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# Olive oil ameliorates the serum biochemical and haematological changes induced by cypermethrin

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**Abstract**
The use of synthetic pyrethroids like cypermethrin has been on a rise in agriculture and other areas of pest control. Though moderately safer than other classes of pesticides pyrethroids are toxic to living beings. Recent studies reveal oxidative stress as the causative factor of the adverse effects by many pesticides. Hence olive oil, a well known antioxidant was chosen in the study to counter act the effect of cypermethrin. Twenty four adult female *Wistar* rats were taken and made into four groups namely control, CYP, OO and CYP+OO. CYP group rats were administered CYP @ 25mg/kg b.wt., OO group rats were administered OO @ 6g/kg b.wt and CYP+OO group rats were administered CYP @ 25mg/kg b.wt and OO @ 6g/kg b.wt. daily for 28 days. Animals were sacrificed on the 28$^{th}$ day of experiment and blood was collected for analysis. Significantly lower values of total erythrocyte count (TEC) and haemoglobin (Hb) were recorded in CYP group in comparison to control, OO and CYP+OO groups. Packed cell volume (PCV) values were significantly lower than control and OO group and non significantly lower than CYP+OO groups. Significantly higher total leucocyte count (TLC) values were recorded in CYP group compared to that of control, OO and CYP+OO groups.
Total protein and serum albumin were significantly reduced, while alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine were significantly increased in CYP group compared to that of control and OO groups. CYP+OO displayed significantly higher values of total protein, significantly lower values of ALT, ALP and creatinine and non significantly higher values of albumin and non significantly lower values of AST and BUN compared to CYP group.

**Keywords:** DHPEA-EDA, hydroxytyrosol, oleocanthal, oleuropein, olive oil, oxidative damage of RBC

## 1. Introduction
Pesticide residues have become a component of the environment in the present scenario owing to their irrational and extensive use. They are omnipresent including water, air and soil. Cypermethrin (CYP) is one of the most commonly used pyrethroid pesticides (Yadav and Devi, 2017) [17]. It belongs to the class II category of pesticides based on hazard classification (WHO, 2020a). In India, CYP is registered by Central Insecticides Board and Registration Committee (CIBRC) for use in brinjal, cotton, sugarcane, rice, sunflower, cabbage, okra and wheat crops (Chandra Bhushan *et al*., 2013) [3]. CYP is highly used in households, agriculture, veterinary and public health. Like other pesticides, CYP remnants were also detected in human and animal food (Neskovic *et al*., 2013) [9]; soil and water (Choudhary *et al*., 2018) [4].
CYP has caused many health hazards which resulted into physiological impact, neurotoxicity, molecular toxicity, haematotoxicity, immunotoxicity and hepatotoxicity (Das *et al*., 2016 and Sharma *et al*., 2018) [5, 15]. CYP has caused significan-($p$<0.05) decrease in total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV) and a significant ($p$<0.05) increase in total leucocyte count (TLC) (Poonam *et al*. 2019) [12]. It also caused a significant ($p$<0.05) reduction in serum total protein (TP) and albumin (ALB), while there was a significant ($p$<0.05) increase in serum urea and creatinine (CRT), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) (Oladele *et al*. 2020; Poonam *et al*. 2019) [10, 12].
Several studies have been made using many natural agents to alleviate CYP toxicity. These include garlic extract, vitamin C, zinc (Zn), green tea extract, moringa tea, vitamin E, *Withania somnifera* and resveratrol, extract of *Bersama engleriana* leaves and sesame oil. Olive oil known as liquid gold having potent anti-inflammatory, antimicrobial and antioxidant properties (Ray *et al*., 2015) [13] was chosen in the present study to mitigate the oxidative stress caused by CYP.

Olive oil (OO) is derived from *Olea europaea* L. *sativa* of family Oleaceae, genus Olea, popularly known as olive tree (Ray *et al*., 2015) [13]. Physical and chemical processing of olive fruit gives OO.OO is proven to have beneficial effects in healing of wounds, heart diseases and tumour therapy Phenolic compounds in OO like oleuropein, hydroxytyrosol and oleocanthal have antimicrobial, antioxidant and sedative properties respectively (Yousefi *et al*., 2018) [18]. Hydroxytyrosol and oleuropein have an orthodiphenolic (contains hydroxyl groups) structure which donates phenolic hydrogen to free radicals and is responsible for their antioxidant properties (Jovanovic *et al*., 1996 and Ray *et al*., 2015) [7, 13].

## 2. Materials and Methods

A total of 24 adult female *Wistar* albino rats weighing between 200-250 g were procured from M/S Jeeva Life Sciences Ltd., Hyderabad. The rats were housed in solid bottom polypropylene cages at the laboratory animal house facility, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar and were maintained in a controlled environment (22 to 24 °C) during the experiment. All the experimental animals were closely observed thrice daily for clinical signs and mortality, if any, during the entire period of study. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (8/24/C.V.Sc., Hyd, IAEC Rats/12.06.2021). CYP was procured from a retail pesticide store in Hyderabad under the t-rade name Vivacyp 10 EC, manufactured by Vi-va Crop Science India, Sonipat, Haryana.

The rats were divided into four groups of six animals each. The group 1 rats served as control, CYP group rats were given CYP orally at the rate of 25mg/kg b.wt./day, OO group rats were given OO at the rate of 6g/kg b.wt./day and CYP+OO group rats were given CYP orally at the rate of 25mg/kg b.wt./day and OO at the rate of 6g/kg b.wt./day for 28 days.

The animals were sacrificed on the 28$^{th}$ day of the experiment. The blood samples were used for estimation of Total Erythrocyte Count (TEC, millions/µL), Total Leucoc yte Count (TLC, thousands/µL), Haemoglobin (Hb, g/dL) con centration and Packed Cell Volume (PCV, %) by using automatic whole blood analyser (ABX Micros ESV 60 manufactured by HORIBA Ltd., Kyoto, Japan).

The blood was also collected into a plain serum vacutainer and allowed to clot for 3-4 h, later centrifuged (REMI COOLING CENTRIFUGE C 24 BL, a be nchtop laboratory centrifuge, India) at 4500 rpm for 10 minutes (min), serum was separated into Eppendorf tubes and stored at 20 °C. The stored samples were used for serum biochemistry analysis by using Erba Mannheim biochemical k its (Transasia Biomedicals Ltd., Solan, Himachal Pradesh, India).

## 3. Results and Discussion

### 3.1 Haematological Parameters

TEC, Hb and PCV were significantly ($p$<0.05) reduced in CYP treated rats compared to that of control. This is in accordance with the findings of the Poonam *et al*. (2019) [12] and in disagreement with the findings of Berroukche *et al*. (2017) [2] where latter observed no significant changes in TEC, Hb and PCV. Significantly ($p$<0.05) higher values of TLC were recorded in the CYP group compared to that of the control group. This is in agreement with that of Poonam *et al*. (2019) [12] and Berroukche *et al*. (2017) [2]. Decrease in TEC could be due to direct damaging effect of CYP on erythrocyte precursor cells in bone marrow (Assayed *et al*., 2010) [1], nephrotoxicity of CYP hypothetically resulting in decreased production of erythropoietin and oxidative damage to erythrocytes (Gabbianelli *et al*., 2002) [6] by CYP, hypothetically resulting in their extra vascular destruction in spleen. Low PCV might be due to decreased TEC. Decreased haemoglobin might be due to inhibition of enzymes involved in the haeme synthesis or could be due to decreased availability of iron as CYP has damaging effect on mucosa of GIT. Inflammatory action of CYP, activating the immune system and thus increasing the recruitment of leucocytes might have resulted in higher TLC count.

TEC and Hb were significantly ($p$<0.05) higher while PCV was non significantly ($p$>0.05) higher in CYP+OO group compared to that of CYP. This might be due to protective action of OO on kidneys against damage caused by CYP. OO also contains a compound dihydroxy phenyl ethanol-elenolic acid dialdehyde (DHPEA-EDA) which protects erythrocytes from the oxidative damage (Paiva-Martins *et al*., 2010) [11]. OO is a good source of iron and thus helps in synthesis of haeme. All these features of OO might have resulted in positive response on haemogram. Oleocanthal is an antiinflammatory agent present in OO which might have counteracted the CYP induced inflammation and thus reducing the TLC count. Haematological parameters in control and OO group were within the range and comparable with each other.

### 3.2 Serum Biochemical Parameters

Significant ($p$<0.05) decrease in total protein and Serum albumin was observed in CYP group compared to that of control. There was a significant ($p$<0.05) increase in the values of serum ALT, AST, ALP, BUN and CRT of CYP group compared to that of control. This is in agreement with that of Oladele *et al*. (2020) [10] and Poonam *et al*. (2019) [12]. It was in contradiction to findings of Mansour *et al*. (2018) [8] where they observed significant ($p$<0.01) reduction in BUN and Sankar and Ramya (2017) [14] where they observed non significant reduction in total protein. CYP causes inflammation and oxidative damage in liver leading to fibrosis thus affecting the protein manufacturing machinery of liver.

This might have resulted in lower protein values. Hepatocytes are directly damaged by ROS and LPO products generated by CYP which results in the leakage of cellular enzymes like ALT, AST and ALP into the extracellular fluid. Increase in BUN and CRT reflects the decreased nephron activity. This might be attributed to the damage caused by CYP to the functional units of kidneys thus, decreasing the extent of filtration apparatus available.

Total protein levels in CYP+OO group were significantly ($p$<0.05) higher when compared to that of CYP group rats. No significant ($p$>0.05) improvement was noticed in albumin levels in CYP+OO group compared to that of CYP group rats. ALT, ALP and CRT were significantly ($p$<0.05) lower in CYP+OO group compared to that of CYP, whereas AST and BUN were non significantly ($p$>0.05) lower in CYP+OO group compared to that of CYP group rats. This might be due to the protective action of olive oil on liver and kidney. Serum biochemical parameters in control and OO groups were normal and comparable with each other.

**Table 1:** Haematological parameters

| | Control | CYP | OO | CYP+OO |
|---|---|---|---|---|
| TEC (millions/µL) | 6.95 ± 0.02[a] | 6.75 ± 0.02[b] | 6.91± 0.03[a] | 6.85 ± 0.008[b] |
| Hb (g/dL) | 14.52 ± 0.12[a] | 13.88 ± 0.07[b] | 14.58 ± 0.05[a] | 14.23 ± 0.04[b] |
| PCV (%) | 46 ± 0.36[a] | 43.25 ± 0.11[b] | 46.17 ± 0.40[a] | 44.33 ± 0.21[c] |
| TLC (thousands/ µL) | 9917 ± 10.5[a] | 17417 ± 11[b] | 10700 ± 8.00[a] | 13317 ± 9.23[b] |

Values are mean ± SE (n=6); One way ANOVA
Means with different superscripts in a row differ significantly at $p<0.05$

**Table 2:** Serum biochemical parameters

| | Control | CYP | OO | CYP+OO |
|---|---|---|---|---|
| Total protein (g/dL) | 7.33 ± 0.12[a] | 6.13 ± 0.19[b] | 7.29 ± 0.10[a] | 7.00 ± 0.08[a] |
| Serum albumin (g/dL) | 3.1 ± 0.15[a] | 2.34 ± 0.23[b] | 3.04 ± 0.18[a] | 2.73 ± 0.08[c] |
| ALT (IU/L) | 13.80 ± 0.26[a] | 27.65 ± 0.5[b] | 12.76 ± 0.76[a] | 21.10 ± 1.18[b] |
| AST (IU/L) | 26.84 ± 2.91[a] | 61.63 ± 2.70[b] | 24.51 ± 2.86[a] | 52.35 ± 2.92[c] |
| ALP (IU/L) | 67.92 ± 2.60[a] | 67.92 ± 2.60[b] | 31.26 ± 1.40[a] | 48.91 ± 1.28[b] |
| BUN (mg/dL) | 20.89 ± 1.50[a] | 20.89 ± 1.50[b] | 14.21 ± 1.40[a] | 20.08 ± 0.45[c] |
| Creatinine (mg/dL) | 0.421 ± 0.02[a] | 0.421 ± 0.02[b] | 0.127 ± 0.01[a] | 0.207 ± 0.01[b] |

Values are mean ± SE (n=6); One way ANOVA
Means with different superscripts in a row differ significantly at $p<0.05$

## 4. Conclusions

CYP, a pyrethroid pesticide is increasingly deployed in the agriculture, household and veterinary fields owing to its ability to efficiently kill pests. However, CYP poses threat to living beings either through direct contact or as residue in food and water. The present study shows the detrimental effect of CYP on blood, liver and kidneys by causing oxidative and inflammatory damage. The above results suggest that OO has promising effect against oxidative stress caused by CYP due to the presence of compounds like hydroxytyrosol, oleuropein, oleocanthal etc. Further studies have to be made to determine the potential antioxidants in food to overcome the effect of pesticides residues.

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