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Studies on Tissue Residual Level of NeemAzal T/S in Stressed African Catfish" *Clarias gariepinus*" with Reference to the Protective Role of Lupine

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Abstract: The current study aimed to clarify the effects of NeemAzal T/S (NA) on certain blood and tissue (liver and white muscle) oxidative stress parameters in *Clarias gariepinus*. Tissue residual level of NA (braine, Gills, liver and white muscles) was also monitored. The antioxidant effects of lupin was also studied. Two doses of NeemAzal T/S; 1/10 LC_{50}: 5.58 ppm (NA1) and 1/5 LC_{50}:11.15 ppm (NA2) and three periods of 10, 20 and 30 days were used. The results revealed that NA induced marked hyperglycemia, significant elevations in total peroxide as well as significant decrease of catalase and superoxide dismutase activities in most experimental periods in blood, liver or white muscle. Tissue residues were detected in all studied tissues, either with low or high doses of NA. Sublethal doses of NA led to oxidative damage in liver and white muscle. In turn, administration of lupine could improve the adverse effect induced by NA exposure which might be due to its antihyperglycemic effect as well as its contents of some natural antioxidants.

Key words: NeemAzal · Oxidative stress · *Clarias gariepinus* · Lupine · Muscle · Liver · Serum · Residue

INTRODUCTION

Fish and other organisms are affected by pesticides which pollute the natural water through agricultural runoff. Pesticides are directly used in agricultural field mainly to get rid of pests and to improve growing of crops. These pesticides finally find their way into water channels and cause harm to aquatic flora and fauna [1]. The use of synthetic pesticides and their entry into the environment has had a destructive influence on the aquatic ecosystem. Synthetic pesticide usage has resulted in the development of resistant pests. In view of the environmental problems caused by the use of synthetic chemicals, there is a growing need for alternative methods of pest control that would minimize this damage [2]. The use of eco-friendly botanical and soft-pesticides is considered to be of considerable importance [3, 4]. Fish and other aquatic organisms are affected by pesticides that pollute surface water through agricultural runoff and are considered a significant bio-indicator of aquatic pollution [5]. Plant origin pesticides are less toxic to fish than synthetic which are apply as piscicide for cleansing the fish culture pond [5, 6].

Neem, *Azadirachta indica*, provides many useful compounds that are used as pesticides and could be applied to protect stored seeds against insects. Extracts of neem are less toxic at low concentration. Concentrations exceeding 3,200 mg/l influence physiological and biochemical disturbances in fish [7]. A standardized variant of NeemAzal T/S with 1% of the active ingredient, azadirachtin, is approved as an insecticide in organic agriculture for use in pest control in potatoes (against Colorado potato beetle) and pear and apple trees (against aphids) [2].

Winkaler *et al*. [8] showed a decrease in liver catalase activity at all neem concentrations and the detoxifying enzyme glutathione-S-transferase was activated in fish exposed to the high dose of 5.0 g L^{-1} accompanied with tissue damage in gill and kidney. The liver is the metabolic center for detoxification and some authors suggest that changes are essential for the metabolism and excretion of toxic substances in fish [9, 10].

Extract of *Azadirachta indica* (neem) leaf induces apoptosis in rat oocytes cultured *in vitro* [11]. In mice, also, crude ethanol extract of neem leaf induced mitosis disruptive changes in metaphase chromosomes of bone marrow cells on days 8, 15 and 35 of treatment [12].

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It has been showed that most sublethal dose of pesticides induced hyperglycemia in most different species of fish such as *Labeo rohita* (Cypermethrin: [13]), *Rhamdia quelen* (Cypermethrin: [14]), *Cyprinus carpio* (2,4Diamin: [15]), *Clarias batrachus* (malathion: [16]) and *Oreochromis niloticus* (Aqueous extracts of Neem: [7], *Clarias gariepinus* (Malathion: [17] and Piaractus mesopotamicus (trichlorfon) [18].

Hyperglycemia induced by pollutions or glucose infusion and glucose load are important factors to increase reactive oxygen species, oxidative stress and metabolic perturbation in fish [19, 20]. In addition, hyperglycemia induced by glucose infusion causes hepatic oxidative stress in rats [21].

There is little knowledge of antioxidant activity in lupines. Lupines are mostly utilized by stock feed manufacturers in compound feed rations. There is increasing utilization in aquaculture [22]. The antioxidant activities were measured in extract from Lupine seeds [23]. It is suggested that lupine seeds may have higher antioxidant activity in lipid-soluble substances [24, 25]. The digestion of legume protein resulted in high amount of arginine, aspartate and glycine. El-Missiry *et al*. [26] found that the amino acid arginine could ameliorate the oxidative stress in alloxan-treated rats. Also, Alpha-, gamma-and delta-tocopherols were found in the lupine oil antioxidant activity was found both in the flours and in the hulls [27].

This study aimed to evaluate the toxicity effects of NeemAzal T/S on powerful antioxidant organ, liver and other organ of less containing antioxidants, white muscle the main bulk of the body mass. In addition, to evaluate antioxidant power of lupine seed supplementation against oxidative stress generated by NeemAzal T/S in these organs in *Clarias gariepinus*. Monitoring of tissue residues of NA in Gills, brain, liver and muscles were also studied.

MATERIALS AND METHODS

Fish and Materials: Healthy sixty six cat fish (*Clarias gariepinus*) of both sexes weighing 32-38 g/fish (12-15 cm length) were collected from the nursery ponds of El-Jomoom Fish Farm, Jeddah, Kingdom of Saudi Arabia. On arrival at the laboratory, fishes were immediately released into special five glass tanks (Aquaria) (40 x 70 x 60) containing dechlorinated tap water and then maintained there for 10 days for acclimatization condition. The fish were divided to three main groups. The first one was subdivided into three subgroups exposed to NeemAzal T/S (NA) for 10 days; control, low dose (NA1) and high dose (NA2). The second group was also subdivided into three subgroups exposed to NA for 20 days; control, NA1 and NA2. The third group was subdivided into five subgroups exposed to NA for 30 days: control, NA1, NA2, NA1+ lupine supplementation (LS) and NA2 + LS. The number of each fish subgroup was six. Air compressor was used for oxygenation of water. Dissolved oxygen concentrations ranged from 6.1 to 6.6 mg L^{-1}. The ambient water temperature was ranged between 20-23 °C; pH was ranged between 7.1-7.4. All fish were fasted for the last 12 h of the experiment. The water medium was changed at 24 hours interval to remove the metabolic-pollutants with the same treatments according to the experimental protocol. Portable oxygen meter (DO-980) and Hanna Instrument HI 2210 Bench top PH meter W/Temperature Compensation meter were used. Fish were fed on artificial feed twice daily a total of 2% of mean body weight of dry pellets of 2.5 mm. Food ratio of dry weight composed of 40 % fish meal, 18% fat, 10 % corn starch, 11% ashes.

Lupine Seed Preparation: Seeds of lupine (*Lupinus termis*) were washed, kept in the incubator at 37°C to dryness for 24 h and ground well and then lupine powder was added by 5 % to the paste of normal ratio of fish diet before dryness. This food containing lupine was prepared to be tested as antioxidant effect as well as against oxidative stress in fish treated with bioinsecticide; Neemazal of different doses and durations.

NeemAzal-T/S: One of the most promising produced neem products. The active ingredient is Azadirachtin (1%), in addition to plant oil and emulsifier [28]. The recorded 96-h LC_{50} of NeemAzal-T/S for the catfish was 55.76 PPM [29]. Therefore, the used NeemAzal-T/S doses used in this experiment were 1/5 LC_{50} (11.15 PPM: high dose) and 1/10 LC_{50} (5.58 PPM: low dose). Before use, the NeemAzal-T/S was emulsified by using emulsifier agent (SiSi-6) and the emulsifiable concentrate was 50%.

Design of the Experiment: Table (1) shows fish groups, subgroups, intervals and treatments involved in the current study.

At the end of the experiment, blood samples were taken from the caudal peduncle by suction and then fish were sacrificed and dissected. Samples of liver and white muscle (under the dorsal fin) were excised.

Table 1: Experimental design

Intervals ▸	10 days			20 days			30 days				
Groups ▸	Group 1			Group 2			Group 3				
Each SG ▸ was 6 fish	SG1	SG2	SG3	SG1	SG2	SG3	SG1	SG2	SG3	SG4	SG5
Treatments ▾											
Cont	+			+			+				
NA1		+			+			+			
NA2			+			+			+		
NA1+ LS										+	
NA1+ LS											+

NA1: *NeemAzal-T/S* of 5.58 PPM, NA2: *NeemAzal-T/S* of 11.15 PPM, SG: Subgroup Healthy six fish were chosen from each SG. n = 6.

Ten percentage homogenates (w/v) were made in phosphate buffer (pH 7.4) using homogenizer (model IKA-WERKE, D118 BASIC, Germany). Then, homogenates were centrifuged at 5000 rpm for 15 min. to separate the homogenate. Blood samples were left to coagulate and centrifuged at 3000 rpm. Separated serum was kept at-40°C till used. All samples were 250 µL-aliquotted into Eppindorff's tubes and stored at-40°C till used to avoid repeated freeze-thaw cycles in different assays, except aliquots of total peroxide were assayed quickly after homogenization of tissues for more accurate estimation.

Biochemical Assays: Catalase activity was measured by method of Aebi [30]. Superoxide dismutase (SOD) activity was determined as described by Misra and Fridovich [31]. Total peroxide (TP) was assayed according to the method of Harma *et al*. [32]. Haemoglobin (Hb) content was also, assayed by cyanomethaemoglobin method [33]. Total protein in the tissue homogenates was measured using the folin reagent [34]. All assays were performed in triplicate.

Residue Analysis

Extraction of Azadirachtin Insecticide: Fish samples (1g) of liver and brain with 10 ml of methanol and 100 ml of methanol to (50g) fish muscle flesh and gills were added and blended in warring blender at high speed centrifuge for 2 min and partition with methanol [35].

Clean Up: The resulting extracts of fish tissues were cleaned with Hexane and reextracted into dichloromethane layer, after evaporation of the dichloromethane layer, the residue were dissolved in ethyl acetate purified on a florsil minicolumn, eluted with ethyl acetate and analyzed using a reversed phase C-18 column UV detection at 210 nm and Acetonitrile /water gradient system was used for the separation of azadirachtin parent compound from its metabolites and another components in the neemazal formulation sample by the method adopted by Sundaram and Curry [36].

Statistical Analysis: The data were expressed as mean + SEM. The results were analyzed statistically using one way ANOVA with Newman-Keuls Multiple comparison test as a post-test. These analyses were carried out using computer statistics prism 3.0 packages (Graph pad software, Inc, San Diego. (A. USA). The minimum level of statistic significance was set at $P < 0.05$.

RESULTS

Serum: Table (2) shows that CAT activity was significantly increased in the 10 days followed by significant decrease in both 20 and 30 days in response to the high dose, while low dose only sig. decreased it in the 30 days response to NA. In turn, oxidative stress parameter, TP level shows significant rise in all period of high dose (Table 3).

Also, at the 30 days, LS significantly curtails the rise rate (in comparison to control) of TP level in serum of fish treated with NA of high dose. There are significant differences between value of TP of LS plus NA group and that of both control and NA group of high dose. In addition, LS normalized the activities of both CAT and SOD in serum of the fish-exposed to NA (Table 2).

Table (4) pointed out that both doses of NA significantly induced hyperglycemia, while treatment with LS in the fish-exposed to NA of both doses induced normal glycemia.

Table 2: Effect of lupine (L) on catalase (CAT) and superoxide dismutase (SOD) activities in liver, muscle and serum of *Clarias garipinus* contaminated with neemazal (doses: NA1=5.58 and NA2 =11.15 ppm)

	After 10 days			After 20 days			After 30 days				
Parameters	Cont	NA1	NA2	Cont	NA1	NA2	Cont	NA1	NA2	NA1+L	NA2+L
Serum SOD activity											
U/min/mg protein	1.09^{a}±0.05	0.77^{b}±0.05	0.99^{a}±0.06	0.99^{ab} ±0.06	0.87^{a}±0.04	1.16^{b}±0.09	0.94^{a}±0.05	0.59^{bc}±0.07	0.42^{c}±0.04	0.79^{ab}±0.06	0.85^{ab}±0.10
Serum CAT activity											
U/min/mg protein	0.96^{a}±0.13	1.02^{a}±0.07	1.67^{b}±0.08	1.52^{ab} ±0.14	1.78^{a}±0.15	1.21^{b}±0.06	1.01^{a}±0.11	0.70^{b}±0.08	0.65^{b}±0.04	1.17^{a}±0.08	0.92^{ab}±0.03
liver SOD activity											
U/min/mg protein	5.70^{a}±0.19	4.31^{b}±0.17	6.33^{a}±0.39	5.79^{a}±0.30	5.14^{a}±0.38	3.29^{b}±0.15	4.38^{a}±0.21	3.02^{b}±0.22	2.86^{b}±0.19	4.06^{a}±0.28	4.18^{a}±0.35
Liver CAT activity											
U/min/mg protein	2.76^{a}±0.12	2.14^{b}±0.11	2.37^{ab} ±0.23	3.02^{a}±0.12	2.73^{ab}±0.27	2.078^{bc} ±0.19	2.63^{a}±0.17	2.51^{a}±0.23	1.37^{b}±0.12	3.22^{c}±0.09	3.37^{c}±0.19
Muscle SOD activity											
U/min/mg protein	3.09^{a}±0.39	6.91^{b}±0.33	6.97^{b}±0.56	5.07^{a}±0.51	10.20^{b}±0.39	8.66^{b}±0.71	2.77^{a}±0.22	5.17^{b}±0.54	4.83^{b}±0.51	3.05^{a}±0.19	3.33^{a}±0.41
Muscle CAT activity											
U/min/mg protein	2.41^{a}±0.07	3.51^{b}±0.13	2.27^{a}±0.03	1.90^{a}±0.11	2.21^{ab}±0.28	0.97^{b}±0.10	1.67^{a}±0.11	1.41^{a}±0.09	1.10^{b}±0.12	1.34^{a}±0.09	1.52^{a}±0.12

Data are presented as means ± SEM. Columns in each period with different letters differ significantly ($P < 0.05$), while those with the same letters do not differ significantly. NA: neemazal, L: Lupine, n=6

Table 3: Effect of lupine (L) on total peroxide in liver, muscle and serum of *Clarias gariepinus* contaminated with neemazal (doses: NA1= 5.58 and NA2 =11.15 ppm)

	After 10 days			After 20 days			After 30 days				
Parameter	Cont	NA1	NA2	Cont	NA1	NA2	Cont.	NA1	NA2	NA1+L	NA2+L
Serum T. peroxide											
nMol/mg protein	2.44^{a}±0.13	2.05^{a}±0.19	3.48^{b}±0.12	1.89^{a}±0.19	1.56^{a}±0.14	3.38^{b}±0.27	2.37^{a}±0.20	1.93^{a}±0.21	4.89^{b}±0.32	2.61^{a}±0.24	3.62^{c}±0.29
Liver T. Peroxide											
nMol/mg protein	20.11^{a}±1.09	23.64^{a}±1.11	44.12^{b}±5.84	24.36^{a}±1.11	54.81^{b}±2.86	46.25^{b}±6.97	29.27^{a}±0.82	81.72^{b}±3.88	62.65^{b}±3.13	37.79^{a}±2.20	49.58^{d}±3.23
Muscle T. peroxide											
nMol/mg protein	14.44^{a}±1.29	33.88^{b}±2.61	28.88^{b}±1.35	15.04^{a}±1.39	27.19^{b}±2.18	28.19 ^{b}±1.36	12.30^{a}±1.27	34.02^{b}±1.20	33.16^{b}±2.87	20.57^{c}±1.39	21.44^{c}±1.19

Data are presented as means ± SEM. Columns in each period with different letters differ significantly
($P < 0.05$),while those with the same letters do not differ significantly. NA: neemazal, L: Lupine,n=6

Table 4: Effect of lupine supplementation (LS) on blood sugar (glycemia) of *Clarias gariepinus* contaminated with NeemAzal T/S (doses:NA1=5.58 and NA2 =11.15 ppm)

	Control	NA1	NA2	NA1+ LS	NA2+ LS
Blood sugar mg/Dl	72.55±1.21	131.76*± 2.62	112.45*±1.10	94.32±1.49	83.43±1.22

Data are presented as means ± SEM. *: Significant at P<0.5.

Table 5: Effect of lupine (L) on residues analysis in brain, liver, gills and muscle of *Clarias garipinus* contaminated with neemazal (doses: NA1= 5.58 and NA2 =11.15 ppm)

Concentrations of treatments	NA1				NA2				NA2+L			
Residues conc. ppm	BrainPpm	LiverPpm	GillsPpm	Muscleppm	Brainppm	LiverPpm	Gillsppm	Muscleppm	Brainppm	Liver Ppm	Gills ppm	Muscle ppm
Time of treatments(days)												
10	ND	0.009±0.06	0.016±0.25	0.001±0.05	ND	0.012±0.08	0.017±0.13	0.002±0.19	ND	0.006±0.11	0.008±0.01	0.001±0.02
20	ND	0.013±0.12	0.016±0.17	0.001±0.93	ND	0.016±0.13	0.026±0.93	0.003±0.08	ND	0.008±0.09	0.008±0.09	0.001±0.09
30	ND	0.012±0.07	0.013±0.02	0.002±0.08	ND	0.013±0.03	0.029±0.08	0.003±0.01	ND	0.009±0.12	0.010±0.09	0.001±0.08

All values = means ± SE (Standard Error) ND: Not Detected

Liver: Table (2) shows that NA significantly decreased catalase (CAT) activity after exposure to low dose for 10 days and to high dose for 20 and 30. NA of the low dose significantly reduced activity of superoxide dismutase (SOD) at periods of 10 and 30 days and high of NA significantly diminished SOD activity in the liver after 20 and 30 days (Table 2). On the other hand, total peroxide showed sig. rise at all periods of the experiment with both doses except of the low dose at the first 10 days which did not show any significant change (Table 3).

At the 30 days, lupine supplementation (LS) significantly enhanced CAT activity in liver of the fish exposed to both doses vs control groups, in fish-exposed to NA (Table 2). LS normalized liver SOD activity in the fish exposed to both doses of NA. TP was significantly reduced after treatment with LS in the fish exposed to both doses of NA in comparison with NA-treated fish showing improvement but TP level in fish treated with NA plus LS was not be normal (Table 3).

Muscle: The current data show that the low dose of the 10 days induced marked rise, while the high dose at the 20 and 30 days significantly decreased CAT activity in white muscle (Table 2). The muscle SOD activity showed marked rise in all periods and both doses (Table 2). On the other hand, pronounced rise in TP level in muscle was showed in response to all periods and both doses (Table 3).

At the 30 days, LS significantly counteracts the increase rate (in comparison to control) of TP level in muscle of fish treated with low and high dose of NA (Table 3). There are significant differences between value of TP of LS plus NA group and that of both control and NA groups. In addition, LS recovered activities of both CAT and SOD in muscle of the fish-exposed to NA (Table 2).

Chronic Toxicity Treatment: The results in Table (5) exhibit that the residue analysis of azadirachtin in the neemazal insecticide formulation with $^1/_5$ LC_{50} (11.15 ppm),$^1/_{10}$ LC_{50} (5.58 ppm) and $^1/_5$ LC_{50} with lupine in brain, liver, gills and muscle flesh of fish at 10, 20 and 30 days after application. Were ND, 0.009, 0.016 and 0.001 µg/g wet tissues after 10 days of treatment and were ND, 0.013, 0.016 and 0.001 µg/g wet tissues after 20 days of treatment and were ND, 0.012, 0.013 and 0.002 µg/g wet tissues after 30 days of treatment with 1/10 LC_{50}, but the residues level of azadirachtin in the tissue after 10 days of treatment were ND, 0.012, 0.017 and 0.002 µg/g wet tissues and were ND, 0.016, 0.026 and 0.003 µg/g wet tissues after 20 days of treatment and were ND, 0.013, 0.029 and 0.003 µg/g wet tissues after 30 days of treatment with 1/5 LC50. While the residues levels of azadirachtin in all tissues were decreased after treatment with both 1/5 LC50 of azadirachtin and lupine plant, The residue concentration were ND, 0.006, 0.008 and 0.001 µg/ g wet tissues after 10 days of treatment and were ND, 0.008, 0.008 and 0.001 µg/ g wet tissues after 20 days of treatment and were ND, 0.009, 0.010 and 0.001 µg/ g wet tissues after 30 days of treatment.

DISCUSSION

Aquatic organisms have developed several cellular defense paths, which under normal metabolic conditions regulate the level of ROS and protect against the deleterious effects of free radicals. Most of the research on oxidative stress in fish has focused on toxicological effects of different xenobiotics on antioxidant systems and LPO [37].

In the last decades, many studies confirmed the severe toxicity of chemical and synthetic pesticide (organophosphates and organochlorides) on the environmental safety on the animals particularly fish [38]. These let researchers to look for a biopesticide of high safety instead of these chemicals [39]. Neem plant (*Azadirachta indica*) has been used, particularly as an antifeedant, antiattractant, or repellent [40]. *Azadirachta indica* Syn, *Melia Azadirachta*, margosa of the family Meliaceae, are widely distributed in Asia, Africa and other tropical parts of the world.

However, the long exposure to low concentrations of the crude extract of neem (*A. indica*) delayed the growth of fish [41]. Little literature was available concerning the effect of NeemAzal-T/S on oxidative stress in animals particularly fish. Liver plays a fundamental role in the uptake, biotransformation and detoxification of foreign compounds [42] in the body and is thus a target organ of xenobiotics. It is also one of the most affected organs by contaminants in water [43, 44] and as a consequence it undergoes different levels of damage. Antioxidative defense renders a significant protection against environmental stress in organisms and maintains the correct redox balance in cells [45]. Exposure to neem extract interfered with the antioxidant defense system of the fish by reducing liver catalase activity [7]. The current study showed that neemazal (NA) induced significant decrease in both catalase (CAT) and superoxide dismutase (SOD) activities in liver. The result showed that reducing effect of NA on CAT and SOD in liver in response to high dose was more than that of low one with high percentage recorded after 30 days.

The reducing effect of NA on CAT and SOD and the significant rise in TP may be due to the toxicity of the NA resulted from the formation of reactive oxygen species (ROS) in liver tissue and cause alterations in the antioxidant systems. These alterations are caused by an imbalance between ROS and the antioxidant activity or damage to biological system [46, 47]. SOD activity in control fish was highest in liver and progressively lower in muscle, the levels of lipid peroxides (LOOH) in control fish were highest in liver and lowest in muscle [48].

Serum TP level showed significant rise in response to both doses of NA. In spite of control values of antioxidant enzyme activities (CAT and SOD) and oxidative stress indicator (TP) in *Clarias gariepinus* were higher in liver more than that of muscle and that of the later were higher than that of serum, the differences between the changes in response to NA were low. In general, the toxicity of the high dose may be more toxic

due to its high effect in reducing activities of CAT and SOD and increasing TP in liver, muscle and serum. The depletion of SOD activity may be due the overproduction of superoxide anion while, reduced CAT may be due to overproduction of H_2O_2 and its consumption during detoxifying H_2O_2 into water.

Pesticide can affect body glucose homeostasis by several mechanisms including such as nitrosative stress, pancreatitis, inhibition of cholinesterase, stimulation of adrenal gland and disturbance in metabolism of liver tryptophan [49]. Blood glucose was increased in *Oreochromis niloticus* exposed to 96-h and 24-h the LC_{50} of neem extract was estimated at 3,200 and 6,800 mg/l, respectively [7]. Also, hyperglycemia was induced in fish *Cirrhinus Mrigala* exposed to LC_{50} dose of 1.035g L^{-1} Neem Leaf Extracts [50]. On the other hand, hyperglycemia induced by glucose infusion causes hepatic oxidative stress and activates a low-grade systemic inflammation in rats in liver [21].

Also, hyperglycemia is one of the important factors that causing overproduction of ROS inducing oxidative stress and lower antioxidants in tissue organs [51, 52]. NA reduces the oxygen uptake of fish fingerlings and causes mortality at a faster rate [53]. Hypoxia induced oxidative stress in fish [48]. Based on these findings, the recoded hyperglycemia in the 30 days and probably hypoxia may be the main factors to induce overproduction of ROS causing oxidative stress and reduced antioxidants in the liver and muscle of *Clarias gariepinus* exposed to NA especially resulted from the highest dose.

Seeds of lupine (*Lupinus termis*) have a hypoglycemic action in diabetic animals [54, 55, 56]. Also, hypoglycemic effect of lupine was recorded in fish either treated by alloxan or loaded glucose [57]. In addition, lupine seeds have an ameliorating effect on glucose tolerance test in the fish *Clarias gariepinus* [58]. Siger *et al*. [59] had suggested that lupine seeds might have higher antioxidant activities, total phenolic compounds, phenolic acids and flavonoid contents.

The current study revealed that lupine supplementation (LS) normalized glycemic level. Based on these findings, the present work suggests that the ameliorating or normalizing effects of LS on the levels of destructive oxidative stress biomarker TP and antioxidant enzyme activities of CAT and SOD, may be associated mainly with its hypoglycemic effect in *Clarias gariepinus* exposed to NA. Also, the hypoglycemic effect of lupine may improve glucose metabolism and probably increasing NADPH necessary for CAT activity which is consider as a powerful antioxidant in tissues. It has been demonstrated that NADPH protects catalase from inactivation [45, 60].

The high uptake and penetration within tissues of pesticides via the integument of tilapia fish was also observed by El-Shemy *et al*. [61]. The highest concentration of azadirachtin treatment was found in the liver, while the lowest was found in fish brain. Penetrability led to higher residue levels of azadirachtin in fish treated with the NeemAzal T/S. These results may be attributed to the lipophilic nature of the pesticides [62, 63].

In conclusion, this study suggest that sublethal doses of NA on fish generate oxidative damage in organ-containing powerful antioxidants like liver or organ-containing less antioxidants like white muscle. In turn, the antioxidant role of lupine seeds against NA induced oxidative stress in fish, *Clarias gariepinus,* may be mainly due to its antihyperglycemic effect rather than to its contents of some antioxidants.

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