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# A potential effect of eucalyptus and clove extracts against toxicity induced by sub-acute exposure of thiacloprid in rats

**Sunil Boghia, Mahender Miland Lakeshar, Vachaspati Narayan and Shwetaanad**

**Abstract**
The present study was carried out to evaluate ameliorating potential of extracts of Eucalyptus, Clove and it's combination (1:1) against toxicity induced by sub-acute exposure of thiacloprid in rats. The main clinical signs observed was piloerection, decreased motility and reactivity, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnea, dyspnea, labored breathing, diarrhoea, narrowed palpebral fissure, closed eyelids and red incrusted snout sub-acute intoxication induced decrease body weight, absolute organ weight and relative organ weight of liver, kidney, spleen respectively which were significantly restored by eucalyptus and clove extract. Moreover, there was significant decrease in TEC, Hb, PCV, MCH, DLC (Neutrophil, lymphocyte) values while TLC increased in thiacloprid treated rats. On the other hand eucalyptus, clove and their combination increase these haematological values except TLC. There was a significant increase in serum biochemical parameter related to hepatic and renal injury like ALT, AST, LDH and creatinine which were restored by eucalyptus, clove and their combination (1:1). Thiacloprid treatment resulted in oxidative stress by increased LPO and decreased GSH, SOD, Catalase levels as compared to control animals which was normalized by Eucalyptus, Clove, combination of eucalyptus and clove co-treatment with thiacloprid partially. Histopathological findings revealed that thiacloprid produced a mild to moderate degenerative changes in liver, kidney, brain and spleen which were minimized by Eucalyptus, clove and combination of eucalyptus and clove co-treated with thiacloprid respectively. In general, it was conducted that eucalyptus and clove are able to protect against thiacloprid induced toxicity.

**Keywords:** Eucalyptus, clove, thiacloprid, sub-acute toxicity

## 1. Introduction
Pesticides are substances that are intended to restraint pests or weeds. The word "pesticide" contains all of the following: herbicide, insecticide, insect evolution regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, produced, bacteriacide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial) and sanitizer. The most common of these are herbicides which account for approximately 80% of all pesticide used (Carolyn Randall *et al*., 2013) [1].
Insecticides are practiced in agriculture, medicine, industry and households. The use of insecticides is considered to be one of the major components behind the increase in agricultural productivity in the 20th century almost all insecticides have the potential to significantly alter ecosystems, many are toxic to humans and others are concentrated in the food chain. Insecticides fall into two categories-inorganic and organic. The organic insecticides are further divided into different groups such as organophosphorus compounds (OP), organochlorine compounds (OC), carbamates (C), pyrethrins/synthetic pyrethroids (SP), neonicotinoids, insect growth regulators (IGR) etc. (Van Emden and Pealall, 1996) [2].
Neonicotinoids are a class of neuroactive insecticides chemically concerned to nicotine. Nicotine is the primary biologically active alkaloid found in tobacco extract and has been used for many years as an insecticide. Thiacloprid is a bio-mimetic insecticide belonging to class of chloro neonicotinoid insecticides. It has a strong contact and stomach poisoning action as well as systemic function. This is a new and rare type of a chloro-neonicotenoid. Half- life period of soil for the thiacloprid is last for 7-21 days which make it harmless for the birds, fish and many kinds of beneficial arthropods because of it's low toxicity to mammals.
Eucalyptus (Myrtaceae), an Australian native, represented by around 700 species is a genus of tall, evergreen and magnificent trees cultivated the world over for its oil, gum, pulp, timber, medicine and aesthetic value.

Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Sellers, C.H 1910) [3].

Eucalyptus is mainly cultivated for the paper and cosmetic industries, while some of them are used in traditional medicine. Certain species of Eucalyptus are even used in modern medicine (Masen AP *et al*., 2000) [4] Many researches were conducted an experiment on the medicinal properties of leaves of eucalyptus were reported to possess antifungal, antibacterial, anti-inflammatory, antioxidant and antihelmintic properties. In addition the beneficial effect of Eucalyptus was demonstrated in rats given toxic doses of thiacloprid However, the therapeutic effect of Eucalyptus against thiacloprid-induced toxicity and oxidative stress has not yet been studied. Therefore, the present study was conducted to evaluate the protective effects of Eucalyptus and clove against thiacloprid induced sub-acute toxicity in rats (Mulyaningsih S. *et al*., 2010) [5].

## 2. Material and Method

### 2.1 Animal model

Adult male albino rats weighing 100-200 gm was used for the study. A total number of 30 rats was used in the study. Rats was housed in polyacrylic cages in a group of 6 rats per cage in the Department of Veterinary Pharmacology & Toxicology. Bedding material (Wheat straw) was changed on alternate days. The animals was provided feed and water ad libitum and maintained at room temperature with a natural light-dark cycle. Rats was acclimatized to laboratory conditions for 7 days before the experiments was conducted.

### 2.2 Herbal extract

**i) Collection of plant materials**

The plant materials was procured from nursery & grocery respectively.

a) Plants used-Eucalyptus & Clove.
b) Plant parts used-leaves and Buds.

**ii) Extraction**

The dried leaves of Eucalyptus and Clove buds was powdered and extract was prepared in rotary vacuum evaporator. The ethanolic extract of eucalyptus leaves prepared according to Azza Dawoud H Dawoud *et al*., (2015) [6] in view of reported highest antioxidant activity. Similarly Clove buds ethanolic extract was prepared.

### 2.3 Toxicological and pharmacological agents

The Thiacloprid (98%) pure was procured from Sigma-Aldrich Company. The Thiacloprid was administered @ 22.5 mg/kg Bwt. The desired concentration of Thiacloprid was made in corn oil. The extract of Eucalyptus and Clove extract was prepared by rotary vacuum evaporator.

### 2.4 Instruments

Various instruments used in the study like refrigerator, centrifuge machine, Rotary vaccum evaporator, weighing balance, Tissue homogenizer, Double beam spectrophotometer was available in the Department of Veterinary Pharmacology and toxicology, CVAS, Navania, Udaipur.

### 2.5 Experimental design

Thirty rats will be randomly divided into five groups (6 rats/group).

- **Group I:** This group serving as control in which corn oil (acting as vehicle of thiacloprid) was administered orally.
- **Group II:** This group serving as Thiacloprid treated group @ 22.5mg/kg B.wt was administered orally.
- **Group III:** In this group thiacloprid along with eucalyptus leaves extract @ 233.4 mg/kg B.wt was administered orally.
- **Group IV:** IN this group thiacloprid along with clove buds extract @ 359.7mg/kg B.wt) was administered orally.
- **Group V:** In this group 50% combination of eucalyptus leaves extract and clove bud extract was administered orally along with thiacloprid.

**Weight**

Body weight of each rat was recorded on day 0 and at an interval of one week till the completion of experiment.

### 2.6 Sample collection

**a) Blood collection:** Experimental rats was anaesthetized 24 hrs after the last treatment/dose. Blood samples was collected directly from the posterior vena cava and heart in tubes containing sodium-EDTA and without sodium-EDTA from all the rats. Serum was separated out and it was stored in -20 °C for various biochemical estimations.

**b) Organ weight:** At the time of sacrifice, vital organs viz. liver, kidney, brain and spleen was excised free from surrounding tissues blotted with tissue paper and examined for gross pathology. All these organs was weighed individually and organ body weight ratio was calculated.

Absolute weight gain (g) = final body weight (g)-initial body weight (g)

Relative organ weight (g% or g/100 gm) = organ weight/body weight X 100

### 2.7 Parameters to be evaluated

#### 2.7.1 Serum biochemical

Serum was separated from the blood and stored at -20 ºC for estimations of the activity of ALT and AST, LDH and serum creatinine. These was estimated by using commercially available diagnostic kits (Span Diagnostics Ltd; India). LDH was estimated by automatic analyzer.

#### 2.7.2 Haematological studies

Effect of thiacloprid, Eucalyptus leaves extract, clove buds extract and their various combinations on various haematological parameters was studied in rats. Blood from individual animal was collected from heart at the time of sacrifice using dry sterilized vials containing anticoagulant, EDTA. Various haematological parameters viz. haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was determined using automated haematology analyzer.

## 3. Results

### General toxicity parameters

### 3.1 Clinical signs

The clinical signs and symptoms which was observed during experimental period of 28 days sub-acute toxicity of thiacloprid and it's amelioration by eucalyptus clove and their combination (1:1), in group 2, 3, 4 and 5 such as, Diarrhoea, dyspnoea, salivation, piloerection and lacrimation except control group.

### 3.2 Body weight

The body weight (g) recorded at weekly intervals and percentage body weight gain calculated after last weighing are presented below:

#### 3.2.1 Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on weekly recorded body weights against toxicity induced by sub-acute exposure of Thiacloprid in male albino rats

Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on weekly recorded body weights against toxicity induced by sub-acute exposure of thiacloprid in rats are presented in the form of mean values of absolute weight (gm) in each group.

#### 3.2.2 Ameliorating potential of eucalyptus, clove and their combination (1:1) extracts on body weight gain & percentage weight gain against toxicity induced by subacute exposure of thiacloprid in male albino rats (mean ± S.E)

Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on body weight gain and percentage weight gain against toxicity induced by sub-acute exposure of Thiacloprid in male albino rats (mean ± S.E) are presented in Fig. (3.2.1) Percentage body weight gain was significantly ($p$<0.05) lower in group 2 as compared to the control group, while in the other groups 3, 4 and 5 percentage weight gain was significantly ($p$<0.05) higher as compared to the group 2.

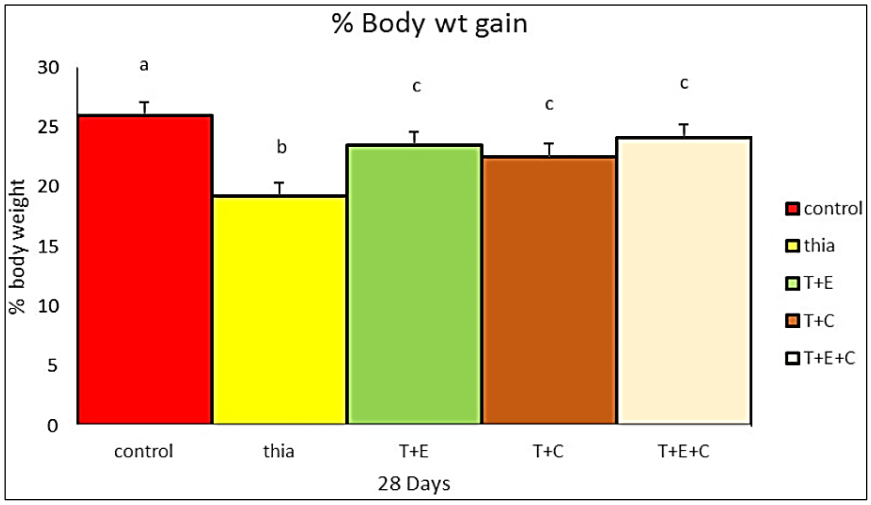


**Fig 1:** Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on body weight gain and percentage weight gain against toxicity induced by sub-acute exposure of thiacloprid in male albino rats (mean ± S.E)

**Table 1:** Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on Relative body weight gain (g/100 g) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats

| S. No. | Group | 28 days |
|---|---|---|
| 1. | Control | 35.5a ± 0.67 |
| 2. | Thiacloprid | 23.16b ± 0.40 |
| 3. | Thiacloprid + Eucalyptus | 30.15ab ± 0.30 |
| 4. | Thiacloprid + Clove | 28.82c ± 1.26 |
| 5. | Thiacloprid + Eucalyptus + clove | 30.33d ± 0. |

#### 3.2.3 Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on absolute organ weights (mg/100g body wt.) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats

The mean values of absolute organs (liver kidney, spleen, brain, lungs, heart, thymus) of male albino rats in all treatment groups is expressed as gm and presented in Fig (3.2.2) respectively statistically significant increase ($p$<0.05) in weight was observed in liver in group 2, 3, 4 and 5 as compared to the control group. Group 3, 4 and 5 lower the liver weight significantly ($p$<0.05) as compared to the group 2 the relative weight gain of kidney was significantly ($p$<0.05) higher in group 2 as compared to the control, while in group 3, 4 and 5 lower the kidney weight significantly ($p$<0.05) was observed as compared to the groups 2. No significant change observed on relative weight of brain ($p$<0.05) in groups 2, 3, 4 and 5. A statically significant ($p$<0.05) decrease was observed in relative spleen weight non-significantly ($p$<0.05) in groups 2, 3, 4 and 5, while in group 3, 4 and 5 higher the relative spleen weight significantly ($p$<0.05) as compared to the group 2. No significant ($p$<0.05) change was observed in the relative weights of thymus, heart and lungs in different treatment groups.

#### 3.2.4 Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on relative organ weights against toxicity induced by sub-acute exposure of thiacloprid in male albino rats

Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on relative organ weights against toxicity induced by sub-acute exposure of thiacloprid in male albino rats and are expressed in (g/100) presented in fig (3.2.3). A statistically significant ($p$<0.05) was higher in liver, and kidney weight was determined in the group 2 as compared to control group. While in groups 3, 4 and 5 significantly ($p$<0.05) lower the liver and kidney weight as compared to group 2, while eucalyptus and clove co-treatment

higher the relative spleen weight significantly ($p$<0.05) as compared to the group treated with thiacloprid alone.

### 3.3 Serum biochemical

#### 3.3.1 Ameliorating potential of extracts eucalyptus, clove and their combination (1:1) on ALT, AST, LDH, (IU/L), creatinine against toxicity induced by sub-acute exposure of thiacloprid male albino rats

Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on ALT, AST, LDH, (IU/L), creatinine against toxicity induced by sub-acute exposure of thiacloprid in male albino rats presented in table (4.3.1) and fig. (4.3.1) respectively. Thiacloprid treatment in group 2, 3, 4 and 5 higher the serum ALT and AST level significantly ($p$<0.05) compared to the control group. Group 3, 4 and 5 lower the ALT and AST values significantly ($p$<0.05) as compared to the group 2. LDH values was also found to be significantly ($p$<0.05) higher in group 2 as compared to control group, while in group 3, 4 and 5 it was lower the elevated LDH level significantly ($p$<0.05) as compared to the group 2. Group 2 also higher the serum creatinine values significantly ($p$<0.05) as compared to group 3, 4, 5 and control.

**Table 2:** Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on ALT, AST, LDH, (IU/L), Creatinine against toxicity induced by sub-acute exposure of thiacloprid male albino rats

| Group | 28 days | | | |
|---|---|---|---|---|
| | ALT (IU/L) | AST (IU/L) | LDH (IU/L) | Creatinine (mg/di) |
| 1. Control | 38.675a ± .47 | 97.31a ± 1.67 | 237a ± 3.67 | 302.21a ± 1.75 |
| 2. Thiacloprid | 79.1d ± .81 | 191.41b ± 3.17 | 288b ± 4.52 | 379.0b ± 1.03 |
| 3. T + EUC | 54.26c ± | 172.70c ± 3.30 | 253.33c ± 2.64 | 364.33c ± 1.80 |
| 4. TH + CL | 58.29c ± 1.76 | 152.84d ± 1.25 | 242.12c ± 2.07 | 366.26d ± 2.95 |
| 5. TH + EUC + CL | 48.62b ± .96 | 153.27d ± 1.37 | 237.90a ± 2.18 | 359.04c ± 0.88 |

### 3.4 Haematological parameters

#### 3.4.1 Ameliorating potential of eucalyptus, clove and their combination (1:1) on haematological parameters (TEC, HB and PCV) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats

Ameliorating potential of extracts of eucalyptus, clove ad their combination (1:1) on haematological parameters (TEC, HB and PCV) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats presented in fig (3.4.1) Group 2 significantly ($p$<0.05) lower the TEC values as compared to control group while in group 3, 4 and 5 higher the TEC values significantly ($p$<0.05) as compared to the group 2. Hb levels was also found to be significantly ($p$<0.05) lower in each groups of thiacloprid treatment 2, 3, 4 and 5 as compared to the control group, while in groups 3, 4 and 5 it was higher the levels non significantly ($p$<0.05) as compared to the group 2. PCV was also found significantly ($p$<0.05) lower in groups 2, 3, 4 and 5 while in group 3, 4 and 5 it was higher the level of PCV significantly ($p$<0.05) as compared to the group 2.

**Table 3:** Ameliorating potential of eucalyptus and clove extracts on haematological parameters (TEC, HB and PCV) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats

| Group | Hematological parameters | | |
|---|---|---|---|
| | TEC ($x10^6$/pL) | Hb (g/dL) | PCV (mm/hr) |
| 1. Control | 6.91a ± 0.11 | 12.88a ± 0.33 | 38.64a ± 0.99 |
| 2. Thiacloprid | 5.62b ± 0.35 | 10.08b ± 0.40 | 30.17b ± 1.23 |
| 3. TH + EUC | 5.95c ± 0.05 | 11.0c ± 0.27 | 32.58c ± 0.82 |
| 4. TH + CL | 6.51 a ± 0.04 | 10.710 ± 0.31 | 32.47c ± 0.94 |
| 5. TH + EUC + CL | 6.72a ± 0.29 | 11.7ac ± 0.50 | 35.17ac ± 1.55 |

## 4. Discussion

### 4.1 Dose of Thiacloprid

The thiacloprid was administered @ 22.5 mg/kg Bwt. The desired concentration was prepared in corn oil the same dose of Thialoprid was used by Birsen Aydin (2011) [31] in rats to generate oxidative stress and other toxicity.

### 4.2 Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on body weight against toxicity against sub-acute exposure of thiacloprid in male albino rats

The weight gain in animals serves as index of growth rate (Palani *et al*., 1999) [8]. It is observable that monitoring of body weight provides information on general health level of animals which can also be an important interpretation of reproductive effects (Aly *et al*., 2009) [7]. A significant reduction in both absolute (g) and relative body weight gain (g/100 g) was observed in the present study in the rats treated with resveratrol and thiacloprid alone in 28 days exposure. Similar findings have been mentioned in the study reports of US EPA (US Environmental Protection Agency) and JMPR (2010) [9], wherein a decrease of body weight gain was reported in male rats given 60 mg/kg/day and 120 mg/kg/day dose of thiacloprid.

Goyal *et al*., (2010) [10] conducted a toxicity study of thiacloprid in the digestive tract of birds, and reported that thiacloprid acts as an irritant to the intestinal membrane, and further opined that this could reduce food consumption. A similar response of the thiacloprid in rat intestine cannot be ruled out and might have resulted in reduced food intake in animals subjected to subacute exposure of thiacloprid. In group 3, 4 and 5 there was significantly increased the weight gain in 28 days trial. This may be due to the hepato-protective effect of eucalyptus and clove or by reduction in protein catabolism caused by thiacloprid.

In present study, reduced body weight gain in eucalyptus administered groups 3 and 5 may be because of anti-obesity effects of eucalyptus and clove on adipocytes in body storage tissues eucalyptus also decreases adipogenesis and viability in maturing preadipocytes, mediated through down-regulating adipocyte specific transcription factors and enzymes and also by genes that modulate mitochondrial function. (Azza H Dawood *et al*., 2015) [6]

### 4.3 Relative organ weight

In toxicological studies, relative organ weights are important criteria for evaluation of organ toxicity (Timbrell, 2000; Carney *et al*., 2004) [32, 35]. Generally in subacute toxicity

studies relative weight of affected organ increases.

**Liver:** A statistically significant increase was observed in weight of liver in thiacloprid treated groups. Our results are in accordance with Bhardwaj *et al*., (2010) [11] where a significant increase in relative liver weight was reported at 20 mg/kg b.wt./day imidacloprid dose. Liver is the main organ of imidacloprid metabolism. Thus, physiologically the liver could be affected directly by thiacloprid during the period of exposure.
In present study, Eucalyptus co-treatment significantly reduced the increased relative liver weight by thiacloprid exposure. Eucalyptus resulted in reduced weight gain by decreasing the levels of hepatic carnitine palmitoyl transferase-Ia and acyl-coenzyme A oxidase (Ribeiro *et al*., 2015) [33]

**Kidney:** Thiacloprid treatment significantly increased the relative kidney weight in comparison to naive. Our findings are in agreement with the study of (Srivastava *et al*., 2006) [12] where a significant increase was observed in the weight of kidney after exposure to liquid mosquito repellent containing allethrin (3.6% w/w). In male rats, long term feeding studies with cypermethrin have shown an increase in kidney weights (Elbeitha *et al*., 2001) [13]. Cypermethrin resulted in alteration in the distribution pattern of oxidoreductase in the kidney of rats. The general loss of oxidoreductases in kidney of rabbits because of cypermethrin toxicity may be suggestive of decreased metabolism of physiological processes due to degenerative changes in various segments of nephron (Bansal *et al*., 2007) [14].
Clove and eucalyptus co-treatment decreased the relative kidney weight in the present study. Our results are in accordance with (Medhat M.J abajid el and S.M., EL-Sayed *et al*., 1981) [36] where clove treatment revealed improvement in kidney weight showing its reno protective nature which is possibly through the anti-oxidant potential of eucalyptus and clove.

**Brain:** In present study, no significant changes was observed in the relative weight of brain indicating thiacloprid as well as eucalyptus have no significant effect on brain.

**Spleen:** Spleen weight was reduced in thiacloprid treated rats as compared to naive. Our results are in agreement with the study of Vohra *et al.*, (2014) [15] where similar results was observed in Swiss albino mice after subacute exposure to imidacloprid.

**4.4 Ameliorating potential of extracts of eucalyptus, clove and their combination (1:1) on Serum biochemical levels against toxicity induced by sub-acute exposure of thiacloprid in male albino rats**
Biochemical analysis performed in our experiment showed that the oral intake of thiacloprid resulted in rise in liver functional enzymes activities in serum of treated animals. The elevation rate was increased significantly with increasing oral intake dose of thiacloprid. Our findings
are in accordance with Hendawi *et al.* (2016) [16] who stated that sub-acute thiacloprid intoxication at 22.5 mg/kg body wt. for 30 days induced a significant increase serum biochemical parameters related to hepatic injury: alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Similarly, a significant increase in ALT, AST and GGT in animals exposed to imidacloprid at 20 mg/kg b.wt./day dose has been reported (Bhardwaj *et al*., 2010) [11].
Qadir *et al.* (2014) [17] studied the effect of imidacloprid on the hematological and serum biochemical profile of fresh water fish *Labeo rohita*. A sub lethal dose of imidacloprid (120 mg/L) was applied under short (2, 4 and 8 days) and long term (16, 32 and 64 days) experimental conditions. Pesticide effect was more pronounced in the short term experiments. There was significant increase in serum ALT and AST enzymes level.
In a separate study, (Mohany *et al.* 2011) [18] reported that oral administration of imidacloprid at 0.21 mg/kg b. wt. for 28 days in male albino rats resulted in elevation of AST, ALT, ALP and MDA levels. Also another study, subacute toxicity of repeated oral administration of imidacloprid in male white Leghorn chicks showed significant increase in AST level at 14 and 28 days of experiment, while no significant change in ALT, total protein, albumin and creatinine was seen (Balani *et al*., 2011) [19].
In the present study, eucalyptus and clove co-treatment with thiacloprid administered rats reduced the serum levels of liver bio-marker enzymes. (Mahdi M. Thuwani *et al*., 2016) [34]. Observed a similar decrease in AST and ALT enzyme activity in the animals that received clove with aqueous extract suggesting that clove had a protective effect on the liver. The administration of eucalyptus has been able to protect against the increased activity of ALT, AST and GGT, thus demonstrating the.

**4.5 Ameliorating potential of extracts of eucalypus, clove and their combination (1:1) on hematological parameters against toxicity induced by sub-acute exposure of thiacloprid in rats.**
The Hb concentration and hematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced. (Suresh *et al*., 2012) [20]. Brar *et al*. (2002) [21] suggested that if the PCV is decreased, the animal is anemic whereas an elevated PCV indicate polycythemia. The decrease in Hb concentration suggested that thiacloprid might have interfered with erythropoiesis. The observed results are in agreement to that of Kaur *et al.* (2005) [22] who reported similar changes after the administration of imidacloprid @ 0.5 mg/kg/day for 150 days in cow calves. Decline in Hb concentration has also been reported by other workers following the exposure to different insecticides in cockerels (Singh *et al.*, 2001) [23], buffalo calves (Sandhu *et al.*, 1991) [24] and rodents (Choudhary *et al.*, 2002) [25]. The fall in Hb concentration on subchronic oral exposure to thiacloprid might have occurred because of the interference with heme synthesis as reported with certain toxins and drugs (Turk *et al.*, 1997) [26]. The increase in leucocyte count during treatment has been attributed to the occurrence of internal haemorrhage (Hassan *et al.*, 1988) [29] and to the effects on bone marrow and/or the pituitary adrenal system. Marked leukocytosis has been reported in cow calves (Thaker *et al.*, 1988) [27] and buffalo calves (Sandhu *et al.*, 1991) [24] following long term administration of imidacloprid, phosphamidon and triazophos, respectively. However, Thaker *et al.* (1988) [27] did not observe any significant alteration in TLC in white leghorn chicks by long term daily oral administration of endosulfan and Malathion.
Our findings showed a significant decrease in RBC, Hb, PCV and MCV, whereas a significant increase in ESR, TLC, neutrophils and lymphocytes was observed. The findings of

this study are in agreement with those of Singla *et al.* (2015) [34] who reported a significant decline in values of PCV, Hb, TEC and significant increase in ESR and TLC in *Gallus domesticus* on thiacloprid administration @ 1 mg/kg/day for 90 days.

## Conclusion

In conclusion, it is suggested that thiacloprid possesses mild to moderate toxicity potential for liver, kidney, brain and spleen in male Albino rats. Eucalyptus and clove both possesses potential to sufficiently ameliorate the toxicity produced by thiacloprid and as such do not have any toxic effect at therapeutic doses in male Albino rats. The exact mechanism of action of acute and subacute toxicity of thiacloprid is not fully unspoken as yet but it can be hypothesized that it produces multiorgan toxicity by enhancing oxidative stress, endocrinal disruption, recruitment of neutrophils and lymphocytes, lipid peroxidation, decreasing antioxidant status and damaging histological structures along with change in hematological profile.

So, it is hypothesized that eucalyptus and clove extracts ameliorate toxicity of thiacloprid by virtue of its antioxidant activity at cellular and molecular levels. The present study suggests the necessity of a further detailed investigation of Eucalyptus and clove extracts for the development of a medicine against oxidative stress inducing neonicotinoids like thiacloprid. Further studies need to be done to find the exact pathway of thiacloprid toxicity and to confirm the findings with other models of toxicity.

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