



RESEARCH PAPER

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Comparative potential of *Moringa oleifera* and *Bougainvillea spectabilis* against pyrethrin (Deltamethrin) induced toxicity in albino rats

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Abstract

Pesticidal induced toxicity proved to impose lethargic effects on mammalian hematological and serological parameters. Study was conducted to evaluate ameliorating potential of *Bougainvillea spectabilis* and *Moringa oleifera* against pyrethrin type-II induced toxicity in albino rats. Experiment carried out for 60 days by selecting 12 healthy male albino rats, in Animal House of Food Science and Technology department of Agriculture University Faisalabad, acclimatized for one week and kept under favorable condition at 23°C ± 3°C temperature. All of these rats divided into 4 groups. T₀ taken as control group feed on simple diet and water receiving no chemicals and plant extract. T₁ received daily dose of deltamethrin (1.55ml) *bougainvillea spectabilis* along with (150mg) per body weight orally, T₂ received deltamethrin (1.56ml) along *Moringa oleifera* plant extract (150ml), T₃ received deltamethrin only about 1.57ml. Each rat act as individual replica. Daily dosage of mixture was offered to all these groups. Autopsy of these rats done after considerable period to analyzed certain changes observed in weight, hematological parameters and serological parameters. RBC, MCV, WBC, HCT, HGB, MCH, MCHC, LYM, LYM% and serological parameters such as AST, ALT, ALP, TSP, urea and creatinine. Results were analyzed by using Anova and Tukey comparison test. Statistical analysis showed highly significant (P<0.01) results of both anti-oxidant against pesticidal induced toxicity.

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Introduction

The environment that considered a key contributor towards human health now exposed to many of the stresses including pesticides having detrimental lethal effects on living organisms and (Bjorling *et al.*, 2008). The pesticide term, covers broad range of compounds including growth regulators, rodenticides, insecticides, fungicides, herbicides and others (Damalas and Eleftherohorinos, 2011). This high risk of diseases is due to many factors including poor knowledge about use of pesticides and direct exposure of chemicals (Gbaruko *et al.*, 2009).

World health organization depicted about 3 million cases of pesticide poison and approximately more than 250,000 deaths (Yang and Deng, 2007). Pyrethins derivatives known as synthetic pyrethroids are highly modified and natural substances obtained from pyrethrum specie flowers widely used in agricultural sector (Luty *et al.*, 2000). Deltamethrin an active neurotoxic belonging to type II pyrethroids, widely used because of its low accumulation tendency in organisms and short period of biodegradation and more toxic to insect's nervous system (Loskowski, 2002).

Recent studies showed some of their negative impacts on urinary, respiratory, neurodegenerative, cerebrovascular and hematological diseases (Mani *et al.*, 2014). Level of their activity can be determined by target specification, penetration and metabolism (EPA, 2001). Deltamethrin poisoning caused neurodegenerative diseases in humans by oxidative stress, enzymes antioxidant activity, greater lipid peroxidation and causing destruction of macromolecules like nucleic acid, lipids and proteins (Abdel-Daim *et al.*, 2013).

Due to deltamethrin, reactive oxygen species (ROS) production enhanced, including hydrogen peroxide, hydroxyl radical and superoxide anion which causing damages in DNA of rats and also harmful to nephritic, reproductive and hepatic system (Hamid and Salah, 2013) hyperglycemia and toxic effects on pancreas following chronic, sub chronic and acute exposure in rats (Kamath *et al.*, 2008).

Exposure of deltamethrin caused reduction in serum testosterone, sperm count, sperm motility, decreased in reproductive organs weight and also increases testes and sperm abnormalities. Lipid peroxide concentration increased significantly in liver, testes and kidney. Some histological changes also occur in kidney and liver and brain of albino rats (Issam *et al.*, 2009). Rich source of secondary metabolites and anti-oxidant are plant based so these are used for several activities for control of oxidative stress. Several medicinal plants species are present which have cytoprotective properties (Anwar *et al.*, 2005).

Bougainvillea spectabilis and *Moringa oleifera* having many antilipidemic, anthelmintic and anti-diabetic activities prevent oxidative stress and myocardial necrosis in albino rats (Krishna and Rajan, 2018). The flowers of *Moringa* tree contain D-glucose, sucrose, wax, quercetin, alkaloids traces along with nine amino acids, the ash rich in calcium and potassium. Some of the flavonoid elements known as kaempferitrin, isoquercitrin, alkaloids, kaempferol and rhamnetin reported in their flowers (Siddhuraju and Becker, 2003).

Bougainvillea spectabilis also contains amylase inhibitor, oxidase and pinitol (hypoglycemic element). *Bougainvillea spectabilis* is a thorny woody plant with flowers. It contains many components. One of these compounds is phytochemicals. Phytochemicals include quinones, saponins, triterpenoids, flavonoids, phenols, sterols, glycosides, tannins, furanoids and small amounts of sugars (Chauhan *et al.*, 2016). Studies showed that *Bougainvillea spectabilis* contain antiulcer antimicrobial, anti-diabetic, anti-inflammatory, amylase inhibitory, anti-hyperlipidemic, radical scavenging, anti-atherogenic, thrombolytic, analgesic, antipyretic, anthelmintic properties (Do *et al.*, 2016).

The aims of the this study was to evaluate the ameliorating potential of *Bougainvillea spectabilis* and *Moringa oleifera* against pyrethrin type-II induced toxicity in albino rats. It also included the parameters the Hematological parameters RBCs, Leukocytes and other blood parameters, Creatinine,

liver functions tests such as the ALT, AST, Alkanine phosphatase to check the liver toxicity.

Materials and methods

Collection of animals

16 healthy male albino rats of about 60 days age with average weight of 250-300g were obtained from animal house of Agriculture University Faisalabad acclimatized for one week and kept under favorable condition at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ temperature.

Chemicals

WHO recognized deltamethrin having 1.5 % W/V, EC purchased from public scientific store, Faisalabad.

Preparation of plant extract

From leaves of *Bougainvillea spectabilis* and *Moringa oleifera* bio-pesticide of plant origin was prepared. For this purpose their leaves were dried under shade for 4-6 days, grinded into fine powder and undergoes ethanol extraction. About 500mg of leaves powder dipped into 70% ethanol and 30% distilled water and allow to stand for 15 days by covering with lids. The mixtures were continually mixed by using of stirrer 3 times in a day, lids of bottles also removed for sometimes to remove excessive gases from bottles. After 15 days mixtures were filtered by filter paper for removal of debris this soaked material than re-filtered for more refined extract, the remaining extract re-soaked for three days and again filtered by using filter paper and dried in oven for over-night. The obtained plant extract kept in glass bottles and covered with lids.

Study design and treatments

16 healthy male albino rats of about 60 days age divided into 4 groups and each group containing three rats.

To: Control group feed on simple diet and water receiving no chemicals and plant extract.

T1: Receiving daily dose of deltamethrin (1.55ml) *bougainvillea spectabilis* along with (150mg) per body weight orally.

T3: receiving deltamethrin (1.56ml) along *Moringa oleifera* plant extract (150ml)

T4: received deltamethrin only about 1.57ml. Each rat act as individual replica.

The trial remain two one month and at the last of month dissection of these rats done for observing morphological, clinical and histological changes in all groups.

Collection of blood samples

The rats were slaughtered and blood from jugger vein collected directly in EDTA valves for hematological and serological studies.

Determination of Hematological parameters

After slaughtering the blood samples were collected in EDTA vials and simple vials which further used for study RBCs, WBCs, HGB, Mean corpuscular volume (MCV), HCT, Mean corpuscular concentration (MCH), Mean corpuscular haemoglobin concentration (MCHC), Lymphocyte concentration (LYM) and Lymphocyte % parameters.

Determination of serological parameters

From blood serum Alkanine alanine transferase (ALT), Aspartate aminotransferase (AST), Alkanine phosphatase, Urea, Creatinine, Total soluble protein (TSP) were studied.

Statistical analysis

The obtained results were analyzed by using Anova and Tukey pairwise comparison test.

Results and discussions

Results showed exposure of deltamethrin caused reduction in body weights of albino rat's also significant hematological changes, including reduction in blood parameters like RBCs, WBCs, Hb and lymphocytes which also effects other blood parameters like MCV, HGB, MCH and MCHC level. However elevation in kidney biomarkers ALT, AST and ALP also observed. Study revealed that both *M. oleifera* and *B. spectabilis* plant extracts showed highly significant ($P < 0.01$) results against deltamethrin induced toxicity, compensate these changes and bring these levels to normal range.

Table 1. Table showing changes observed in body weights of rats.

Treatment	Control	T1(M+D)	T2(B+D)	T3 (D)
Initial body weight	272.66 ±2.0548	270.33 ±1.2472	272 ±2.4494	290 ±1.6329
Final body weight	317.3 ±4.1096	284.6 ±2.0548	281.33 ±1.6996	246.66 ±1.2472
Liver(g)	10.566 ±0.5249	8.066 ±0.3399	11.033 ±0.3399	10.4 ±0.1632
Liver index (%)	3.323 ±0.1357	2.826 ±0.0987	3.92 ±0.1451	4.213 ±4.213
Kidney(g)	1.3 ±0.2943	1.033 ±0.1247	1.3 ±0.2943	1.733 ±0.1699
Kidney index	0.35 ±0.0216	0.363 ±0.0418	0.46 ±0.1098	0.696 ±0.0634

M= *Moringa oleifera*, B= *Bougainvillea spectabilis*, D= Deltamethrin

Hematological Parameters

By observing hematological parameters certain changes are observed. In To (Control group) values of all blood parameters are in normal ranges, T3 showed certain elevation and reduction in these parameters, T1 and T2 showed the compensate values of blood parameters by using of anti-oxidants along with pesticide.

Table 2. Table showing blood parameters in control and treated groups of albino rats.

Parameters	To	T1(B+D)	T2(M+D)	T3(D)
RBCs	8.1866 ±1.123	6.02 ±0.3453	8.70 ±0.1800	3.7033 ±0.3324
WBCs	20.3 ±0.9486	25.2 ±1.172	25.4 ±25.4	43.5 ±1.4983
MCV	59.91 ±0.536	52.55 ±0.6388	54.46 ±0.7505	32.1 ±1.5049
HCT	41.74 ±0.4901	39.97 ±0.5969	40.20 ±0.5065	26.25 ±1.1489
HGB	15.76 ±0.4501	12.48 ±1.0210	15.07 ±0.1040	5.788 ±0.4557
MCH	20.13 ±0.7513	18.41 ±0.7051	18.59 ±0.6109	7.58 ±0.8386
MCHC	34.33 ±0.2098	33.18 ±0.6847	30.16 ±0.6300	16.13 ±1.2096
LYM	72.02 ±1.3138	61.96 ±1.1905	61.7 ±0.6337	22.76 ±2.2201
LYM%	61.48 ±0.7873	65.81 ±0.4636	68.13 ±0.7468	81.16 ±1.14389

Values are mean± S. deviation for control and treated groups

Table 3. ANOVA table showing variations in all blood parameters in all control and treated groups.

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	827.2504	1	827.2504	18.60745	0.000	3.906849
Columns	64688.72	8	8086.09	181.8815	0.000	2.003251
Interaction	4151.036	8	518.8794	11.67122	0.000	2.003251
Within	6401.954	144	44.45802			
Total	76068.96	161				

Serological parameters

Induction of deltamethrin caused elevation in certain kidney biomarkers like AST, ALT, ALP, blood urea and creatinine level. Both of these anti-oxidant compensate these changes and bring them at normal level.

Table 4. Table showing serological parameters for control and all treated groups.

Parameters	To	T1(B+D)	T2(M+D)	T3(D)
AST	124.6 ±0.9428	139 ±0.8164	131.3 ±0.4714	1966 ±4.5460
ALT	24.3 ±0.4042	39.06 ±0.3858	28.93 ±0.3091	57.07 ±0.6599
ALP	102.33 ±1.5273	119.6 ±0.4714	116.6 ±1.2472	158.33 ±1.5275
Urea	21.76 ±0.1247	30.2 ±0.4546	22.96 ±0.4109	41.1 ±0.6976
Creatinine	0.693 ±0.01247	1.983 ±0.0169	1.65 ±0.0787	2.163 ±0.00471
TSP	21.76 ±0.1247	1.983 ±0.0169	1.65 ±0.0787	41.1 ±0.6976

Values are mean± S. deviation for control and treated groups

Table 5. ANOVA table showing variations in serological parameters for all groups.

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	7378.829	1	7378.829	55.75643	0.000	3.960352
Columns	273123.6	4	68280.89	515.9489	0.000	2.485885
Interaction	3266.661	4	816.6652	6.170943	0.0002	2.485885
Within	10587.23	80	132.3404			
Total	294356.3	89				

Study revealed that both *M. oleifera* and *B. spectabilis* plant extracts showed highly significant ($P < 0.01$) results against deltamethrin induced toxicity, compensate these changes and bring these levels to normal range. Ethanol extract of *Bougainvillea spectabilis* prevents oxidative stress and myocardial necrosis in albino rat's similar results recommended by (Rakam and Raja, 2018).

Moringa along with deltamethrin restore many changes, *Moringa* improved body weights, function of liver and reduce oxidative stress induced by deltamethrin, compensate level of AST, ALP, ALP, urea and creatinine similar observation shown by (Refaie *et al.*, 2017). This could be due to the anti-inflammatory and antioxidant activities of the *Moringa oleifera* leaves which had been shown to be attributable to the presence of polyphenols and tannins similar studies also reported by (Leon *et al.*, 2015).

Approximately in agricultural sector about 85% of pesticides are used. World health organization depicted about 3 million cases of pesticide poison and approximately more than 250,000 deaths (Yang and Deng, 2007). Deltamethrin is type II pyrethroids which is widely used because of its low accumulation tendency in organisms and short period of biodegradation (Loskowski, 2002). Deltamethrin is fast active neurotoxic pyrethroids and designed chemically to be more toxic to the nervous system of insects. However, deltamethrin initially thought as more safe insecticide due to its less toxicity and less persistent in environment, but the recent studies also shows some off their negative impacts on urinary, respiratory, neurodegenerative, cerebrovascular and hematological diseases (Mani *et al.*, 2014).

Rich source of secondary metabolites and anti-oxidant are plant based so these are used for several for actively control of oxidative stress. Several medicinal plants species are present which have cytoprotective properties. *Moringa oleifera* belongs to family Moringaceae commonly known as drumstick tree or horseradish (Anwar *et al.*, 2005). Along with *M. oleifera* another plant belong to family Nyctaginaceae known as *Bougainvillea spectabilis* having many antilipidemic, anthelmintic, anti-oxidant and anti-diabetic activities. Ethanol extract of *Bougainvillea spectabilis* prevents oxidative stress and myocardial necrosis in albino rats (Rakam and Raja, 2018).

Study was conducted to determined protective effect of plant extracts against deltamethrin induced toxicity in albino rats. Experiment was conducted in Animal House of Food Science and Technology department of

Agriculture University Faisalabad. Experiment carried out for one month and histological changes were determined. 16 healthy male albino rats of about 60 days age with average weight of 250-300g were obtained from animal house of Agriculture University Faisalabad acclimatized for one week and kept under favorable condition at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ temperature. All of these rats divided into 4 groups. Certain changes in body weights, histological parameters and serological changes were observed.

Conclusion

Pesticidal (deltamethrin) induced toxicity caused reduction in body weight and hematological parameters while caused elevation in certain kidney biomarkers like AST, ALT, ALP, urea and creatinine. Studies showed that anti-oxidant properties of both *Moringa oleifera* and *Bougainvillea spectabilis* restored these changes. The obtained results showed that *M.oleifera* proved as strong anti-oxidants rather than *B. spectabilis* due to its anti-inflammatory and anti-oxidants properties.

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