0.01$). After that, during the withdrawal period from this dose (L), the percentage of change of the hormone decreased to 21.5% after 30 days after withdrawing the treatment dose with statistically significant ($P<0.01$).

Table 4: Level of Adrenaline (ng/L) in blood of male rabbits after treatment with (1/10 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	56.7±6.77	64.5±7.08 *	+13.8
	30	58.1±5.46	75.6±3.24 **	+30.1
	45	39.9±4.75	54.6±6.40 **	+36.8
	60	43.7±4.20	69.1±7.67 **	+58.1
Withdrawal Period	75	37.7±4.66	55.1±7.48 **	+46.2
	90	42.3±5.35	51.4±1.12 **	+21.5

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001.

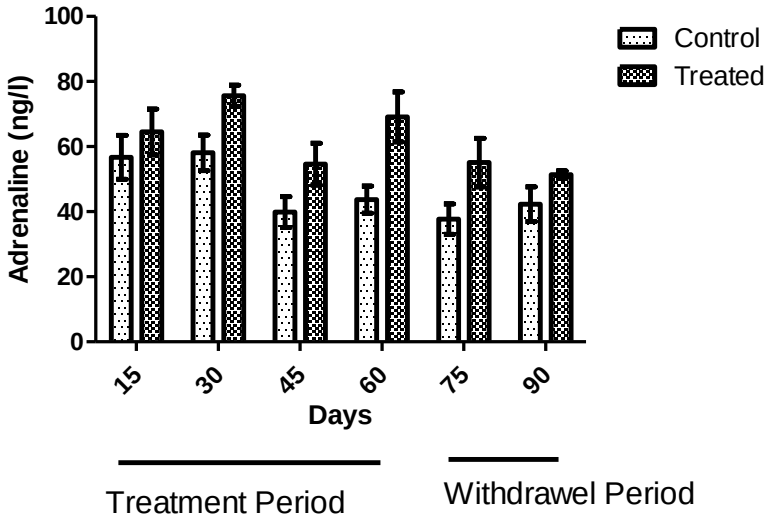

Fig. 7: Level of adrenaline hormone (ng/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

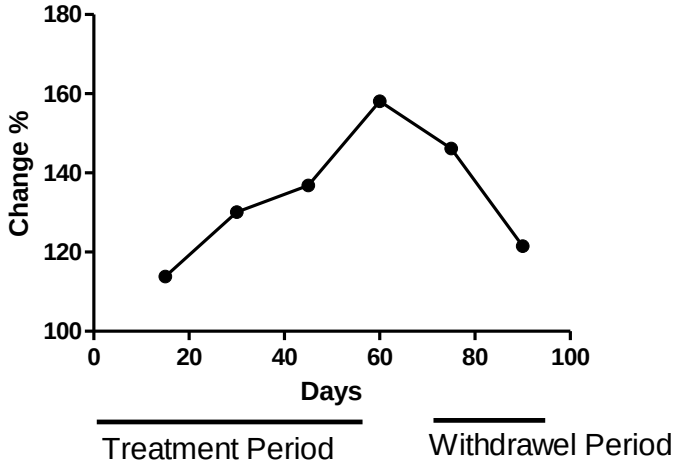

Fig. 8: Percentage change in level of adrenaline hormone (ng/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

5- Low Dose Effect (1/10 LD50) on Noradrenaline:

The results shown in (Table 5 and Fig. 9) show that the level of the hormone after giving low doses (L) 1/10 LD50 has been exposed to a very slight increase after 15, 30 days from the beginning of the experiment, and this increase was recorded by 3.6% and 0.5% on Respectively (Fig. 10), these changes were non-significant from the statistical point of view. The last two periods of treatment with the dose, which are at 45 and 60 days of treatment with this dose, the change in the level of the hormone was clear with an increase compared to the control group under the same conditions and in the same period, where the percentage increase in its level from 54.7% to 100%, respectively, and statistically it was from Significant ($P<0.05$ to $P<0.01$). During the withdrawal period, the percentage of change in the level of the hormone at 15 days was 4.2% and at 30 days 3.9% and statistically, it was recorded that there was no significant change.

Table 5: Level of Noradrenaline (ng/L) in Blood of male rabbits after treatment with (1/10 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	35.9±4.19	37.2±4.22	+3.6
	30	38.9±2.15	39.1±5.36	+0.5
	45	31.8±3.40	49.2±4 *	+54.7
	60	29.8±2.37	59.6±5.92 **	+100
Withdrawal Period	75	37.8±3.72	39.4±4.11	+4.2
	90	36.2±3.70	37.6±4.80	+3.9

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001

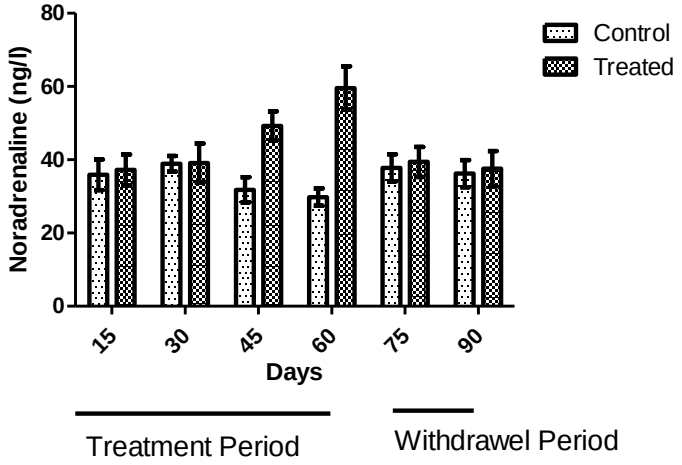

Fig. 9: level of adrenaline hormone (ng/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

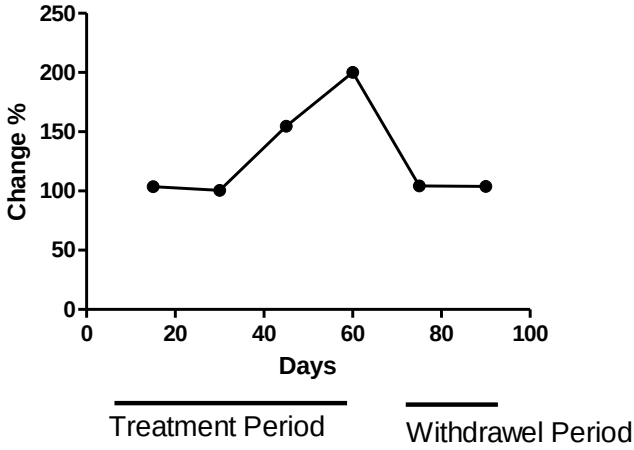

Fig. 10: Percentage change in level of noradrenaline hormone (ng/l) in serum of male rabbits after treatment with (1/10 LD50) of neem oil for 60 days followed by 30 days withdrawal.

I-High Dose Effect (1/4 LD50):

The high dose group of the experimental animal received this prescribed dose, which was (1125 mg/kg body weight) for a period of 60 days at specific time periods in the research plan, where samples were taken from the blood of this group after each scheduled period of 15, 30, 45 and 60 days from The start of administering the neem oil according to the schedule prepared in the experiment plan, as well as tracking the stages of withdrawal after stopping the administration of the neem oil at 60 days for another 30 days.

1- High Dose Effect (1/4 LD50) on Blood Glucose Level:

The effect of a high dose of neem oil on the blood sugar level of the experimental animal (H) 1/4 LD50 during the prescribed treatment periods is more clear than the effect of the low dose compared with the control group (Fig. 11), where the percentage of change was recorded as a significant decrease in the blood glucose level of 19.4 % To 32.6% during those treatment periods from the start of the first 15 days after treatment to the end of the last after 60 days of treatment with significant values ($P < 0.01$) (Fig. 12), as for the withdrawal phase From this dose during its 15-day and 30-

day recovery periods from treatment with this dose, a decrease in the blood glucose level of the experimental animal was observed to 23.7% after 15 days and to 6% after 30 days compared to the percentage of the last treatment dose and statistically it was of Significant (P <0.001, P <0.01) (Table 6).

Table 6: Level of sugar (mg/dL) in Blood of male rabbits after treatment with (1/4 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	103±0.81	83±1. 63 ***	-19.4
	30	97±2.16	61±4.89 ***	-37.1
	45	96±5.35	65±5.09 **	-32.3
	60	95±1.41	64±2.71 **	-32.6
Withdrawal Period	75	97±1.41	74±1.41 ***	-23.7
	90	100±1.41	94±1. 63 **	-6

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at p<0.05, 0.01, 0.0001.

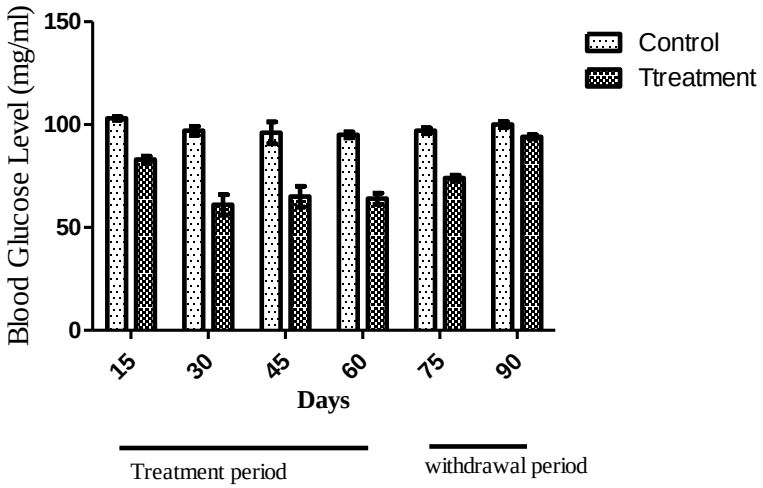

Fig. 11: Blood glucose level of (mg/ml) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

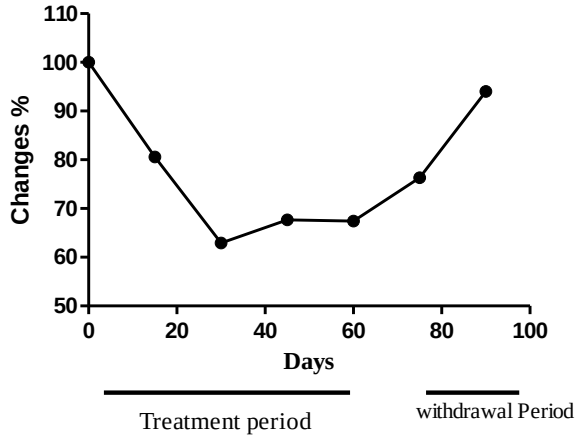

Fig. 12: Percentage of change in blood glucose level of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

2- High Dose Effect (1/4 LD50) on Insulin:

As for the results of the high dose of neem oil 1/4 LD50 on insulin, it was a gradual increase in its level in the blood serum of the experimental animal from the results of 15 days to the results of the last after 60 days from 33.7% to 88% expert significant increase. The effect of the withdrawal period from the high therapeutic dose (H)1/4 LD50 recorded a decrease in the percentage of the insulin level from its mean during the treatment period to 81% after 15 days of withdrawal and to 43.6% after 30 days of withdrawal from this dose. This indicates that the hormone level began to return to a level close to the normal level before the dose treatment and in both cases compared to the control group not exposed to the neem oil treatment (Table 7, Fig. 13 and Fig. 14).

Table 7: Level of insulin (IU/ml) in Blood of male rabbits after treatment with (1/4 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	8.3±1.41	11.1±1.68 *	+33.7
	30	8.2±2.18	12.3±1.58 *	+50
	45	7.8±1.44	13.5±1.19 **	+73.1
	60	8.5±1.41	16±2.16 **	+88
Withdrawal Period	75	7.9±1.36	14.3±1.64 **	+81
	90	7.8±1.33	11.2±1.67 *	+43.6

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001.

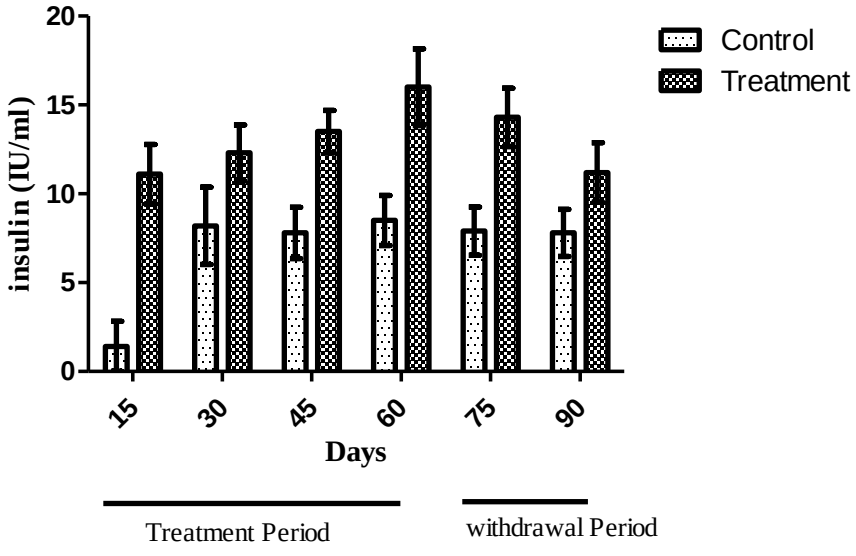

Fig. 13: Level of insulin (IU/ml) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

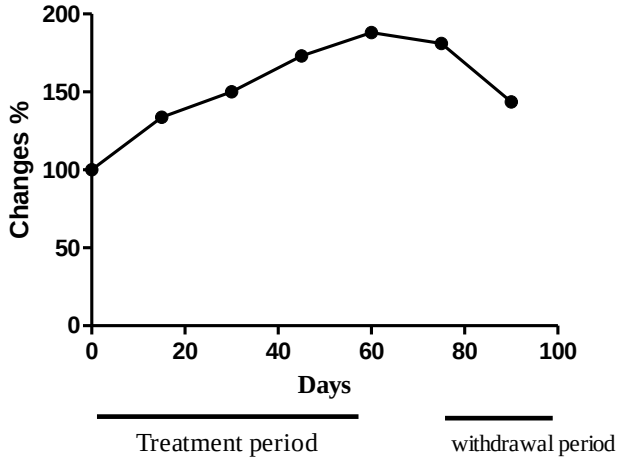

Fig. 14: Percentage of change in level of insulin (IU/ml) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

3- High Dose Effect (1/4 LD50) on Cortisol:

The high dose of neem oil 1/4 LD50 had an effect on the level of cortisol for the group of experimental animals that received this dose and there was a clear increase in its level in the blood serum of the experimental animal compared to its level in the same period and under the same conditions for the control group (C), where the percentage of The increase in cortisol level from the results of first angina to any of the results of last angina ranged from 179% to 400% and statistically, the increasing levels were statistically significant ($P < 0.05$ & $P < 0.001$) (Table 8, Fig. 15).

The effect of the two periods of withdrawal from this dose, which is 15 days and 30 days after 60 days of treatment with the neem oil, was that the level of the hormone in the blood serum of the experimental animal began to decrease from its level that was recorded after 60 days of treatment with the neem oil at this dose, where the percentage of decrease reached 256.1% After 15 days of withdrawal and to 85.3% after 30 days (Fig. 16).

Table 8: Level of Cortisol (nmol/L) in Blood of male rabbits after treatment with (1/4 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	5.8±2.12	16.2±1.64 **	+179
	30	7±2.60	27±1.33 ***	+285
	45	6.7±1.62	33.4±2.10 ***	+398.5
	60	8±2.16	40±2.16 ***	+400
Withdrawal Period	75	6.6±1.01	23.5±3.26 **	+256.1
	90	6.8±1.47	12.6±1.87 **	+85.3

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05, 0.01, 0.0001$.

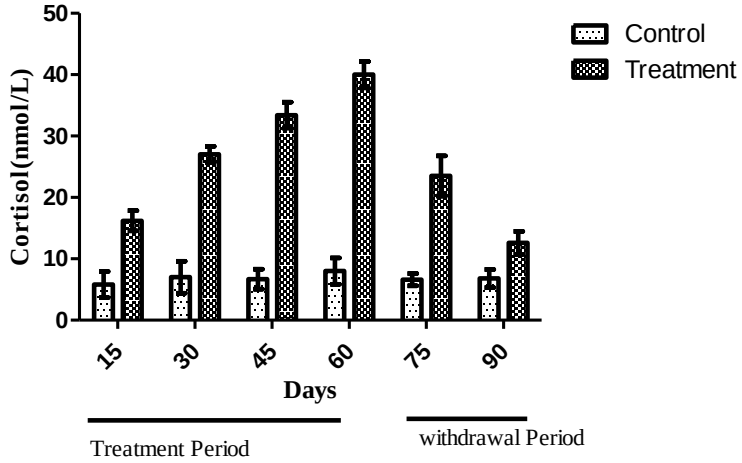

Fig. 15: Level of cortisol (nmol/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

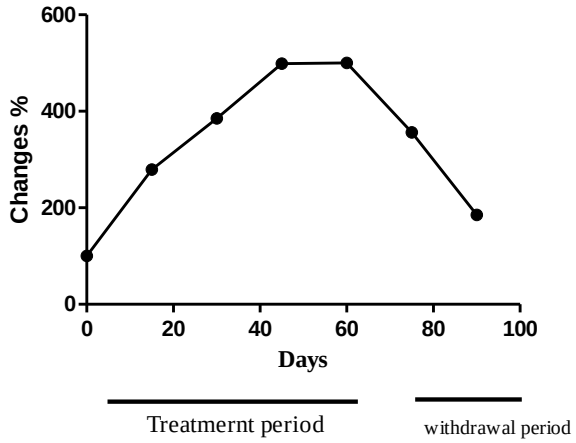

Fig. 16: Percentage of change in level of cortisol (nmol/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

4- High Dose Effect (1/4 LD50) on Adrenaline:

As for the effect of the high dose of neem oil (H) 1/4 LD50 at the same period and under the same conditions, the results showed that there was an increase in the level of the adrenaline hormone in the blood serum of the experimental animal more clear than the effect of the low dose, where the percentage increase from 20.6% to 60.6% from the first 15 days to the 60 days of treatment with this dose and statistically it was from Significant ($P<0.05$) to Significant ($P<0.01$) (Table 9, Fig. 17 and Fig. 18). As for the two recovery periods from this dose, its effect was clear on the level of this hormone at 15 days of treatment, which increased to 108% from the rate of the last treatment dose at 60 days, and then decreased significantly after 30 days of treatment to 64.8%, and this explains that the effect of the withdrawal period began to appears in the second period after 30 days of stopping this dose of the neem oil and statistically, it was Significant ($P<0.01$).

Table 9: Level of Adrenaline (ng/L) in Blood of male rabbits after treatment with (1/4 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	56.7±6.77	68.4±8.10 *	+20.6
	30	58.1±5.46	85.6±9.36 **	+47.3
	45	39.9±4.75	59.9±6.40 **	+50.1
	60	43.7±4.20	70.2±7.91 **	+60.6
Withdrawal Period	75	37.7±4.66	78.4±7.60 **	+108
	90	42.3±5.35	69.7±6.64 **	+64.8

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at $p<0.05$, 0.01, 0.0001

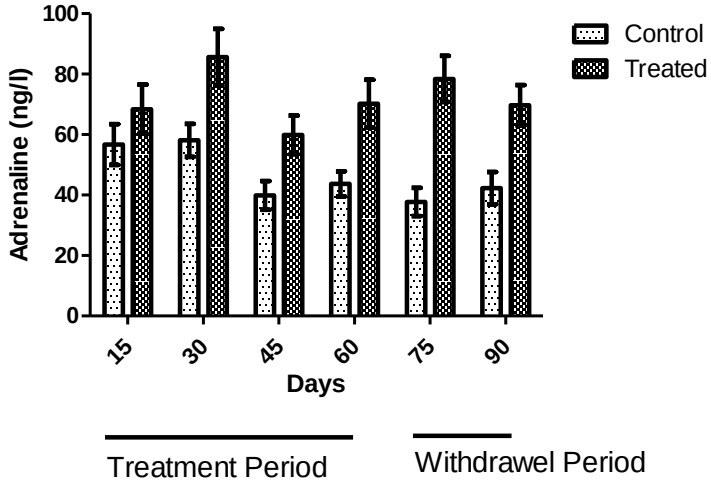

Fig. 17: Level of adrenaline (ng/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

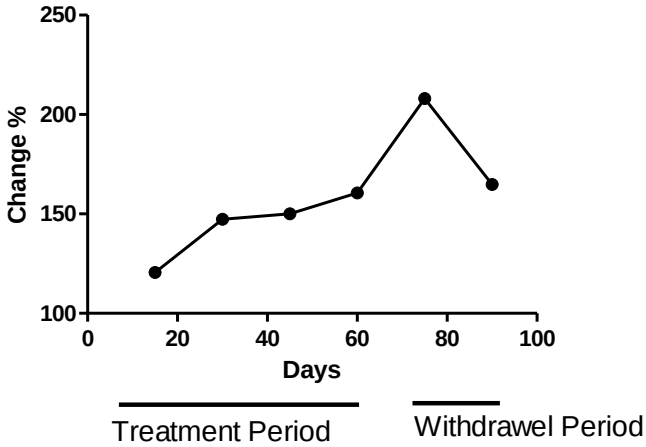

Fig. 18: Percentage of change in level of adrenaline (ng/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

5- High Dose Effect (1/4 LD50) on Noradrenaline:

According to the observed results of the effect of the high dose (H) 1/4 LD50 of neem oil on this hormone under the same conditions and the same time periods prescribed for this group receiving this dose, the results showed a significant increase in the level of this hormone in the serum of the experimental animal compared with the results of group (C). The control group, where the percentage increase was gradual from 17.8% to 200% after 15 days and after 60 days of treatment (Table 10, Fig. 19 and Fig. 20), and statistically it ranged between the Significant (P<0.001to P<0.05). As for the two withdrawal periods from this high dose, the results showed a significant decrease in the percentage of the hormone in the blood of the experimental animal to 23.3% after 15 days and to 12.4% after 30 days of treatment compared to the last percentage recorded after 60 days of treatment during the treatment period, it was statistically significant (P<0.05) to non-significant.

Table 10: Level of Noradrenaline (ng/L) in Blood of male rabbits after treatment with (1/4 LD 50) of neem oil for 60 days followed by 30 days withdrawal.

Period	Days	Control Mean±SD	Treated Mean±SD	% Change
Treatment Period	15	35.9±4.19	42.3±3.95 *	+17.8
	30	38.9±2.15	47.4±4.88 **	+21.9
	45	31.8±3.40	67.4±5.41 ***	+111.9
	60	29.8±2.37	89.4±4.31 ***	+200
Withdrawal Period	75	37.8±3.72	46.6±4.33 *	+23.3
	90	36.2±3.70	40.7±3.17	+12.4

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significance at p<0.05, 0.01, 0.0001

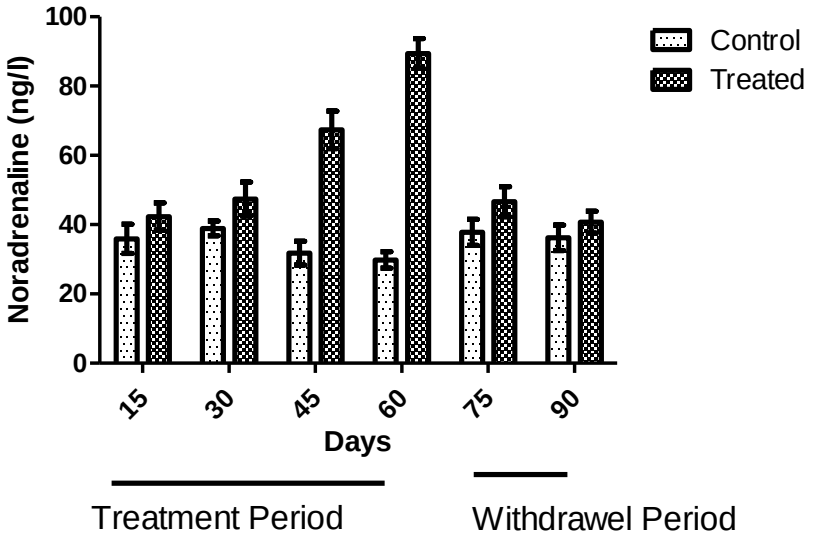

Fig. 19: Level of noradrenaline (ng/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

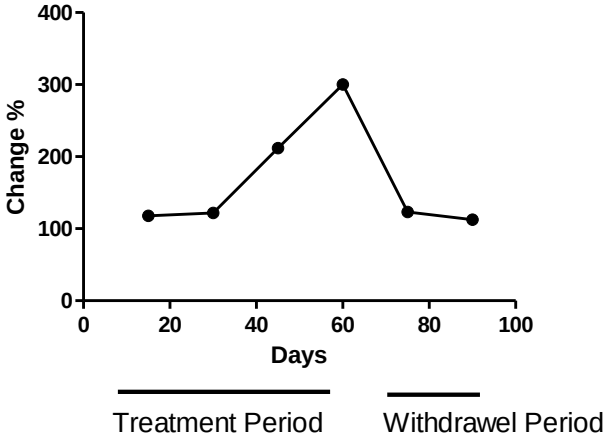

Fig. 20: Percentage of change in level of noradrenaline (ng/l) in serum of male rabbits after treatment with (1/4 LD50) of neem oil for 60 days followed by 30 days withdrawal.

DISCUSSION

The study aimed to record the hypoglycemic effect of neem oil on rabbits by measuring blood glucose levels and two metabolic hormones, insulin and cortisol. 90 rabbits were used and divided into three groups each of 30 rabbits. The applied doses were a low dose (450mg/kg body weight) which was equivalent to 1/10 LD50 and a high dose equivalent to 1/4 LD50 (1125 mg / kg body weight). The doses were induced every 5 days through 60 days, then followed by withdrawal period for 30 days, 5 rabbits were slaughtered from each group for each trial period according to the following times 15, 30, 45, 60 days (treatment period) and 15, 30 days (withdrawal period) after that.

When we induced the low dose of neem oil to the animals, a decreased blood glucose level was observed in the treated group compared to the control group and the highest decrease was observed after 60 days of treatment, also the percentage of change of blood glucose level decreased significantly by longer treatment period, but during withdrawal period it was observed that blood glucose level started to increase again compared with control group, these results indicated that, neem oil has a rule in decreasing blood glucose level and have a hypoglycemic effect. Our results are in agreement with previous studies on the neem extract which showed that neem extract contains bioactive compounds like azadirachtin, meliacin, nimbidin and other glycoside constituents which may be responsible for its hypoglycaemic activity (Chattopadhyay, 1999, Iman *et al.,* 2012 and Khosla, 2000). Several studies on neem have shown its hypoglycemic effect (Hamdy *et al.*, 2008a), however, until now the available information is still scarce regarding its mechanism.

In accordance with the present study, it has been found that by measuring insulin hormone as in the decided protocol, it was found that insulin hormone level raised after treatment with neem oil compared to control and the percentage of change in the level of insulin hormone showed a direct proportional with the time of treatment, this result explains the hypoglycemic effect of neem oil, it may be concluded that neem oil can enhance the release of insulin from β cells and increase the absorption of glucose as an action of sulphonyle urea (Bajaj and Srinivas, 1999). These results are concurrent with previous studies which showed that neem extract may play a significant role in the management of type-2 *diabetes mellitus* by improving the expression of insulin signaling molecules and GLUT4 protein to enhance and

oxidation in the skeletal muscle (Satyanarayana *et al.,* 2015). Previous study recorded a significant increase of serum insulin in diabetic rats, they concluded that pancreas still able to produce insulin hormone even with type 2 diabetes if its cells are stimulated (Ian *et al.*, 2008).

Glucocorticoids are well known to play an important role in the regulation of most essential physiological processes (Atanasov and Odermatt, 2007). High levels of cortisol are associated with dysregulation of the hypothalamic-pituitary-adrenal axis and increased volume of the adrenal glands (Pasquali *et al.,* 2006 and Godoy *et al.,* 2006). It is known that cortisol further increases blood glucose levels by decreasing the rate of glucose uptake by most cells, except the brain (Larry *et al.*, 2010). according to this study, it was found that a low dose of treatment with neem oil has resulted in a significant rise in the cortisol level compared to the control group, also treated with a high dose of neem oil resulted in a significant increase in cortisol level, other study introduced that serum cortisol level showed a non-significant change in diabetic rat and treated group, exhibit hypoglycemic activity without altering the serum cortisol concentration (Gholap and Kar 2004). In our vision, we can interpret the increase in cortisol level resulted from neem oil as a stressful stimulus, such that stressful stimuli activate the hypothalamus-pituitary-adrenal cortex axis (HPA) and lead to an increase in cortisol secretion. Increased cortisol levels are associated with physiological, social, or psychological stress. Cortisol functions to initiate various bodily adaptations to counteract stressors (Adam and Kumari 2009).

Adrenaline and noradrenaline hormones are synthesized in the adrenal medulla ganglia. They work through the theory of adenosine monophosphate (c-AMP) and the effect of these hormones on the extraction of glucose from glycogen in hepatocytes is done by stimulating the action of cyclic c-AMP to obtain the enzymes necessary for this function. The results obtained during this study showed that the level of both hormones was exposed to an increase during the periods of treatment with the neem oil.

The metabolic effects of adrenaline are similar to the effects of the hormone glucagon, as both works to stimulate the breakdown or decomposition of starch to release glucose into the bloodstream, as well as both stimulating the breakdown of fats to release fatty acids from fatty tissue during fasting, which helps to raise the rate of blood sugar and fatty acids in the blood (Nawale *et al.,* 2006). This happens when the sympathetic adrenal system is stimulated when stress, contradicts the results of this study, but it may be parallel to it in terms of an increase in the level of the hormone adrenaline and noradrenaline, considering that the experimental animal was exposed to an abnormal condition by giving it the high and low doses of neem oil and thus the level of hormones increased, but what contradicts us is the increase in blood sugar due to the increase in the level of hormones. Ghariani, 2009, reported that neem oil had effects on the liver and kidneys that led to an imbalance in the metabolic process Thus, the level of sugar in the blood decreased due to the increase in the level of the hormone insulin. However, the alterations in liver and kidney functions after exposure to neem oil have been reported (Hamdy *et al.,* 2008 a; Hamdy *et al.,* 2008 b; Hamdy *et al.*, 2008 c).

In conclusion, pretreatment with neem oil prevented the rise in blood glucose levels as compared to control. A high dose of it (1/4 LD50) is more effective than the low dose (1/10 LD50) in decreasing blood glucose level and increasing both hormones, the raising of serum cortisol, adrenaline and noradrenaline may result from stress or inflammation during the treatment, so

increasing hormones level need more experiments to interpret this result.

Ethical Approval:

All applicable international, national, and institutional guidelines for the care and use of animals were followed. We respected the welfare of animals and excluded situations when animals were in pain.

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