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Effect of garlic extract on mortality and biochemical parameters of fresh water fishes *Heteropneustes fossilis* against Cypermethrin

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ABSTRACT

To find out the effect of garlic extract (GE) on mortality different concentrations of GE (1ml/l, 2ml/l, 5ml/l & 10ml/l) were administered along with the LC_{50} values of cypermethrin after 24, 48, 72 & 96 h exposures and 10 ml/l of GE was found to be effective at which no mortality occurred. To analyze the effect of GE on biochemical parameters after acute exposure of cypermethrin, fishes were divided into 4 groups of 10 fishes each. Ist group served as control, IInd, IIIrd and IVth group were treated with toxicant (24h LC_{50}), GE (10ml/l) and toxicant + GE respectively. Same protocol was employed using 48-96 h LC_{50} values & 10ml/l GE. For chronic toxicity experiments fishes were divided into 4 groups of 10 fishes each. Ist group taken as control, IInd group contained 1/10th of 96 h LC_{50} of CYP, IIIrd group contained 10ml/l GE and in IVth group GE (10ml/l) + CYP (1/10th of 96 h LC_{50}) for 15 days. Experiments were also carried out after 30 and 45 days exposure by same protocol. After acute and chronic exposure periods blood samples were collected, centrifuged and serum was separated to analyze biochemical parameters. Increased level of SGOT, SGPT, ALP, ACP, Creatinine, and Blood Glucose were observed during both acute and chronic exposure. Activity of Total protein was found to be decreased following acute and chronic exposure. Level of Uric acid increased in acute exposure but decreased during chronic exposure. However, garlic extract supplementation showed a remarkable reduction to these changes and all the parameters tends to become normalize. Our data indicate that garlic is a powerful antioxidant against cypermethrin induced toxicity.

Keywords: Cypermethrin, Garlic extract, *Heteropneustus fossilis*, LC_{50}.

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INTRODUCTION

The widespread use of pesticides worldwide has resulted in severe health hazards and environmental pollution. Pesticides applied to the land may be washed into waters bodies and their residues may kill or negatively influence the life of aquatic organisms[1]. Cypermethrin is a synthetic pyrethroid widely used for the pest management but it shows toxicity for many aquatic organisms specially fishes [2]. Pyrethroids are directly absorbed by the gills into the blood stream [3]. Several studies reported the acute and sub acute toxicity of cypermethrin and its effect on biochemical parameters on fishes [4,5].

Antioxidants present in food are good source of health-promoting factor. Naturally occurring antioxidants are present in whole grains, fruits and vegetables. Garlic is a medicinal plant which has been used in Indian ayurvedic medicine for many years [6]. Garlic consist of Sulphur containing compounds such as allicin, alliin, dially disulfide, ajoene, dithiin and S-allylcysteine. The taste and smell of garlic is pungent due to presence of sulphur compounds. Allicin found in raw garlic is a powerful antibiotic and antifungal compound which improves some immune functions [7] . Garlic consumption can reduce blood pressure; prevent heart disease; high cholesterol, artherosclerosis and cancer [8-10]. Garlic has also hepato-protective functions in rats[11] and antimicrobial properties in fishes[12] . Some studies have been carried out on the protective effects of garlic following pesticides exposure on fishes and rats [13,14] . Sahu *et al*, 2007 studied the effect of garlic on immunity and survival of *Labeo rohita*[15]. Thanikachalam *et al*., 2010 reported the effect of garlic peel on growth and heametological parameters in African catfish *Clarius gariepinus*[16]. El-Banne *et al*., (2009) also reported effect of garlic on antioxidant properties and some biochemical parameters on rats exposed to chlorpyrifos [17]. There is a lack of knowledge on how to overcome the adverse effect of cypermethrin using medicinal plant in Bundelkhand region. So, this study was aimed to evaluate the protective effects of garlic extract as a cheap, available and potent medicinal plant against cypermethrin caused adverse effects in *Heteropneustus fossilis*.

MATERIALS AND METHODS

Live and healthy fishes with average weight 80-90g were purchased from local market in Jhansi district of Bundelkhand region, transported to laboratory conditions and acclimatized for 10-15 days. The infection was removed by keeping the fishes in 0.2% potassium permanganate solution for 2-4 minutes. During acclimatization and chronic studies fishes were fed with commercial diet but feeding was stopped to fishes 24 h before acute toxicity experiment.

Cypermethrin

Jackpot 25 (Cypermethrin 25% EC) insecticide manufactured by Crystal Crop Protection Private Limited, Delhi was used in all the experiments.

Garlic

Garlic extract was prepared daily according to the requirement. For preparing extract garlic cloves (30g) were peeled, crushed and grinded in 60 ml distilled water then filtered with sieve [13].

Experimental design

For mortality experiments fishes were divided into 5 groups of 10 fishes each. Different doses of garlic extract viz 1ml/l, 2ml/l, 5ml/l, 10 ml/l were selected. All the groups were treated with cypermethrin of 24 h LC_{50} (0.00066ml/l) concentration. 2nd, 3rd 4th and 5th groups were also treated with 1ml/l, 2ml/l, 5ml/l & 10ml/l garlic extract respectively and mortality was recorded after 24 h. Similar experiments were repeated after administration of different concentrations of toxicant (48, 72 & 96 h) with above mentioned doses of garlic extract.

For acute and chronic studies fishes were divided into 4 groups of 10 fishes each and were treated as below:

Group Ist : Control

Group IInd : Cypermethrin (CYP)

Group IIIrd : Aqueous extract of garlic (GE)(10 ml/l)

Group IVth : Cypermethrin (CYP) + GE (10 ml/)

For acute toxicity 24 LC_{50} concentration (0.00066 ml/l) were used in IInd and IVth group. Same protocol was repeated for 48 (0.00044 ml/l), 72 (0.00033 ml/l) and 96 (0.00022 ml/l) h. For chronic toxicity bioassay 1/10th of 96 h (0.00022 ml/l) LC_{50} concentration of cypermethrin was used in IInd and IVth groups separately for 15, 30 and 45 days.

Blood collection and biochemical studies

After completion of experiments blood samples from all the fishes were collected, centrifuged at 3000rpm for 20 min and serum was separated and analyzed for serum Total protein, SGOT, SGPT, Alkaline phosphatase, Acid phosphatase, Creatinine, Uric acid and Blood Glucose using colorimetric diagnostic kit through biochemical auto-analyzer.

Statistical Analysis - Data were analyzed using Student's 't' test and differences with a P- value < 0.05 was considered as statistically significant and results were expressed as Mean ± S.D.

([a]) Significant when compared to control group ($p < 0.05$).

([b]) Significant when compared to cypermethrin treated group ($p < 0.05$).

RESULTS AND DISCUSSION

The previously estimated LC_{50} values of cypermethrin were found to be 0.00066, 0.00044, 0.00033, and 0.00022 at 24, 48, 72 and 96 h respectively [18] .The effect of garlic extract on mortality is shown in fig-1. 70% mortality occurred after 24 h at 1ml/l garlic extract whereas 60%, 60% and 50% mortality was observed after 48, 72 and 96 h respectively. On increasing the concentration of garlic i.e. 2ml/l, 5ml/l, and 10ml/l the mortality was reduced. The results revealed that no mortality occurred after co-administration of cypermethrin at 10 ml/l of garlic extract (Fig-1.).

Exposure of *Heteropneustus fossilis* to cypermethrin shows influential changes in fish behavior, fish showed abrupt and sluggish movements in various directions. Mucous secretion, jumping and hitting against the walls of aquarium was also observed. Body colour changed to light due to pesticide and it was remarkably different from control group. As the cypermethrin was given with GE the frequency of abnormal behaviour decreased.

The changes in biochemical parameters in the test fishes recorded after the treatment of cypermethrin and garlic extract are presented in Table 1,2 & 3 during acute and chronic toxicity. The significantly ($p < 0.05$) increased levels of SGOT, SGPT, ALP, ACP, and Blood Glucose were observed and the level of Total protein was found to be decreased in CYP treated fishes when compared to control group. The IVth group shows the ameliorative effect of GE when administered along with toxicant.

Table- 4 shows changes in kidney markers i.e. Creatinine and Uric acid after both acute and chronic exposure of CYP and GE. The level of Creatinine was found to be increased as compared to untreated control group. There was an increased level of Uric acid during acute exposure but significantly decreased level in chronic exposure. The kidney markers were found to be restored after supplementation of GE. Comparative analysis with acute and chronic exposures revealed that the percent change in IVth group was decreased in chronic exposure.

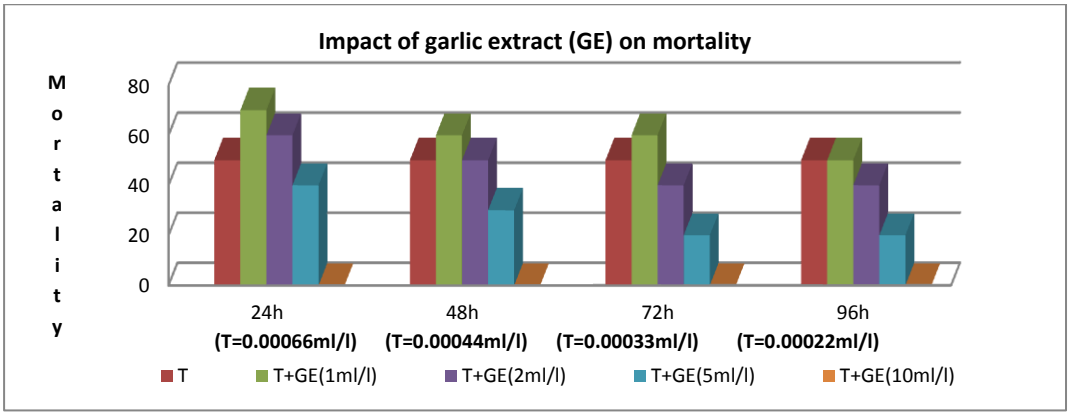

Figure 1: Impact of garlic extract (GE) on mortality

Table 1: Effect of Garlic extract (GE) on CYP induced acute toxicity in *Heteropneustes fossilis.*

S.NO.	PARAMETERS	24h				48h			
		C	T	GE	T+GE	C	T	GE	T+GE
1	S.G.O.T	28.93 ± 2.38	43.83 **a** ± 1.54	30.67 **b** ± 1.77	26.73 **b** ± 6.38	31.07 ± 1.53	45.85 **a** ± 2.30	31.79 **b** ± 2.96	38.65 **ab** ± 2.86
	% Change with C		51.50	6.01	-7.60		47.57	2.31	24.39
2	S.G.P.T	33.23 ± 2.08	69.06 **a** ± 3.39	36.3 **b** ± 2.90	46.33 **ab** ± 3.80	33.88 ± 2.76	70.9 **a** ± 4.30	37.43 **b** ± 2.64	48.06 **ab** ±1.21
	% Change with C		107.82	9.23	39.42		109.26	10.47	41.85
3	S.PROTEIN	5.57 ±0.24	5.11 **a** ±0.05	5.62 **b** ±0.20	5.84 **b** ±0.07	5.62 ±0.22	5.24 **a** ±0.08	5.64 **b** ±0.21	5.87 **b** ±0.07
	% Change with C		-8.25	0.897	4.84		-6.76	0.35	4.44
4	ALP	253.91 ±4.79	272.66 **a** ±5.77	252.09 **b** ±8.89	260.93 **b** ±1.22	261 ±10.06	274.43 **a** ±6.33	263.16 **b** ±3.01	267.24 ±3.55
	% Change with C		7.38	-0.71	2.76		5.14	0.82	2.39
5	ACP	3.63 ±0.03	3.95 **a** ±0.04	3.67 **b** ±0.04	3.80 **ab** ±0.01	3.65 ±.04	3.98 **a** ±0.03	3.72 **b** ±0.02	3.83 **ab** ±0.02
	% Change with C		8.81	1.10	4.68		9.04	1.91	4.93
6	BLOOD GLUCOSE	74.66 ±3.78	96.66 **a** ±1.52	81 **b** ±2	86.66 **ab** ±2.08	78.66 ±5.05	100.33 **a** ±1.40	81.33 **b** ±3	90.33 **ab** ±3.05
	% Change with C		29.476	8.49	16.07		27.54	3.39	14.83

Table 2: Effect of Garlic extract (GE) on CYP induced acute toxicity in *Heteropneustes fossilis.*

S.NO.	PARAMETERS	72h				96h			
		C	T	GE	T+GE	C	T	GE	T+GE
1	S.G.O.T	32.10 ± 2.20	47.96 **a** ± 1.34	32.32 **b** ± 2.63	37.57 **b** ± 2.60	33.00 ± 3.41	49.97 **a** ± 3.09	38.26 **b** ± 3.06	40.9 **b** ± 1.32
	% Change with C		49.40	0.68	17.04		51.42	15.93	23.93
2	S.G.P.T	34.76 ± 1.29	51.43 **a** ± 2.99	39.67 **b** ± 1.58	42.87 **ab** ± 2.28	35.14 ± 2.48	54.16 **a** ± 9.73	38.4 **b** ± 2.31	44.53 **ab** ± 2.31
	% Change with C		41.95	14.12	23.33		54.12	9.27	-26.72
3	S.PROTEIN	5.66 ±0.07	5.30 **a** ±0.08	5.74 **b** ±0.08	5.96 **ab** ±0.07	5.78 ±0.07	5.34 **a** ±0.09	5.83 **b** ±0.04	5.99 **ab** ±0.06
	% Change with C		-6.36	1.41	5.30		-7.61	0.86	3.63
4	ALP	268.77 ±4.02	294.73 **a** ±6.67	272.93 **b** ±1.90	276.07 **b** ±2.40	275.33 ±3.05	298.8 **a** ±6.17	276.71 **b** ±2.82	277.69 **b** ±3.70
	% Change with C		9.65	1.54	2.71		8.52	0.50	0.85
5	ACP	3.71 ±0.01	4.02 **a** ±0.04	3.75 **b** ±0.03	3.87 **ab** ±0.01	3.72 ±.02	4.03 **a** ±0.02	3.73 **b** ±0.02	3.86 **ab** ±0.06
	% Change with C		8.35	1.07	4.31		8.33	0.26	3.76
6	BLOOD GLUCOSE	90.33 ±2.08	102.33 **a** ±4.50	81.66 **ab** ±1.52	94 **b** ±8.18	86.66 ±3.05	106.33 **a** ±1.52	84 **b** ±3	94.66 **ab** ±3.05
	% Change with C		13.28	-9.59	4.06		22.69	-3.06	9.23

Table 3: Effect of Garlic extract (GE) on CYP induced chronic toxicity in *Heteropneustes fossilis*.

S. N.	PARA-METERS	15 days Exposure				30 days Exposure				45 days Exposure			
		C	T	GE	T+GE	C	T	GE	T+GE	C	T	GE	T+GE
1	S.G.O.T	29.32 ± 2.07	87.54**a** ±3.88	27.30**b** ±3.08	33.39**b** ±2.76	28.51 ± 1.95	81.82 **a**±2.77	34.63**b** ±2.68	31.45**b** ±2.50	26.39 ±2.24	76.42**a** ±2.75	33.06 **b** ±3.37	28.32**b** ±2.66
	% Change with C		198.56	-6.88	13.54		182.98	21.46	10.31		189.57	25.27	7.31
2	S.G.P.T	46.92 ±2.27	100.76 **a** ±2.92	55.37**ab** ±2.14	51.22**b** ±1.88	43.22 ±2.88	95.85 **a** ±3.33	35.69**b** ±3.72	46.71**b** ±1.73	40.22 ±2.27	89.83**a** ±2.99	33.57 **b** ±3.80	42.90**b** ±2.64
	% Change with C		114.74	18.0	9.16		121.77	-17.42	8.07		123.34	-16.53	6.66
3	S.PROTEIN	5.04 ±0.02	4.16**a** ±.03	5.11**b** ±0.04	5.08**b** ±0.03	5.14 ±0.02	4.25**a** ±.03	5.17**b** ±.0.03	5.15**b** ±0.02	5.23 ±0.2	4.45**a** ±0.03	5.27**b** ±0.03	5.26**b** ±0.03
	% Change with C		-17.46	1.38	0.79		-17.31	0.58	0.19		-14.91	0.76	0.57
4	ALP	243.23 ±2.81	289 **a** ±2.72	222.38 **ab** ±3.75	248.01 **b** ±2.43	236.80 ±2.82	287.12**a** ±3.72	216.61**a b** ±3.87	240.21 **b** ±1.31	232.42 ±3.52	281.83 **a**±2.80	207.64**ab** ±2.56	236.83 **b** ±3.78
	% Change with C		18.81	-8.51	1.96		21.25	-8.52	1.44		21.25	-10.66	7.20
5	ACP	3.85 ±0.02	4.45**a** ±0.02	3.73**ab** ±0.03	3.89**b** ±0.02	3.71 ±0.01	4.29**a** ±0.02	3.54**ab** ±0.02	3.81**ab** ±0.03	3.58 ±0.03	4.11**a** ±0.02	3.31**a b** ±0.02	3.45**ab** ±0.03
	% Change with C		15.58	-3.11	4.93		15.63	-4.58	2.69		14.80	-7.53	-3.63
6	BLOOD GLUCOSE	91.33 ±1.52	104.33 **a** ±2.51	85.66**b** ±3.51	93.33**b** ±1.52	86.66 ±1.52	99.66 **a** ±1.52	82.33**b** ±3.21	87.33**b** ±1.52	79.33 ±2.51	93.66**a** ±3.05	78.66 **b** ±3.05	80.33**b** ±2.51
	% Change with C		14.23	-6.20	2.18		14.23	-6.20	2.18		18.06	0.84	1.26

Table 4: Effect of GE on Kidney markers in *Heteropneustus fossilis* during CYP induced acute and chronic toxicity

EXPOSURE TIME			S.CREATENINE		S.URIC ACID	
			Mean ± S.D.	% Change with C	Mean ± S.D.	% Change with C
ACUTE	24	C	0.86 ±0.04		4.63 ±0.03	
		T	1.43 ±0.04**a**	66.27	5.39 ±0.02**a**	16.41
		GE	0.88 ±0.01**b**	2.32	4.68 ±0.03**b**	1.07
		T+GE	1.19 ±0.02**ab**	38.37	5.05 ±0.04**ab**	9.07
	48	C	0.89±0.04		4.66 ±0.02	
		T	1.42 ±0.04**a**	59.55	5.41 ±0.02**a**	16.09
		GE	0.95 ±0.03**b**	6.74	4.67 ±0.04**b**	0.21
		T+GE	1.20 ±0.03**ab**	34.83	5.05 ±0.02**ab**	8.36
	72	C	0.94 ±0.03		4.74 ±0.02	
		T	1.49 ±0.04**a**	58.51	5.45 ±0.02**a**	14.97
		GE	1.05 ±0.04**ab**	11.70	4.69 ±0.11**b**	-1.05
		T+GE	1.17 ±0.02**ab**	24.46	5.05 ±0.04**ab**	6.54
	96	C	0.92 ±0.04		4.73 ±0.03	
		T	1.50 ±0.05**a**	63.04	5.49 ±0.04**a**	16.06
		GE	1.05 ±0.03**ab**	14.13	4.73 ±0.03**b**	0.21
		T+GE	1.25 ±0.03**ab**	35.86	5.11 ±0.03**ab**	8.03
CHRONIC	15	C	0.75±0.03		4.58±0.03	
		T	1.65±0.03**a**	120	3.89±0.03**a**	-15.06
		GE	0.64±0.02**ab**	-14.66	4.76±0.03**ab**	3.93
		T+GE	0.78±0.02**b**	4	4.32±0.03**ab**	-5.67
	30	C	0.63±0.04		4.44±0.03	
		T	1.56±0.02**a**	147.61	3.77±0.04**a**	-15.09
		GE	0.56±0.02**b**	-11.11	4.46±0.05**b**	0.45
		T+GE	0.65±0.03**b**	3.17	4.21±0.05**ab**	-5.18
	45	C	0.43±0.03		4.25±0.03	
		T	1.40±0.02**a**	225.58	3.75±0.03**a**	-11.76
		GE	0.45±0.03**b**	4.65	4.36±0.03**b**	2.58
		T+GE	0.46±0.03**b**	6.97	4.07±0.04**ab**	-4.23

Toxicity caused by various agents (toxins, metals, dioxin and pesticides) is considered as threat for many organisms which leads to death. CYP, an organophosphorus insecticide is known to cause oxidative stress and various metabolic disorders [19]. To overcome these problems, it is necessary to find out the solutions against this threat. In the context of above, nature can provide us many substances that can attenuate the oxidative stress. Most of these substances are found in some medicinal plants and garlic is one of them. In

the present study, toxicity of CYP on some biochemical parameters and a protective role of garlic were investigated.

Our results showed that the mortality rate of fishes decreased on increasing the concentration of garlic extract and no mortality was observed at 10ml/l of garlic extract along with different LC_{50} concentration of CYP. This shows the vital role of garlic as an antioxidant which increases the survival rate of fishes intoxicated by cypermethrin [20].

Protective effect of garlic may be associated with its antioxidant properties [21]. Free radicals have been produced during stressed conditions adversely they can alter biological processes. These free radicals can be prevented or reduced by dietary natural antioxidants [22]. Al-Shaikh (2012) indicated that cypermethrin alone decreased the antioxidant enzyme activity, while the treatment of cypermethrin with garlic increased the level of these enzymes[13]. Garlic with cypermethrin results in improvement of biochemical parameters as suggested by several authors [13, 23].

Increased concentration in the activity of SGOT, SGPT, ALP and ACP enzymes in the serum of CYP treated group for both acute and chronic exposures periods was observed in this study which could be attributed to the toxic effect of nitroso compounds, formed in the acidic environment of the stomach, causing enzymatic changes due to cellular damage. When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of SGOT and SGPT indicates the utilization of amino acids for the oxidation reactions to liberate energy [24]. Also, the elevation in ALP and ACP level suggests an increase in lysosomal mobilization and cell necrosis due to pesticide toxicity. The analysis of total protein has showed significant decrease in protein content in *Heteropneustes fossilis* after acute and chronic exposure to CYP in this study. The decrease in protein content might be attributed to the destruction of the cells and consequent impairment in protein synthesis machinery [25]. To fulfill the energy demand transaminases activity increased which modulate the activity of protein as shown in the present work. All the parameters tend to become normalize after long term exposure comparatively to acute exposure with co-administration of GE and CYP. These results show the role of GE in stabilizing the cell membrane and protect the liver from free radicals.

Uric acid and creatinine are waste products of protein metabolism that need to be excreted by the kidney. A marked increase in serum creatinine, as noticed in this study, confirms an indication of functional damage to the kidney [11]. Increased creatinine may be due to changes in the threshold of tubular reabsorption, renal blood flow and glomerular filteration rate [26]. Increased in uric acid noticed in this study after acute exposures while decreased activity of uric acid was observed after chronic exposure. This is a classical sign that the kidney was adversely affected by CYP administration [27]. Garlic extract showed a clear improvement in the kidney functions by modulating creatinine and uric acid, perhaps due to antioxidant properties of garlic in scavenging free radicals leading to reduced level of lipid peroxidation. Garlic improved the antioxidant mechanism due to the ability of Diallyl disulfide & Diallyl trisulfide in modulating the oxidative stress and detoxifying enzyme system [28, 29].

Our results clearly showed that there was a significant increase in blood glucose after acute and chronic exposure. Stress is an energy demanding process and the fishes mobilizes energy substrate to cope with stress. The increase in blood glucose might indicate destructed carbohydrate metabolism due to enhance break down of liver glycogen [26]. Addition of garlic extract restored the damage caused by CYP.

CONCLUSION

Results of the present study clearly indicate that administration of the garlic combats toxicity induced by CYP in the fishes. Medicinal plants serve as therapeutic alternative, in overcoming the occurred adverse effects caused by pesticides.

REFERENCES

1. Rahman O, Sadhu DN, Effects of pesticides on aquatic and aerial oxygen consumption in an air- breathing murrel fish, *Channa gachua*, Nature Environment and Pollution Technology, 2009; 8(3):603-608.
2. Singh SK, Singh SK, Yadav RP, Toxicological and biochemical alterations of cypermethrin (Synthetic pyrethroids) against fresh water teleost fish, *Colisa fasciatus* at different season, World Journal of Zoology, 2010; 5(1):25-32.
3. Neelima P, Sunitha K, Govinda Rao K, Krishna Ch,Chandra Sekhara Rao J, Acute Toxicity of Cypermethrin (25%EC) and Its Effects on Behavioural Changes in *Cyprinus carpio* (Linn.), International Journal of Zoological Investigations, 2016; 2(1):35-47.
4. Das BK, Mukherjee SC, Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences,Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,2003;134 (1):109-121.
5. Vasantharaja C, Pugazhendy K, Venkatesan S, Meenambal M, Prabhakaran S, Jayachandran K, Acute toxicity of cypermethrin and its impact on biochemical alteration in the fresh water fish *Cirrhinus mrigala* (Hamilton) and Protective effect of *Cardiospermum helicacabum* (Linn.), International Journal of Pharmaceutical & Biological Archives, 2012; 3(1):146-152.
6. Naik GH, Priyadarshani KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK and Mohan H, Comparative antioxidant activity of individual herbal components used in ayurvedic medicine, Phytochemistry, 2003; 63(1):97-104.
7. Ban JO, Yuk DY, Woo KS, Kim TM, Lee US, Jeong HS *et al*., Inhibition of cell growth an induction of apoptosis via inactivation of Nf- kappaB by a sulfur compound isolated from garlic in human colon cancer cells, Journal of Pharmacological Science, 2007; 104 (4):374-383.
8. Mirunalini S, Kumaraguruparan R, Subapriya R and Nagini S, Garlic oil Enhances Hepatic and Blood Antioxidant during Hamster Buccal Pouch Carcinogenesis, Pharmaceutical Biology, 2004; 42(3):240-245.
9. Shukla Y and Kalra N. Cancer chemoprevention with garlic and its constituents, Cancer Letters, 2007; 247(2):167-181.
10. Tsai Chai-Wen, Chen Haw- Wen, Sheen Le-Yen and Lii Chong-Kuei, Garlic: Health benefits and actions, Bio Medicine,2012; 2:17-29.
11. El Shenawy SM, Hassan NS, Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats, Pharmacological reports, 2008; 60 (2):199-208.
12. Kumolu- Johnson CA, Ndimele PE and Olasehinde FI, Preliminary study on the antioxidative and antimicrobial effects of fresh garlic (*Allium sativum*) on the shelf life of Hot-smoked Catfish (*Clarias gariepinus*), Jounal of fisheries and aquatic sciences, 2013; 8(1): 253-256.
13. Al-Shaikh TM, Protective effect of garlic against cypermethrin induced lung toxicity in adult male mice: Biochemical and Histopathological studies, Life Science Journal, 2012; 9(4):4017-4025.
14. Frank F, Yanshun Xu, Qixing J, Wenshui X, Protective effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on physiochemical and microbial attributes of liquid smoked silver carp (*Hypopthalmichthys molitrix*) wrapped in aluminium foil during chilled storage, African Journal of Food Science, 2014; 8(1):1-8.
15. Sahu S, Das BK, Mishra BK, Pradhan J, Sarangi N, Effect of Allium sativum on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*, Journal of Applied Ichthyology, 2007; 23(1):80-86.
16. Thanikachalam K, Kasi M, Rathinam X, Effect of garlic peel on growth, hematological parameters and disease resistance

against *Aeromonas hydrophila* in African catfish *Clarias gariepinus*(Bloch) fingerlings, Asian Pacific Journal of Tropical Medicine, 2010; 3(8):614-618.
17. El- Banne, SG, Attia AM, Hafez AM, El- Kaza SM, Effect of garlic consumption on blood lipid and oxidant/ antioxidant parameters in rat males exposed to chlorpyrifos. Slovak Journal of Animal Science, 2009; 42(3):111-117.
18. Singh A, Zahra K, LC_{50} assessment of cypermethrin in *Heteropneustes fossilis*: Probit analysis. International Journal of Fisheries and Aquatic studies. 2017; 5(5):126-130.
19. AL-Ghanim KA, Effect of Cypermethrin toxicity on enzyme activities in the freshwater fish *Cyprinus carpio.* African Journal of Biotechnology, 2014; 13(10):1169-1173.
20. Salah MA, Nashwa M, Abdel A, Mohamed F, Effect of Garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*, 8th International symposium on Tilapia in aquaculture, 2008; 277-296.
21. Rahman MM, Fazlic V, Saad NW, Antioxidant properties of raw garlic (*Allium sativum*) extract, International Food Research Journal, 2012; 19(2):589-591.
22. Hassan HA, Hafez HS Zeghebar FE, Garlic oil as a modulating agent for oxidative stress and neurotoxicity induced by sodium nitrite in male albino rats, Food and chemical Toxicology, 2010; 48(7):1980-1985.
23. Assayed ME, Khalaf AA, Salem HA, Protective effects of garlic extract and vitamin C against in vivo cypermethrin- induced cytogenic damage in rat bone- marrow, Mutation Research, 2010; 702(1):1-7.
24. Fazlolahzadeh F, Keramati K, Nazifi S, Shirian S, Seifi S, Effect of Garlic (*Allium sativum*) on Hematological parameters and Plasma activities of ALT and AST of *Rainbow trout* in temerature stress, Australian Journal of basic and Applied Sciences, 2011; 5(9):84-90.
25. Malla FA, Asssessment of Total protein concentration in liver of fresh water fish, *Channa punctatus* (Bloch) with special reference to an organophosphate insecticide Chlorpyrifos, International journal of pharmaceuticals and biological science, 2012; 3(2):567-571.
26. Hassan AH, El- Agmy SM, Gaur RL, Fernando A, Raj MHG, Ouhtit A. In vivo evidence of hepato- and reno- protective effect of garlic oil against sodium nitrite- induced oxidative stress. International Journal of Biological Sciences, 2009; 5(3):249-255.
27. Ghalehkandi GJ, Ebrahimnezhad Y, Nobar RS, Effect of Garlic (Allium sativum) aqueous Extract on serum values of Urea, Uric-Acid and Creatinine compared with Chromium Chloride in Male Rats. Annals of Biological Research, 2012, 3 (9):4485-4490.
28. Saravanan G, Prakash J, Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. Journal of Ethnopharmacology, 2004; 94(1):155-158.
29. Popova MA, Popove CS, Effect of chemical agents on some enzyme activities and the stability of membrane structures. Bulgarian Journal of Veterinary Medicine, 2005; 8(3):163-171.