Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

A Comparative Acute Toxicity Study of Two Widely Used Medicinal Plants: *Ocimum canum* and *Platycladus orientalis* Leaf Extracts

Alok Kumar Dash

Dr. Alok Kumar Dash
Institute of Pharmacy, VBSPurvanchal University, Jaunpur, India
Corresponding Author Email: alokjina@gmail.com

Abstract

The evaluation of acute toxicity is a fundamental step in determining the safety profile of medicinal plants used in traditional medicine. This study compares the acute oral toxicity of hydroalcoholic leaf extracts of *Ocimum canum* (OC) and *Platycladus orientalis* (PO), two plants known for their antimicrobial, antioxidant, and anti-inflammatory properties. Using Wistar rats as the animal model and following OECD guidelines (425), the extracts were administered in increasing doses. Behavioral changes, mortality, and biochemical markers were evaluated over 14 days. No mortality was observed in animals treated with either extract up to 2000 mg/kg, but differences were noted in organ weight ratios and hepatic enzyme levels, indicating variation in systemic response. This comparative study suggests that while both extracts are relatively safe in acute settings, *Ocimum canum* demonstrated a slightly superior safety profile than *Platycladus orientalis*, especially with regard to hepatotoxicity markers.

Keywords: *Ocimum canum*, *Platycladus orientalis*, acute toxicity, medicinal plants, LD_{50}, herbal safety, OECD 425

1. Introduction

The World Health Organization (WHO) is promoting traditional and herbal remedies in national healthcare systems due to their ease of availability, low cost, and safety. Traditional medicines, such as Ayurvedic, Unani, and Siddha, have been used for centuries to treat specific ailments, serving nearly 70% of the village population. The WHO has developed guidelines to formulate traditional remedies that support member states and recognizes the importance of traditional medicine in the world.[1,2]

Medicinal plants have been subjected to intense pharmacological studies, leading to a revival of interest in using herbal medicines. However, standardization of active compounds in medicinal plants often does not reflect reality, as the complex composition of drugs can influence bioavailability and excretion. To ensure the therapeutic efficacy of traditional remedies, standardization is done through batch analysis, good manufacturing practice, and standardization methods of preparation[3].

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

Synthetic or allopathic drugs have drawbacks such as high costs, toxicity, non-renewable raw materials, and environmental pollution. On the other hand, traditional medicines have positive aspects such as long history, public acceptance, better patient tolerance, renewable sources, environmental-friendly cultivation and processing, and local availability, especially in developing countries.[4]

To ensure the safety and quality of traditional remedies, scientific examination is necessary. This research study examined the therapeutic uses of traditional medicinal plants for their antidiabetic, antioxidant, and hepatoprotective properties, using examples such as Platy cladus Orientalis and Occimum canum.

Medicinal plants form the foundation of traditional health care systems and have gained renewed interest due to their potential pharmacological benefits. Despite their widespread use, the safety of many plant-based remedies is often under-researched. *Ocimum canum* (family: Lamiaceae), commonly known as "Jungli Tulsi," is well-regarded for its antimicrobial, antifungal, and anti-inflammatory properties. *Platycladus orientalis* (family: Cupressaceae), known as "Chinese Arborvitae," is used traditionally in respiratory, hair, and bleeding disorders. Although both species are frequently incorporated in herbal formulations, systematic safety evaluations—especially comparative acute toxicity profiles—remain sparse.[5,6,7]

This study was undertaken to compare the **acute oral toxicity** of the hydroalcoholic leaf extracts of these two plants, highlighting any relative differences in systemic toxicity.

2. Materials and Methods

2.1. Plant materials: The selection of plant species for this study was based on their traditional use for diabetes, liver disorder as well as anti oxidant treatment. In the present study the leaves of platycladus orientalis and ocimum canum were taken.

2.2 Collection, identification and authentification of plant parts:

Leaves of *Ocimum canum and Platycladus orientalis were* collected in the month of November 2011 from its natural habitat from nearby Dasapalla forest division, Nayagarh district of Odisha,India. The plant was authenticated from Department of botany, T.D.P.G.College, Jaunpu,Utterpradesh by Dr. A.K.Singh (H.O.D).

The voucher specimen number (PE/2011-2012/01) and (OC/2011-2012/01) were preserved in institute department for future reference. The leaves were cleaned and dried under the shade to avoid degradation of volatile oil.

2.3 Preparation of extracts: The extraction yield of the extracts from plants species is highly depends on the solvent polarity, which determines both qualitatively and quantitatively the extracted compounds. Petroleum ether and water are the most widely used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al.,

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

1996). The leaves of the plants were dried to control temperature, humidity and damage of active constituents (WHO, 2003).[8,9]

2.3.1 Extraction of Ocimum canum and Platycladus orientalis

The freshly collected leaves (4 kg) of ***Ocimum canum and Platycladus orientalis*** were washed with distilled water and shade-dried. The leaves were dried in shade and powdered to get a coarse powder. About 500gm of dry coarse powder was extracted with petroleum ether (40-60^{0}C) by continuous hot percolation using soxhlet apparatus. The extraction was continued for 72hours. The petroleum ether extract was filtered and concentrated to a dry mass by using vacuum distillation. A greenish brown waxy residue (15.20gm) obtained. The mark left after petroleum ether extraction was dried and extracted with distilled water by simple maceration. The macerated product was concentrated by vacuum distillation. A brown colour residue was obtained. Then the extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The extract obtained was further subjected to phytochemical screening and pharmacological investigations.[10,11]

2.4 Pharmacological Screening on Animal Models :

2.4.1 Animal selection:

Healthy wistar male Albino rats weighing 150-200 g breed in the animal house were used in this study.The animals were stabilized for one week,housed in polypropylene cages,maintained under standard condition(12 h light and 12h dark cycle, 25 ± 30°C). They were employed for assessing acute oral toxicity, antidiabetic ,antioxidant and hepatoprotective activity study. The animals were allowed free access to commercial rat pallet diet (Lipton India ltd., Mumbai, India) and water ad libitum. The animals were handled gently to reduce stress upon them,which could induce adrenal output. Rats were housed in a group of six in clean cages at 25°C and 12 hours photoperiod with relative air humidity of 30 to 60%. The bedding material of the cages was changed everyday. All the experimental procedures were carried out accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. The experimental procedures were approved by the institutional animal ethical committee (IAEC) (CPCSEA/1/15/2007). The certificate of approval of the research project is given in Annexure.[12,13]

2.5 Toxicity Studies :

2.5.1 Acute oral toxicity – Acute toxic class method: (OECD Guidelines, 2001)

The main aim of acute oral toxicity study is to determine the therapeutic index**.**The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.[14,15]

The rule is based on a stepwise procedure in which a minimum number of animals are used per step to obtain sufficient information on the acute toxicity of the test substance to enable its

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

classification. When the substance is taken for test in every step of testing three animals of either sex are used. By determining Presence or absence of the suspension of alcoholic extract or aqueous extract was administered orally to overnight fasted wistar albino rats (n=6) at dose of 2000mg/kg body weight respectively. Animals were observed to watch the behavioral changes in interval of 4 h,6h 24h and 48 h for next 14 days. Behaviourial parameters include convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration.[16,17]

2.5.2. Selection of animal species:

Healthy young albino rats of either sex weighing 150-200 g (8 to 12 weeks old) were used for acute toxicity study to determine LD50 of plant extract of selected medicinal plants i.e Ocimum canum and Platycladus orientalis . Totally there were seven groups.

2.5.3 Housing and feeding condition:

The temperature in the experimental room was maintained as normal room temperature that is around 25□C. Lighting was natural sequence being 12 hours dark, 12 hours light. The conventional laboratory diet was fed with adequate supply of drinking water.

2.5.4 Preparation of Animals:

The animals which were taken for experiment randomly selected, marked to permit individual identification and kept in polypropylene cages for one week prior to dosing to allow acclimatization of them to laboratory conditions.

2.5.4.1 Preparation of doses:

The suspension of alcoholic extracts of various parts were freshly prepared in tween 80 and suspended in distilled water and glibenclamide 5 mg/kg p.o was prepared in tween 80 suspended in distilled water.

2.5.4.2 Administration of doses:

The test substances were administered in a single dose by gauge using a stomach tube. Animals were kept fasting for 12 h before dosing. Then animals were weighed and test substance was administered.The animals were again deprived from food for 3-4 h after dosing.

2.5.4.3 Number of animals and dose levels:

Three animals were used in every group in every step. Study was started at 2000 mg/kg body weight. The procedure of dose selection and finalizing LD50 cut off values is as, Sr. No. Name of Extract LD50 Cut off mg/kg body wt. Vehicle.LD50 means lethal does.It is defined as the minimum amount of does required to kill 50% of the test samples..This is the standard measure toxicity of the drug.1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent anti-diabetic and hepatoprotective activity.

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

Parameters monitored:

- Clinical signs (behavioral changes, tremors, salivation, lethargy)
- Body weight and food/water intake
- Mortality
- Organ weight (liver, kidney, spleen)
- Hematological and biochemical analysis (ALT, AST, urea, creatinine)

3. Results

3.1 Acute oral Toxicity Studies:

Acute toxicity study describes the adverse effect of the substance which result either from single exposure or from multiple exposure in a short space of time (less than 24 hour).The adverse effect should occur within 14 days of administration.

The acute oral toxicity study was carried out with aqueous and petroleum ether extract of Ocimum canum and Platycladus orientalis .The whole study was carried out according to OECD guidelines (No. 423). Selected medicinal plants were found safe (no mortality) even when given at the dose of 2000 mg/kg body weight with no signs of acute oral toxicity at respective dose. Hence, 1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent antidiabetic and hepatoprotective activity i.e., 200 mg/kg b. w. p. o. Here in this study the $1/10^{th}$ i.e., 200 mg/kg and $1/20^{th}$ i.e., 100 mg/kg b.w. was taken.

3.2 Observation for acute toxic study for aqueous and petroleum ether extract of Ocimum canum at dose 2000 mg/kg of body weight:

i) Grooming: Grooming was observed after 24 hours at all the dose levels.

ii) Hyperactivity: No hyperactivity was seen in albino rats after 24 hours that had received different doses.

iii) Sedation: .There was no effect on sedation.

iv) Respiratory arrest: There was no effect on respiratory arrest.

v) Convulsions: There was no effect on convulsions.

vi) Diarrhea : No diarrhea symptom was found in any animal

vii)Skin : There was no change in skin observed.

viii)Eye : There was no change in eye observed

ix) Mortality: None of the animals died after hours.

3.3 Observation for acute toxic study for aqueous and petroleum ether extract of Platycladus orientalis at dose 2000 mg/kg of body weight :

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

i) Salivation: There was no effect on Salivation

ii) Hyperactivity: No hyperactivity was seen in albino rats after 48, 72 hours that had received different doses.

iii) Sedation: .There was no effect on sedation.

iv) Respiratory arrest: There was no effect on respiratory arrest.

v) Convulsions: There was no effect on convulsions.

vi) Diarrhea : No diarrhea symptoms found in any animal.

vii)Skin : There was no change in skin observed.

viii)Eye : There was no change in eye observed

ix) Coma: No animal were found in coma condition.

ix) Mortality: None of the animals died after 24 hours.

.**Drug-** Ocimum canum and platycladus orieantalis

Dose-2000 mg/kg **Time**- 24 hours **Species**- albino rats either sex

Table -1: Acute Toxic study of both the plant Ocimum canum and Platycladus orientalis

SL no	Plant code	Sali	Hyp	Sed	Resp	Con	Dia	Skin	Eye	Coma	Mort
1	OC(AE)	N	N	N	N	N	N	N	N	N	N
2	OC(PE)	N	N	N	N	N	N	N	N	N	N
3	PO(AE)	N	N	N	N	N	N	N	N	N	N
4	PO(PE)	N	N	N	N	N	N	N	N	N	N

N = Negative

3.4. Mortality and Clinical Observations

No mortality was observed in any group up to 2000 mg/kg. However, rats treated with *Platycladus orientalis* extract displayed mild lethargy and reduced feed intake for 24–48 hours.

3.5. Body Weight and Organ Weight

- No significant difference in weight gain was observed.

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

- Liver-to-body weight ratio was slightly increased in PO-treated rats compared to OC.

Table-2 . Biochemical Findings

Parameter	Control	OC (2000 mg/kg)	PO (2000 mg/kg)
ALT (U/L)	32 ± 4	34 ± 5	49 ± 6*
AST (U/L)	45 ± 5	48 ± 5	63 ± 7*
Urea (mg/dL)	22 ± 3	23 ± 2	27 ± 4
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.2

*Statistically significant ($p<0.05$) compared to control.

These results suggest mild hepatotoxicity in the PO group, while OC showed minimal systemic disturbance.

36 Observations:

Initially the animals were observed after dosing at least once during the first half an hour,periodically during the first 24 h. In all cases death was observed within first 24 h. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and somato motor activity and behavior pattern. Attention was also given to observation of tremors and convulsions.

4. Discussion

The safety of herbal drugs is paramount in promoting their mainstream use. *Ocimum canum* extract demonstrated no observable toxicity in the acute setting and maintained near-normal biochemical and organ parameters. In contrast, *Platycladus orientalis* extract, while not lethal, exhibited mild hepatocellular stress as indicated by elevated transaminases. This could be attributed to terpenoids and essential oils present in higher concentrations, which may be metabolized to reactive intermediates.

Previous studies support the hepatoprotective and antioxidant nature of *Ocimum canum*, while certain compounds in *Platycladus orientalis*, although beneficial, may induce hepatic stress when consumed in high doses [16]

The findings suggest that although both plants are safe within therapeutic limits, careful dosing and long-term safety assessments are warranted, especially for PO formulations.

The safety of herbal drugs is paramount in promoting their mainstream use. Ocimum canum extract demonstrated no observable toxicity in the acute setting and maintained near-normal biochemical and organ parameters. In contrast, Platycladus orientalis extract exhibited mild hepatocellular stress as indicated by elevated transaminases, possibly due to terpenoids and essential oils present in higher concentrations.

ISSN: 0975-3583,0976-2833 VOL 8, ISSUE 4, 2017

Previous studies support the hepatoprotective and antioxidant nature of Ocimum canum, while certain compounds in Platycladus orientalis may induce hepatic stress when consumed in high doses. The findings suggest that careful dosing and long-term safety assessments are warranted, especially for PO formulations.Both Ocimum canum and Platycladus orientalis leaf extracts are relatively safe in acute settings up to 2000 mg/kg. However, Ocimum canum exhibits a better safety profile based on behavioral, biochemical, and organ weight parameters. Further sub-chronic and chronic studies are necessary to evaluate long-term safety and therapeutic index.

5. Conclusion

The acute oral toxicity study of Ocimum canum and Platycladus orientalis was conducted according to OECD guidelines. The plants were found safe (no mortality) even when given at a dose of 2000 mg/kg body weight. The study observed no signs of acute toxicity in any group up to 2000 mg/kg. However, rats treated with Platycladus orientalis extract displayed mild lethargy and reduced feed intake for 24–48 hours. No significant difference in weight gain was observed, and the liver-to-body weight ratio was slightly increased in PO-treated rats compared to OC.

The results suggest mild hepatotoxicity in the PO group, while OC showed minimal systemic disturbance. Observations showed that death was observed within the first 24 hours in all cases. Additional observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system activity and behavior pattern, and attention was also given to observation of tremors and convulsions.

References

1. World Health Organization. WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. Geneva: WHO; 2003.
2. World Health Organization. WHO Traditional Medicine Strategy: 2014–2023. Geneva: WHO; 2013.
3. Jhansee Mishra, Alok Kumar Dash and Deepak Kumar Dash (2014 Pharmacognostical, physiochemical and phytochemical comparative study of the constituent of anantidiabetic polyherbal formulation International Journal of PhytopharmacyVol. 4 (1), pp.33-36, Jan-Feb 2014
4. Rates SMK. Plants as source of drugs. Toxicon. 2001;39(5):603–13.
5. Ehiagbonare JE. Antibacterial and phytochemical analysis of Ocimum canum leaf. J Med Plant Res. 2010;4(4):282–5.

6. Venu G, Prasad NR, Karthikeyan R. Protective effect of Ocimum canum leaf extract against H_2O_2-induced DNA damage and apoptosis in HepG2 cells. Toxicol Rep. 2011;1:162–71.
7. Kim SJ, Kim JH, Kim HJ, et al. Anti-inflammatory and cytotoxic effects of components from Platycladus orientalis leaves. Arch Pharm Res. 2006;29(4):293–9.
8. Jackson JE, et al. Effect of solvent on the extraction of bioactive compounds. J Nat Prod. 1996;59(6):646–51.
9. OECD. Guidelines for the Testing of Chemicals: Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method. Paris: OECD Publishing; 2001.
10. CPCSEA. Guidelines for Laboratory Animal Facility. Ministry of Environment & Forest, Govt. of India; 2007.
11. Jhansee Mishra, Alok Kumar Dash and Deepak Kumar Dash (2014). Development and evaluation of polyherbal antidibetic Fast dissolving Tablet. International Journal of Pharmaceutical Science and Health Care. DOI: 10.7409/ijpp .4 (2):106-116
12. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother. 2011;2(2):74–9.
13. OECD. (2008). *Guidelines for the Testing of Chemicals, Section 4: Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure*. OECD Publishing.
14. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*, 4(2), 145–160.
15. Alok Kumar Dash, Jhansee Mishra(2015) A comparative study on phytochemical investigation and pharmacological screening of Platycladus orientalis and Ocimum canum with review of literature. International Journal of Phytopharmacy Vol. 5 (6), pp.130-138, Nov-Dec 2015.
16. Kim, S. J., Kim, J. H., Kim, H. J., et al. (2006). Anti-inflammatory and cytotoxic effects of components from *Platycladus orientalis* leaves. *Archives of Pharmacal Research*, 29(4), 293–299.
17. Venu, G., Prasad, N. R., & Karthikeyan, R. (2011). Protective effect of *Ocimum canum* leaf extract against H_2O_2-induced DNA damage and apoptosis in HepG2 cells. *Toxicology Reports*, 1, 162–171.