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# Protecive effect of *Curculigo orchioides* on hemato-biochemical alterations induced by cypermethrin subacute toxicity in wistar rats

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**Abstract**
In the present study, protective effect of *Curculigo orchioides* (*CO*) was evaluated against cypermethrin induced hemato-biochemical alterations in male Wistar rats. A total of 24 adult male Wistar rats were divided into four groups of six rats each. Group I (control) received normal diet and water *ad-libitum*, Group II received cypermethrin (12.5 mg/kg bw) OD, orally in distilled water for 28 days, Group III (Treatment 1) received cypermethrin 12.5 mg/kg bw and *CO* ethanolic extract (100 mg/kg bw), orally dissolved in distilled water and, Group IV (Treatment 2) received cypermethrin 12.5 mg/kg bw and *CO* ethanolic extract at the dose of 200 mg/kg bw orally for 28 days. From the results of the study it is observed that cypermethrin exposure caused significant ($P \leq 0.05$) decrease in Hb, PCV and TEC values and treatment group showed significant ($P \leq 0.05$) improvement in Hb, PCV and TEC values. MCV, MCH, and TLC values were increased significantly ($P \leq 0.05$) in cypermethrin treated rats whereas *CO* showed restoration to normal MCV, MCH, MCHC and TLC values as compared to control group rats. The serum AST, ALT, ALP and creatinine increased significantly ($P \leq 0.05$) in cypermethrin alone treated group, however, in *CO* treatment groups AST, ALT and ALP values seen comparable to normal levels. In conclusion the findings of present study indicated that cypermethrin showed significant alterations in the hematobiochemical parameters in male Wistar rats. Thus in conclusion the extract of *CO* treatment efficiently protected the cypermethrine induced hematobiochemical alterations in Wistar rats.

**Keywords:** *Curculigo orchioides*, cypermethrine, hematobiochemical, Wistar rat

## Introduction
Exposure to pesticides is a proven factor in impairment of hepatic, renal and reproductive function in animals including humans. Pesticides are persistent organic pollutants, which causes hematobiochemical alteration in human. Cypermethrin is a synthetic pyrethroid insecticide extensively used as an ectoparasiticide in animals, agriculture and public health programmes [1]. As per WHO it is classified as Class II category of moderately hazardous chemicals [2]. Although considered nontoxic to mammals, several reports indicated the adverse effect of cypermethrin on hepatic and renal system [3]. They accumulate in biological membranes stimulate reactive oxygen species resulting in oxidative damage in mammals [4]. *Curculigo orchioides* (*CO*), Gaertn. (family Amaryllidaceae) known as Kali Musli, used as a male sexual tonic in Ayurvedic system of medicine. It is a well-known herb in traditional Indian system of medicine and has been designated as a Vajikaran Rasayan [5]. In ancient text it is reported to rejuvenate physiological functions and enhance metabolism. Pharmacological studies showed that it has marked hepatoprotective, anti-oxidant, adaptogenic, anti-inflammatory, anticonvulsant, sedative, androgenic and immune-promoting activities [6] [7]. From the literature reviewed no substantial work has been reported on protective effect of *CO* on cypermethrin induced subacute toxicity.
Therefore, in present study the protecive effect of *Curculigo orchioides* was investigated on hemato-biochemical alterations induced by cypermethrin subacute toxicity in Wistar Rats.

## Materials and Methods
### Animals
Twenty four male rats of 9-10 weeks of age weighing 130-180 g were procured from National Institute of Bioscience, Pune. Before to start experiment all experimental animals were acclimatized for a week to the new environment under identical managemental and hygienic

condition with *ad-lib* feed and water in laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola. The experimental protocol on laboratory animals was approved from IAEC (312/CPCSEA) PGIVAS, Akola.

**Extract**

*Curculigo orchoides* 60 % ethanolic root extract (cold) was used in this study. The roots of *C.O.* were obtained from local supplier and the roots were authenticated from expert taxonomist Dr. S. P. Rothe, Principal, Maherbanu College of art and Sciences, Akola. The freshly prepared root powder (100 g) was immersed in 400 ml hydro-alcoholic solution (40% distilled water + 60% ethanol) in a flask stoppered with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The contents of the flask were filtered through Whatman No.1 filter paper and was kept for evaporation of solvent at room temperature (36-38$^0$C). The semisolid extract obtained was stored in airtight screw cap vials and kept in desiccator until further use.

**Test Chemicals**

The technical grade cypermethrin was procured from M/s. Ganesh Chemical Industries, District-Valsad, Gujarat (India).

**Experimental Protocol (Sub acute toxicity study, OECD 407)**

In the experimental study were divided equally into four groups and received treatment as follows

| Group | Particulars | Treatment | No of days |
|---|---|---|---|
| I | Healthy Control | Normal saline | 28 days |
| II | Cypermethrin | Cypermethrin 12.5 mg/kg bw p.o. once daily. | 28 days |
| III | Cypermethrin + *C.orchioides* | Cypermethrin 12.5 mg/kg bw + *C.O.* 100 mg/kg bw ethanolic extracts once daily. | 28 days |
| IV | Cypermethrin + *C. Orchioides* | Cypermethrin 12.5 mg/kg bw + *C.O.* 200 mg/kg bw ethanolic extracts once daily. | 28 days |

**Parameter studied**

**Biochemical**

Biochemical parameters viz. AST, ALT, ALP and creatinine were estimated using AGD Diagnostic kits supplied by AGD Biomedicals Pvt.Ltd., Akola on Electrolyte Auto analyzer AGD3100.

**Hematological**

For hematobiochemical estimation blood sample from six rats of each group were collected from the inner canthus of the rats at the end of 4$^{th}$ week of experiment. Hematological parameters viz. Hb, PCV, TEC, TLC, MCV, MCH, MCHC were estimated as per the standard methods [8] using hemato-autoanalyser.

**Gross and histopathology**

Six rats from each group were sacrificed at the end of 4$^{th}$ week of experiment. Detailed necropsy examination was conducted on each rats and gross pathological observations were recorded. The tissues of liver, kidney, and brain were collected in 10% neutral buffer formalin solution for histopathological examination as per the method described by Luna [9].

**Statistical analysis**

The data of the present research work was analyzed as per the method of Snedecor and Cochran [10].

**Results and Discussion**

The per cent extractability of *Curculigo orchioides (CO)* roots was found to be 8.3%. The haematological values related to erythrocytes were analysed (Hb, PCV, TEC, MCV, MCH and MCHC) in different groups at the end of 4$^{th}$ week. The mean values of Hb, PCV and TEC showed significant ($P$≤0.05) differences between treatment and control group rats. Results showed remarkable decrease in Hb, PCV and TEC values in rats treated with cypermethrin alone as compared to controls. The *CO* treated groups III and IV showed dose dependant and significant ($P$≤0.05) improvement in Hb, PCV and TEC values as compared to cypermethrin control group rats. MCV, MCH, and TLC values were found to be increased significantly ($P$≤0.05) in cypermethrin alone treated rats. The MCHC values of cypermethrin control group also increased numerically among different groups. The MCV, MCH, and TLC values from *CO* (100 mg/kg bw) and cypermethrin combination group found to differ significantly from group I and Group II. However, *Curculigo orchioides* (200 mg/kg bw) and cypermethrin combination treated rats showed restoration in MCV, MCH, MCHC and TLC values as compared to cypermethrin control group rats.

**Table 1:** Haematological values related to erythrocytes and leucocytes in different groups (n =6)

| Groups | Hb (g/dL) | PCV (%) | TEC ($10^6$/cu mm) | MCV (fL) | MCH (Pg) | MCHC (%) | TLC ($10^3$/cu mm) |
|---|---|---|---|---|---|---|---|
| I | $12.36^{a}$ ±0.36 | $40.17^{a}$ ±1.47 | $7.67^{a}$ ±017 | $52.38^{b}$ ±1.36 | $16.92^{c}$ ±0.60 | 32.39 ±1.34 | $10.76^{d}$ ±0.28 |
| II | $8.73^{c}$ ±0.25 | $25.67^{c}$ ±1.43 | $3.92^{d}$ ±0.17 | $65.46^{a}$ ±1.79 | $22.42^{a}$ ±0.79 | 34.41 ±1.64 | $21.48^{a}$ ±1.16 |
| III | $10.66^{b}$ ±0.18 | $34.50^{b}$ ±1.20 | $5.42^{c}$ ±0.21 | $64.37^{a}$±4.22 | $19.82^{b}$ ±0.77 | 31.11 ±1.24 | $17.61^{b}$ ±0.40 |
| IV | $11.13^{b}$ ±0.19 | $36.67^{ab}$ ±1.20 | $7.02^{b}$ ±0.17 | $52.21^{b}$ ±0.50 | $11.42^{d}$ ±0.68 | 30.54 ±1.16 | $13.60^{c}$ ±0.26 |
| Significance/NS | * | * | * | * | * | NS | * |
| CD (0.05) | 0.754 | 3.930 | 0.529 | 7.097 | 2.111 | - | 1.898 |

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels *$P$≤0.05, NS= Non significant

The mean serum total protein, mean albumin and mean values of serum globulin of experimental treated rats decreased significantly ($P$≤0.05) in cypermethrin alone and also in *CO* combination groups ( group III and IV ) when compared with control group. The significant ($P$≤0.05) decreased total protein, albumin and globulin was recorded in cypermethrin control group II rats. However, in the treatment groups *CO* (200mg/kg) and cypermethrin combination treatment group showed least decrease in mean values of serum protein, albumin and globulin as compared to controls. The mean

serum A:G ratio in control and different treatment groups at the end of 4th week found to be non-significant.

**Table 2:** Serum total protein, Albumin, Globulin, A: G ratio in different treatments groups at the end of 4th week (n =6)

| Groups | Total protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) | A:G Ratio |
|---|---|---|---|---|
| I | $7.49^{a}$±0.14 | $3.84^{a}$±0.06 | $3.65^{a}$±0.09 | 1.05±0.02 |
| II | $6.19^{c}$±0.20 | $3.04^{c}$±0.05 | $3.15^{b}$±0.19 | 0.98±0.06 |
| III | $6.52^{c}$±0.07 | $3.32^{b}$±0.08 | $3.20^{b}$±0.07 | 1.10±0.07 |
| IV | $6.94^{b}$±0.07 | $3.74^{a}$±0.05 | $3.21^{b}$±0.12 | 0.93±0.12 |
| Significance/NS | * | * | * | NS |
| CD (0.05) | 0.383 | 0.181 | 0.369 | - |

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels * $P \leq 0.05$, NS= Non significant

The mean values of serum AST, ALT and ALP in different treatment groups differ significantly ($P \leq 0.05$) from control group. Remarkable and significant ($P \leq 0.05$) increased AST, ALT, ALP and creatinine were observed in *CO* treated rats (group III and IV) as compared to group II. Observations indicated significant ($P \leq 0.05$) improvement in serum AST, ALT and ALP level in cypermetherin and *CO* combination treated rats (group III and IV) as compared to group II. In these combination treatment groups these serum biochemical values indicated retrieval towards normalcy. At the end of 4th week of experiment, significantly increased ($P \leq 0.05$) serum creatinine was observed in group II (4.29±0.09) and group III (3.61±0.07) with respect to control group rats (3.11±0.01). The rats treated with cypermethrin alone indicated severe nephrotoxicity and *CO* treatment group indicated significant ($P \leq 0.05$) restoration of creatinine values in Wistar rats.

**Table 3:** Serum AST, ALT, ALP and creatinine in different treatments groups (n =6)

| Groups | AST(IU/L) | ALT(IU/L) | ALP(U/L) | Creatinine (mg/dL) |
|---|---|---|---|---|
| I | $35.01^{d}$±0.31 | $40.22^{d}$±0.49 | $34.28^{d}$±0.58 | $2.92^{d}$±0.04 |
| II | $43.39^{a}$±0.44 | $51.88^{a}$±0.36 | $46.30^{a}$±0.36 | $4.29^{a}$±0.09 |
| III | $40.49^{b}$±0.47 | $48.75^{b}$±0.58 | $38.78^{b}$±0.31 | $3.61^{b}$±0.07 |
| IV | $37.01^{c}$±0.47 | $45.06^{c}$±1.05 | $36.98^{c}$±0.28 | $3.11^{c}$±0.01 |
| Significance/NS | * | * | * | * |
| CD (0.05) | 1.220 | 1.985 | 1.172 | 0.181 |

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels *$P \leq 0.05$, NS= Non significant

In histopathological observations the section of liver of cypermethrin treated rats showed alteration of perivascular lymphoid aggregation, increased sinucidal spaces, focal areas of necrosis, vacuolar and granular changes in hepatocytes and blood vessels congestion. The *CO* (200mg/kg) treatment normalizes histological changes in liver architecture induced by cypermethrin. Section of kidney from group II cypermethrin treated rats showed glomerular degeneration, increase in urinary/glomerular space, vacuolar and granular changes in tubular epithelium and intertubular hemorrhages. *Curculigo* extract group IV showed comparatively normal renal parenchyma. Sections of testes from cypermethrin alone treated rats showed necrosis of sertoli cells, arrested stages of spermatogenesis, degenerative changes in seminiferous tubules and in leydig cells whereas *CO* treated rats showed comparatively normal histoarticular structure. Section of the brain from group II cypermethrin treated rats showed mild to moderate neuronal degeneration in the cytoplasm of the brain and increase in Virchow-Robin spaces while *C.O.* treated rats showed comparatively normal parenchyma.

Our findings are in agreement with Abdou and coworkers [11] and Sahar *et al.,* [12] reported significant increase in liver serum enzymes in male rats given cypermethrin 12.5 mg/kg bwt. Aminotransferases are intracellular enzymes considered as specific indicators of hepatocellular necrosis. AST is present in both cytosol and mitochondria while, ALT is found primarily in the cytosol of the hepatocytes which is considered as more sensitive indicator of liver cell injury than AST [13]. As AST is mainly a mitochondrial enzyme, significant increase in AST activity due to cypermethrin treatment in the present study could be correlated to hepatic damage. Due to damage to liver cells, these enzymes are released into the circulation. The results thus suggested that administration of cypermethrin induced degenerative changes in liver due to an increased permeability of cell membrane leading to leakage of these enzymes into the circulation and ultimately increasing levels of these enzymes The increase in these enzymes in cypermethrin treated male rats may be due to liver dysfunction with alteration in the permeability [14].

From the results it is concluded that administration *Curculigo orchioides* hydroethanolic extract showed significant protective effect against hematobiochemical alterations in cypermethrin induced subacute toxicity in Wistar rats which might be due different phytochemicals like cuculignin A and curculigol producing free radical scavenging effect.

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