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Research article

Pharmacological evaluation of essential oils of *Ocimum sanctum*, *Prunus persica* and *Zingiber officinale*

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Abstract

The present study comprises pharmacological trials with the essential oils of *Ocimum sanctum*, *Prunus persica* and *Zingiber officinale,* which have been found to exhibit a fungitoxic effect on plant pathogenic fungi. Analysis of the blood and serum of albino rats fed with an oil-supplemented diet for sixty days did not reveal significant variations in the levels of hemoglobin, total blood glucose, protein, cholesterol, urea, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase nor in differential leucocyte counts, in comparison to rats fed with a normal rat diet. These essential oils thus can be recommended as safe antifungal agents because the oils did not show any adverse alteration on different pharmacological parameters that have been observed in the present piece of work.

Key words: *Ocimum sanctum*, *Prunus persica*, *Zingiber officinale*, fungitoxicity, animal toxicity, pharmacology, essential oils

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1. Introduction

Plant essential oils in general have been recognized as an important natural source of pesticides. The volatility, ephemeral nature and biodegradability of essential oils make them especially advantageous in exploitation as pesticides [1]. These specific characters of essential oils have regenerated interest for their formulation as an alternative source of agrochemicals over the synthetic ones. These are volatile natural complex secondary metabolites characterized by a strong odor and have a generally lower density than that of water [2, 3]. At present, approximately 3000 essential oils are known, 300 of which are commercially important, especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries [3] apart from pesticidal potential [4, 5].

However, recently there are some reports on side effects on respiratory tracts or skin allergy with some of the natural pyrethroids [6]. Therefore, evaluation of safety limit of a product is essential if it is recommended for commercial application in plant protection. Although, there are many reports on *in vitro* fungitoxic properties of essential oils, but reports on

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their *in vivo* efficacy and pharmacological evaluation is very limited.

The essential oil of *Ocimum sanctum, Prunus persica* and *Zingiber officinale* has been found to possess prominent biological activity against different plant pathogenic fungi [7]. However, the safety limit profile of these essential oils has not yet been reported. The present piece of work deals with pharmacological investigations with the essential oil of *O. sanctum, P. persica* and *Z. officinale* so as to find out their safety limits if recommended as botanical pesticides.

2. Materials and Methods

Animals

A total of 24 adult healthy male albino rats (Charles Foster Strain) weighing 125 ± 10g were used in the present study. These animals were kept in polypropylene cages (3 rats per cage) under identical animal house conditions and were fed standard rat feed (Hindustan Lever Ltd, India) and water *ad libitum.*

The rats of cage i and ii were fed only with standard rat feed and were served as control sets. Rats of cage iii and iv were given the feed fumigated with the essential oil of *O. sanctum* (200 ppm) while rats of cage v and vi received a feed fumigated by the essential oil of *P. persica* (100 ppm) and cage vii and viii were given the feed fumigated by the essential oil of *Z. officinale* (100 ppm).

Isolation of essential oils

The essential oils were isolated from the leaves of *O. sanctum, P. persica* and from rhizomes of *Z. officinale* by hydrodistillation. 250 g of fresh leaves/ rhizomes were cut into small pieces and then thoroughly washed with sterilized water. The essential oil was isolated by Clevenger's apparatus. The isolated fraction showed two distinct layers-an upper oily layer and a lower aqueous layer. Both the layers were separated and the moisture from the oily layer was removed by adding anhydrous sodium sulphate.

Fumigation of rat feed

The fumigation of the rat feed was done following procedures of Shaaya *et al*. [8] Varma and Dubey [9]. The animal diet was fumigated with a lethal concentration of the above mentioned oils separately in closed plastic containers. A piece of cotton soaked with 0.1 ml, 0.5 ml and 0.2 ml of *Ocimum, Prunus* and *Zingiber* oils respectively was kept in a plastic container (volume 1000 ml) containing 500 gm of animal feed. The rats of control and treatment groups were fed with requisite diet 75 gm/cage/day up to 60 days. The animals were observed continuously for physiological and behavioral responses.

Sub acute toxic experiments on rats

After 60 days, the animals were sacrificed and their blood was collected in different vials.

Serum collection

At the end of 60 days blood from each rat was collected by cardiac puncture. The serum was separated by centrifugation at 2500 rpm for 10 minutes and stored at -4° C.

Blood analysis

A small amount of blood was taken in separate vials containing EDTA and used for the determination of total and differential WBC count and hemoglobin percentage using standard procedures.

Biochemical analysis

The biochemical analysis was done following Tewari *et al.* [10]. The serum

separated from the blood was used to analyze the levels of total protein and albumin, cholesterol, urea, glucose, alkaline phosphatase, SGOT and SGPT using various reagent kits. These kits were manufactured by Sigma Diagnostic (India). Pvt. Ltd., Baroda and Marketed by Qualigens Fine Chemicals a division of Glaxo India Ltd.

3. Results and Discussion

Visual effects observed

Animal death was not recorded during any of the treatment periods in either control or treated groups, nor did the animals show any changes in the normal behavioral activities.

Effect on the TLC, DLC and Haemoglobin content

The TLC for control animals was recorded as 10.78 X 10^3. The TLC of test animals treated with *Ocimum, Prunus* and *Zingiber* oil was found to be 9.35X10^3, 10.41X10^3 and 10.65 X10^3 respectively. The eosinophil content in control was 3.875 while it was 3.65 in *Ocimum* oil treated animals, 3.725 in *Prunus* treated animals and 3.31 in *Zingiber* oil treated animals. The monocyte content was 0 in treated as well as in control sets. The lymphocyte content in control, *Ocimum, Prunus* and *Zingiber* oil treated animals was found to be 44.33, 48.41, 49.57 and 54.45 respectively. The neutrophils content in *Ocimum, Prunus* and *Zingiber* oil treated animals was found to be 45.48, 56.12 and 42.00 respectively, while in control it was 48.35. The haemoglobin content (gm %) in control, *Ocimum, Prunus* and *Zingiber* oil treated animals was recorded as 11.196, 10.75, 11.198 and 10.15 respectively (Table 1).

Effect on Biochemical parameters of serum

Blood glucose (mg/dl) level in control, *Ocimum*, *Prunus* and *Zingiber* oil treated animals was 48.64, 46.77, 43.03 and 48.90 respectively. The protein (gm%) level in control was 8.676 while in, *Ocimum*, *Prunus* and *Zingiber* oil treated animals its value was 6.9, 7.916 and 7.516 respectively. The cholesterol (mg %) level in *Ocimum*, *Prunus* and *Zingiber* oil treated animals was 58.018, 83.833 and 88.458 respectively while in control set it was 64.093. The urea (mg/dl) level in control, *Ocimum*, *Prunus* and *Zingiber* oil treated animals was recorded as 44.536, 36.891, 39.143 and 36.608 respectively. Similarly the activity of enzyme SGOT (u/ml) in control, *Ocimum*, *Prunus* and *Zingiber* oil treated animals was found to be 106.35, 104.00, 100.45 and 97.97 respectively. The SGPT (u/ml) enzyme activity for control was 44.416 and in the *Ocimum*, *Prunus* and *Zingiber* oil treated animals it was 46.226, 45.498 and 49.016 respectively. The alkaline phosphatase (IU/litr) activity for control, *Ocimum*, *Prunus* and *Zingiber* oil treated animals was 25.233, 24.455, 25.551 and 29.065 (Table 1).

Discussion

The most important suggested areas of essential oil use are in medicine and in cosmetics. Besides, volatile oils of many plants are known to have antimicrobial activity [11, 12]. Complex oil presents a greater barrier to pathogens adaptation than would a more simple mixture of monoterpenes [13]. The complicated mixture of monoterpenes and sesqui-terpenes in the whole oil represents the strongest barrier to fungal infection. During our earlier investigations we have reported the antifungal activity of essential oils of, *Ocimum sanctum, Prunus persica* and *Zingiber officinale* in control of grey mould of grapes [7]. Therefore, it became an

urgency to test the safety limit of these essential oils.

As the results suggests, the present study show that there was no significant variation in blood and serum analysis of control and treatment groups. This confirms that the *Ocimum*, *Prunus* and *Zingiber* oils which have been reported for as efficacious fungitoxicant are also non toxic to test animals and do not induce any adverse effects to the blood, liver function, kidney function, protein, carbohydrate and lipid metabolism of the animals. Though our findings are based on the limited parameters and are not sufficient to declare them as non-mammalian toxic and much more parameters like residual toxicity and mode of action are still required to recommend them as a safe fungitoxicant. Therefore, these oils may be recommended to exploit them as potent and nontoxic bioactive plant products, which besides checking the biodeterioration of food commodities, are not harmful to rats. Thus, because of their non-mammalian toxicity these plant products constitute novel fungitoxicants and may be recommended as nontoxic botanical products in control of fungal deterioration of food commodities.

Table 1. Effect of plant essential oils on various blood parameters of rats

Parameters	Control	*O. sanctum*	*P. persica*	*Z. officinale*
1.TLC	$10.78X10^3$	$9.35X10^3$±0.39 t =.106	$10.41X10^3$±0.55 t =0.690	$10.65X10^3$ ±0.81 t =0.908
2.DLC a. Eosinophil	3.875	3.65 ±0.61 t = 0.801	3.725±0.43 t= 0.838	3.31±0.35 t = 0.426
b. Monocyte	0	0	0	0
c. Lymphocyte	44.33	48.41±1.8660 t=0.107	49.57±1.02 t =0.011	54.45±1.28 t =0.002
d. Neutrophils	48.35	45.48 ±2.59 t =0.375	56.12±2.35 t=0.024	42.00 ± 2.17 t=0.043
3.SGOT(u/ml)	106.35	104.00±5.15 t =0.740	100.45 ±5.21 t=0.416	97.97±5.72 t =0.281
4.SGPT(u/ml)	44.416	46.226±2.29 t=0.560	45.498±3.52 t =0.795	49.016±2.07 t =0.136
5. Haemoglobin Gm (%)	11.196	10.75±0.55 t =0.750	11.198±0.82 t =0.999	10.15±0.41 t =0.449
6. Albumin (gm%)	3.48	3.371±0.26 t =0.828	3.681±0.40 t=0.743	3.59±0.32 t=0.842
7. Glucose (mg/dl)	48.64	46.77±2.78 t =0.589	43.03±2.28 t=0.085	48.90±3.65 t =0.950
8. Protein (gm %)	8.676	6.9±0.64 t=0.055	7.916±0.46 t =0.288	7.516±0.32 t =0.082
9. Cholesterol mg (%)	64.093	58.018±6.65 t=0.415	83.833±5.54 t=0.014	88.458±4.28 t=0.002
10. Urea (mg/dl)	44.536	36.891±1.96 t =0.032	39.143±1.73 t =0.098	36.608±2.26 t =0.038
11. Alkaline Phosphatase u/lit	25.233	24.455±1.54 t =0.780	25.551±1.66 t=± 0.911	29.065±1.18 t =0.169

±Standard error; Tabulated value of t at 1 % level of significance and 6 degree of freedom is 3.71 Difference is not significant

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