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Chronic toxicity studies of aqueous leaf extract of Indian traditional medicinal plant *Ocimum tenuiflorum* (Linn.) in rats

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ABSTRACT

In the present study, the aqueous leaf extract of Ocimum tenuiflorum (Linn.) was subjected to toxicity assessment in wistar albino rats at different dose levels of 100, 500 and 1000mg/kg body weight. No major changes in body weight were observed at the end of 30 days of daily oral administration. Biochemical parameters such as the levels of Protein, Urea, Creatinine, Uric acid, Aspartate Transaminase [AST], Alanine Transaminase [ALT], Lactate Dehydrogenase [LDH] in serum were found to be well within the normal limits. The levels of marker enzymes in the vital organs did not show any statistical significance between control and treated groups of animals. Histopathological examination of the major vital organs (liver, heart, kidney, lung and brain) revealed no significant pathological alterations in the treated group of rats. In conclusion, the present study shows that the aqueous leaf extract of Ocimum tenuiflorum is safe and non-toxic at the doses tested.

Key words: *Ocimum tenuiflorum* (Linn.), Chronic toxicity, Aqueous leaf extract, Herbal medicines, Phytochemicals.

INTRODUCTION

The herbal and natural products of folk medicine have been used by men since the beginning of the human race. Plants continue to be a major source of medicine, as they have been throughout human history [1]. In India, the use of different parts of medicinal plants to alleviate specific ailments was in practice form ancient times [2]. However 80% of the world's population use plant as their primary source of medication [3]. *Ocimum tenuiflorum* L. (Syn : *Ocimum sanctum* L) [4] known as "Queen of Herbs" is an aromatic plant in the family Lamiaceae which is native throughout the Old World tropics and widespread as a cultivated plant and an escaped weed.

Literature reports state *Ocimum* to have potent hypoglycemic [5], anti-hyperlipidemic [6], anti-lipid peroxidative [7], anti-oxidant [8], anti-ulcer [9], cardioprotective [10], neuroprotective [11], hepatoprotective [12], hypotensive [13], analgesic [14], anthelminthic [15], anti-bacterial [16], anti-cataract [17], anti-fertility [18], anti-inflammatory [19], anti-stress [20], anti-thyroid [21], anti-toxic [22], anti-tussive [23], radioprotective [24], wound-healing [25] activities.

Bioactive components of *Ocimum tenuiflorum* are found to be eugenol, methylchavicol, linalool, isoeugenol and methyl isoeugenol [26] and the most abundant phytochemical in the aerial plants of *Ocimum tenuiflorum* L has been reported to be methyl eugenol (36.9%).

MATERIALS AND METHODS

Plant Material

The plant material of *Ocimum tenuiflorum* used in this study were collected from Sembakkam, Tambaram, Tamilnadu, India. Authentication of plant materials were carried out by Professor P. Jayaraman, Plant Anatomy Research Center, Tambaram, Chennai – 600 045. Following identification of voucher specimen, the plants were deposited in herbarium(PARC / 2008 / 287).

Fresh leaves of the collected plant samples were cleaned, washed, shade dried and used for extraction.

Figure 1 : *Ocimum tenuiflorum*

Table 1 : Phytochemical Analysis of aqueous leaf extract of *Ocimum tenuiflorum*

Phytoconstituents	Extract
Phenols	+
Terpenoids	+
Tannins	+
Flavonoids	+
Reducing sugar	+
Alkaloids	-
Glycosides	-

+ = present - = absent

Preparation of plant extract

50g of fine powdered leaves of *Ocimum tenuiflorum* L. was mixed with 300ml of distilled water and boiled in a low flame for 2 hours. The extract was then filtered, the filtrate was then dried at 60ºC in an oven. The fine dried material was stored in labeled sterile bottle at - 10ºC which were utilized for further studies.

Screening for phytochemicals

The aqueous extract of the leaves of *Ocimum tenuiflorum* L. plants were screened for the presence of phytochemicals according to the standard protocols [27].

In vivo toxicity studies

Experimental Animals

Adult female albino rats of Wistar strain, weighing between 100- 150g obtained from Saveetha University, Chennai were used for the experimental studies. Animals were acclimatized to the animal house conditions for a week. Water and diet was given *ad libitum*.

The animal room was well ventilated with 12 hours light and dark cycle, throughout the experimental period. The animals were provided with commercial rat feed supplied by Hindustan Lever Ltd, Mumbai. The experimental protocol used in this study was approved by the Institutional Animal Ethical Committee – [IAEC3-1-2010].

Chronic Toxicity Studies

Grouping of Animals

Animals were divided into 4 groups.
Group I: The animal in this group served as control and were fed with pellet food and water a*d libitum*.
Group II: Animals were treated with aqueous extracts of plant (100mg / kg body weight) orally for one month.

Group III: Animals were treated with aqueous extracts of plant (500mg / kg body weight) orally for one month.
Group IV: Animals were treated with aqueous extracts of plant (1000mg / kg body weight) orally for one month.

Collection of Rat Blood and Tissues

On the 31st day (ie) at the end of the experimental period for chronic studies, animals in all the groups were sacrificed by cervical decapitation after anaesthetizing them with ketamine (22mg / kg body wt). Blood was collected in heparinized tube for the analysis of hematological parameters. The collected blood sample was centrifuged for 10 minutes at 4000 rpm at 4ºC to obtain the serum for biochemical estimations. Organs such as liver, heart, kidney, lung and brain were excised and washed with ice cold saline.

Histopathological Studies

Sections of liver, heart, kidney, lung and brain tissues were perfused with 10% formalin and stored in the same and used for histopathological studies. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 µm thickness and stained with haematoxylin and eosin (H&E). The sections were then viewed under light microscope (Nikon microscope ECLIPSE E400, model 115, Japan).

Statistical Analysis

All the results are expressed as Mean ± Standard deviation (SD). Comparison of group with the control were performed using the SPSS software package for windows (version 20).The significant difference between the groups are considered at $p<0.05$ level by Independent – t – Test [28].

RESULTS

Table 2: Body weight changes in experimental rats treated with *Ocimum tenuiflorum* aqueous extract

Particulars	**Initial**	**Final**
Group I	143±14.75	226±20.10
Group II	152±15.97	240±21.19
Group III	153±15.43	253±20.97
Group IV	151±15.38	276±20.50

Values are expressed as Mean ±SD of 6 rats in each group. Group I-Control, Group II-100 mg/kg b.w, Group III-500 mg/kg b.w & Group IV-1000 mg/kg b.w.

Table 3: Biochemical parameters in serum of control and *Ocimum tenuiflorum* aqueous extract treated rats

Parameters	Group I	Group II	Group III	Group IV
Urea	8.87±0.89	8.97±0.77	9.13±0.81	9.33±1.03
Creatinine	0.40±0.03	0.47±0.05	0.50±0.05	0.53±0.06
Uric acid	1.35±0.14	1.23±0.16	1.32±0.17	1.54±0.25
Protein	6.33±0.59	6.38±0.51	6.32±0.72	6.40±0.72
AST	13.43±1.37	13.53±1.29	13.73±1.3	12.01±1.20
ALT	8.34±0.83	8.99±0.90	9.52±1.05	9.95±1.01

Values are expressed as Mean ±SD of 6 rats in each group. Group I-Control, Group II-100 mg/kg b.w, Group III-500 mg/kg b.w & Group IV-1000 mg/kg b.w. Units : Urea–mg/dl, Uric acid–mg/dl, AST–IU/L, ALT–IU/L, Protein–g/dl and Creatinine–mg/dl.

Table 4: Levels of marker enzymes in vital organs of experimental rats treated with or without aqueous leaf extract of *Ocimum tenuiflorum*

Vital Organs	Marker enzymes	Group I	Group II	Group III	Group IV
Liver	ALP	20.44±2.45	20.33±2.10	21.09±2.17	20.40±2.01
Liver	ALT	9.12±0.92	10.26±1.02	10.44±1.18	11.35±1.14
Liver	LDH	7.05±0.71	8.24±0.83	9.34±0.96	11.48±1.02
Heart	AST	10.32±1.02	10.64±1.08	12.53±1.23	12.44±1.28
Heart	LDH	10.34±1.78	11.49±1.12	12.34±1.19	12.95±1.32
Kidney	ACP	9.32±0.64	9.65±0.95	10.24±1.01	10.53±1.14
Lung	LDH	10.15±1.34	11.77±1.15	12.47±1.28	12.46±1.30
Lung	ICD	10.39±0.95	10.45±1.65	11.32±1.14	11.40±1.18
Lung	MDH	9.23±0.99	10.62±1.21	10.94±1.14	11.52±1.24
Brain	AchE	0.37±0.03	0.43±0.05	0.54±0.13	0.64±0.04

Values are expressed as Mean ±SD of 6 rats in each group.
Group I-Control,Group II-100 mg/kg b.w, Group III-500 mg/kg b.w& Group IV- 1000 mg/kg b.w. Units : ALP–IU/L, ALT – IU/L, LDH – IU/L, AST – IU/L, ACP –IU/L, ICD (nmoles/min/mg), MDH (nmoles of NADH oxidized/min/mgptn), AchE–(nmoles/min/mg).

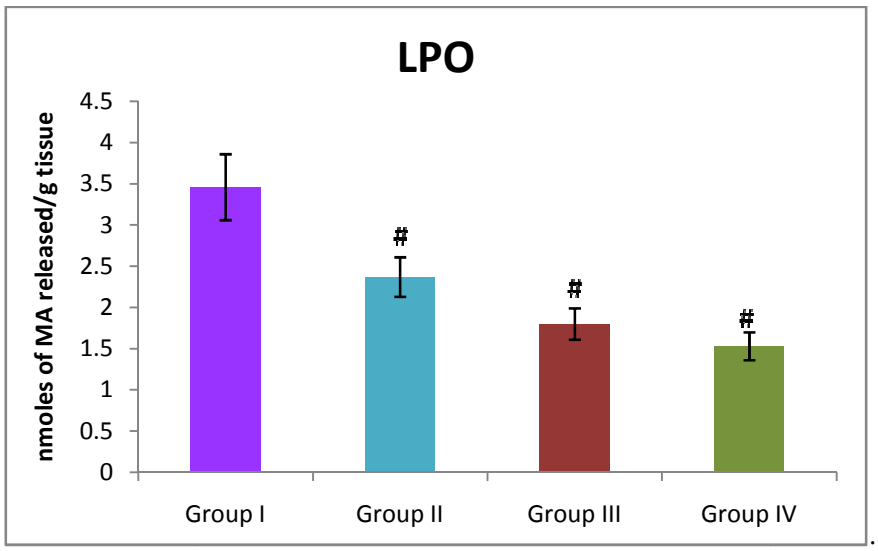
Table 5: Levels of enzymic and of non-enzymic antioxidants in the lung of *Ocimum tenuiflorum* aqueous leaf extract treated rats

Parameters	Group I	Group II	Group III	Group IV
Superoxide dismutase (SOD)	0.29±0.02	0.40±0.03	1.47±0.14#	1.59±0.15#
Catalase (CAT)	0.71±0.06	1.56±0.16#	3.64±0.33#	5.91±0.68#
Glutathione peroxidase (GPx)	1.72±0.12	2.03±0.21#	4.64±0.45#	5.68±0.35#
Ascorbic acid	0.11±0.01	0.58±0.05#	1.45±0.15#	3.66±0.37#
α-Tocopherol	2.89±0.27	3.01±0.29#	3.82±0.39#	6.19±0.60#
Reduced glutathione (GSH)	0.98±0.10	1.42±0.11	3.00±0.31#	5.85±0.59#

Values are expressed as Mean ±SD of 6 rats in each group.

Group I-Control,Group II-100 mg/kg b.w, Group III-500 mg/kg b.w& Group IV- 1000 mg/kg b.w. Units: SOD - u/mg protein; CAT - µ moles of hydrogen peroxide consumed/min/mg protein; GPx - µg of GSH consumed / min / mg protein; Ascorbic acid- µg/mg protein;α-Tocopherol- µg/mg protein; GSH – mg / g tissue. The symbol #represents statistical significance at p<0.05 when compared with Group-I.

Figure 1 : Levels of LPO in the lung of *Ocimum tenuiflorum* aqueous extract treated rats



Values are expressed as Mean ±SD of 6 rats in each group. Group I-Control,Group II-100 mg/kg b.w, Group III-500 mg/kg b.w& Group IV-1000 mg/kg b.w.

Chronic toxicity studies were carried out by oral administration of the leaf extracts of *Ocimum tenuiflorum* for a period of 30 days. **Table 2** depicts the body weight changes in the control and treatment groups with respect to *Ocimum tenuiflorum* leaf extract administration. The average body weight of control group was found to be 143 gm nearly. A 58.04 % increase in the body weight was observed in the control animals after 30 days. Similar increases in the body weight were observed in all the extract fed groups.

Table 3 represents that the biochemical parameters in serum sample. All the biochemical parameters were found to be well within the normal limits in all the treated groups. A slightly increased level of urea and creatinine was observed in the serum of Group-IV animals but it was not statistically significant. An elevation in the ALT activity was also observed in Group-IV animals when compared with that of the control group.

Table 4 represents the level of marker enzymes in homogenate of vital organs of experimental rats fed with aqueous leaf extract of *Ocimum tenuiflorum*. Liver ALP activity was found to be similar in all the experimental groups. However, a marked non-significant increase in the liver ALT and LDH activities were observed in the 1000mg/kg body weight treated group. A maximum of 20.54 % increase in the AST activity was seen in the heart of Group-III animals, accompanied by a 25.24 % increase in the LDH activity in the hearts of Group-IV animals (1000mg/kg body weight), when compared with their respective controls. However all the enzymatic activities of the vital organs were well within the normal limits with no statistically significant elevation in any of the treated groups.

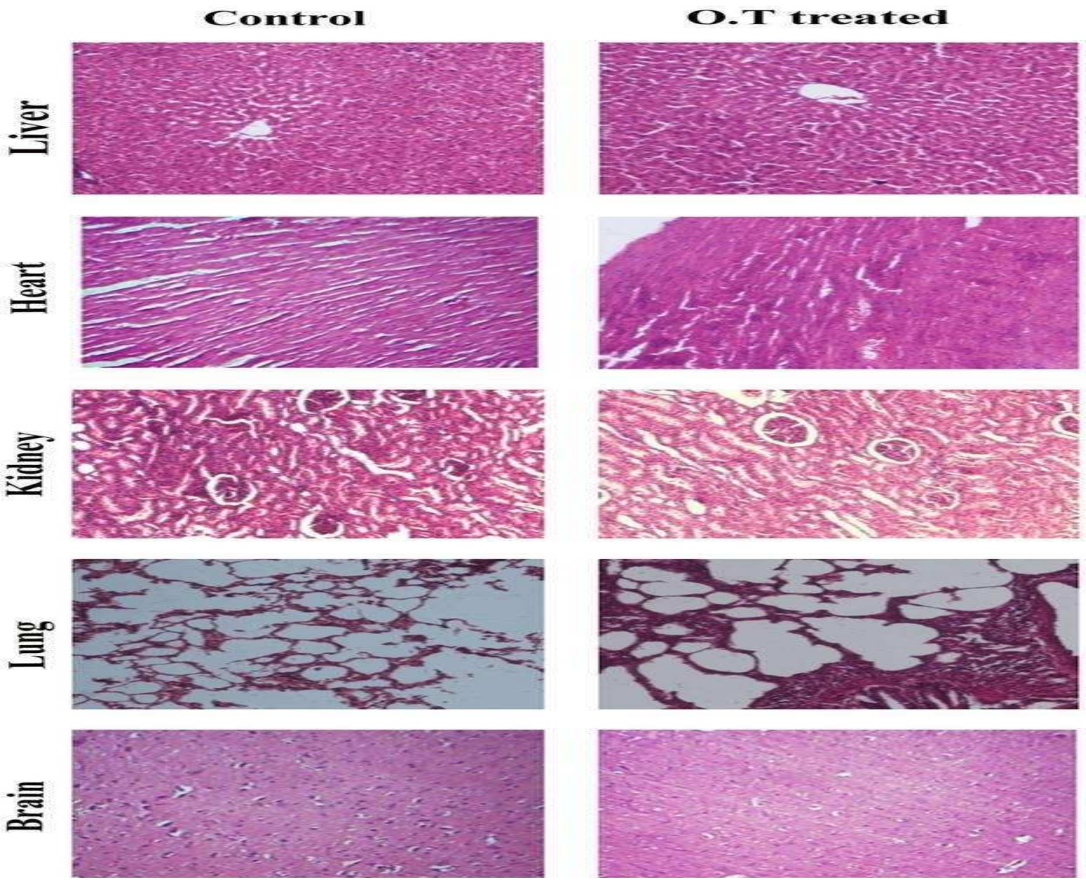
Table 5 represents the levels of enzymic and non-enzymic antioxidants in lung homogenate. The activity of SOD was found to be 0.29 units/mg protein in the lung of control group. There was an increasing trend in the SOD activity as the dosage of the administration increased nearly a 5.5 fold in the activity of SOD was observed in the lung of Group-IV animals when compared with that of the control group. A similar dose dependent activity of catalase, glutathione peroxidase was also observed in the lung of *Ocimum tenuiflorum* leaf extracts treated rats when compared with that of the normal rats. *Ocimum tenuiflorum* administration exerted a tremendous increase in the enzymic antioxidants treatment group (Group-IV animals) when compared with control group. A 114.19% increase

in the α-tocopherol levels was also observed in the Group-IV animals as compared with control. There was an increasing trend in the reduced glutathione levels in all the treated groups as the dosage level of the exract increases.

Figure 1 portrays the levels of lipid peroxidation in the lung of the experimental group treated with or without *Ocimum tenuiflorum* leaf extracts. The levels of lipid peroxidation were found to be decreased as measured by TBARS method in the lung of the control animals. As the dosage of the treatment of *Ocimum tenuiflorum* increased a concomitant significant decrease in the lipid peroxide levels were also observed. A 55.78% decrease of lipid peroxides in the Group-IV animals (1000mg/kg body weight) was observed.

Histopathological sections of kidneys showed normal structural features suggesting the preserved renal integrity of the chronic treatment groups. The glomeruli and renal tubules namely the proximal and distal convoluted tubules exhibit the normal architecture indicating the absence of renal toxicity. Similarly sections of lung tissues of control and the treated groups showed normal alveoli even at the higher dose level of chronic treatment groups of *Ocimum tenuiflorum.* Brain appears to be normal in the treated group of animals compared with control (Fig-2).

Figure-2 Histological sections of rat, liver, heart, kidney, lung, brain from the control and maximum dose (1000mg/bw) treated groups 10x magnification



DISCUSSION

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [29]. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [30] there is a constant need for new and effective therapeutic agents [31]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [32&33].

Plant extracts have been used for many thousands of years in food preservation, pharmaceuticals alternative medicine and natural therapies [34]. The extracts of higher plants can be very good source of antibiotics [35] against various fungal and bacterial pathogens. Eventhough, herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care [36] lack of scientific validation and

toxicity evaluation has attenuated its usage as choice of treatment. Therefore, it is necessary to investigate these plants scientifically which have been used in traditional medicine to improve the quality of healthcare.

The claim that natural plant products are safe should be accepted only after the plant product passes through toxicity testing using modern scientific methods [37]. To determine the safety of drugs and plant products for human use, toxicological evaluations are carried out on various experimental animals to predict toxicity. There are other several comprehensive toxicological studies on some natural extract [38,39,40,41&42].

When a drug is used, it is expected to benefit the recipient. At the same time, it is a fact that there is no drug which is totally free from harmful effects. A drug can be used after a careful weighing of *pros and cons*, the benefit risk reaction. Hence, toxicity assessment of aqueous leaf extract of *Ocimum tenuiflorum* plant species was carried out in order to find, whether the drug has any adverse effect on the organs such as liver, heart, kidney, lung and brain. Chronic toxicity studies in animals are of value in predicting potential toxic effects of a substance or a plant extract from which the response may be correlated with human. It also gives an idea about the organ system involvement [37].
In the present study, the evaluation reports indicate that, reduction in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [43&44]. The evaluation of the chronic toxicity at the doses of 100, 500 and 1000 mg/kg/day for 30 days presented no signs of body weight changes, behavior changes and toxic signs. Hence, we could substantiate that the drug does not bring out any pathological effects on the growth rate of the animals.

There is no significant change in hematological parameters like hemoglobin, RBC, platelets, reticulocytes, monocytes, basophils, MCV, MCH, MCHC, PCV in the extract treated animals. Hematological changes such as anemia are often accompanied with bone marrow toxicity [45&46]. According to [47] anemia that results after administration of agent can be a result of lysis of blood cells. However no such anemia is observed after chronic treatment with the extracts suggesting that there is no lysis of blood cells. The observed values of blood parameters within the normal range show that the drug is non-toxic in nature.

The white blood cells are found to be unaltered in all the experimental groups when compared with that of control in the chronic studies using aqueous extract of *Ocimum tenuiforum*. [48] showed the aqueous extract of *Ocimum sanctum* reduced tuberculosis and increased the neutrophil and lymphocyte counts with enhanced phagocytic activity. Analysis of blood parameters with respect to animal studies has a high relevance and predictive value for humans [45&46]. This indicates its safe usage in humans. Chronic exposure of the animals to the plant extract *Ocimum tenuiflorum* even at higher dosage when tested for the biochemical parameters such as protein, urea, uric acid; AST, ALT and creatinine do not show any significant difference in their levels when compared with the control animals.

The normal values of the biochemical parameters *Viz* Urea and Creatinine suggest that the extract do not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts. Transaminases, LDH, and alkaline phosphatases are good indices of liver, heart and kidney damage respectively [49]. The activities of aspartate transaminase, alanine transaminase and the levels of protein, urea, uric acid and creatinine shows that the function of the liver, heart and kidney are not affected by the oral supplementation of extracts.

Ocimum tenuiflorum do not show any changes in aspartate transaminase, alanine transaminase and total protein which portrays that the function of the liver is not affected by the extract. However, methyl eugenol, the major plant phytochemical constituent of the aqueous extract is shown to form DNA and protein adducts and to cause hepatotoxicity and carcinogenicity in rodents [50&51]. When administered, 3 methyl eugenol is also shown to cause hepatocyte cytological alterations like cytomegaly, kuppfer cell pigmentation, chronic inflammation and atropy of the liver [52]. But, in the current study, we have not observed such changes in the liver proving the protective effect of the other constituents in the plant extract.

No marked changes in the histomorphology of heart and cardiac marker enzymes are observed when rats are fed with different concentrations of aqueous leaf extracts of *Ocimum tenuiflorum.* Our studies are supported by the studies of [53&54] which clearly shows the cardioprotective effect of the juice of *O. tenuiflorum* leaves has greater cardiac stimulant and cardiac tonic drug. The observation of the current study portrays that the oral administration of the aqueous extract of *Ocimum tenuiflorum* did not cause any mortality nor altered the biochemical and histopathological indices in chronic studies. This indicates that the plant extract is not harmful at the level tested and can be safely used as an antimicrobial agent.

CONCLUSION

Chronic administration of aqueous leaf extract of *Ocimum tenuiflorum* (Linn.) shows no significant adverse effects on the parameters such as heamatological, biochemical and also body weight changes. The level of the marker enzymes in the vital organs were also found to be normal. Histopathological studies also showed appearance of normal architecture of the vital organs of the *Ocimum tenuiflorum* treated rats and did not induce any toxic effects at different doses. It may be concluded that the aqueous leaf extract of *Ocimum tenuiflorum* may be considered as relatively safe and non-toxic.

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