

Comparative study on some selected species of *Ocimum* genus on free radical scavenging activity and hepatoprotective activity against CCl₄ induced intoxication in rats

Myron Green¹, Rajeswara Rao Pragada^{2*}, Sambasivarao Ethadi², Bettaiya Rajanna¹

¹Alcorn State University, Mississippi, USA

²AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India

Email: *raoethadi@gmail.com

Received 9 July 2013; revised 10 August 2013; accepted 6 September 2013

Copyright © 2013 Myron Green *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

In this study, we compared the *in vitro* antioxidant property among the selected *Ocimum* species (*O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum*) and hepatoprotective activities of their extracts against CCl₄ induced intoxication. The results suggested that the four species of *Ocimum* genus showed variability in Phenolic content and *in vitro* antioxidant activity against DPPH, superoxide and hydroxyl radicals in the following manner: *O. sanctum* > *O. americanum* > *O. basilicum* > *O. gratissimum* respectively. Based on serum AST, ALT, ALP and T. Bil levels, the alcoholic extracts of *Ocimum* species showed a significant dose dependent (250 mg and 500 mg and 750 mg/kg p.o.) and a protective effect against CCl₄ induced hepatotoxicity in albino rats. The results further revealed that the potential hepatoprotective activity of *Ocimum sanctum* among the *Ocimum* species.

Keywords: *Ocimum sanctum*; *Ocimum americanum*; *Ocimum basilicum*; *Ocimum gratissimum*; Antioxidant Activity; Carbon Tetrachloride; Hepatotoxicity

1. INTRODUCTION

The liver is the most important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate substances and synthesize useful principles [1]. A major cause of these disorders is due to exposure to different environmental pollutants, viruses, and xenobiotics e.g., paracetamol, carbon tetrachloride,

thioacetamide, alcohol, etc. Reactive oxygen species are implicated in liver damage [2] through covalent binding and lipid peroxidation and have been shown to augment fibrosis as seen from increased collagen synthesis [3]. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues [4].

Ocimum sanctum (Family: Lamiaceae) is globally known for its immense medicinal properties, especially in India, and is popularly known as Tulsi. It is a holy plant common to most Indian households. *Ocimum sanctum* is known to possess anti-inflammatory, antimicrobial, anti-diabetic, hepatoprotective, cardioprotective, antioxidant, adaptogenic, anticancer and radioprotective activities. There are other species in the same genus (*Ocimum*) and its influence on hepatoprotective activity is not established scientifically. Hence, the selected four species *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum* were subjected to the estimation of total phenolic content, antioxidant activity and evaluated for hepatoprotective activity.

2. MATERIALS & METHODS

2.1. Plant Material and Extraction

The plants *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum* were collected in Visakhapatnam region, A.P, India and authenticity of the plants were confirmed by Taxonomist Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam. Freshly collected whole plants were dried under shade and were made into coarse powder. 400 gms of *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum* were macerated in 2.5 lts of 95% v/v ethanol. The alcoholic extracts thus obtained were dried under reduced pressure at a room tem-

*Corresponding author.

perature not exceeding 40°C to get the crude extracts.

2.2. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-Deoxyribose, Nitroblue tetrazolium, Silymarin purchased from Sigma Chemicals Co. (St. Louis, MO, USA), Folin-Ciocalteu reagent, Riboflavin, Hydrogen peroxide (H₂O₂), Carbon tetrachloride (CCl₄) from Loba Chemie Pvt Ltd. (Mumbai, India). AST, ALT, ALP and Total bilirubin assay kits from Span diagnostics Pvt Ltd. Gujarat, India. All other chemicals and reagents used were of analytical grade.

2.3. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200 - 250 gms were used in this study. The animals were maintained under standard laboratory conditions at an ambient temperature of 25°C ± 2°C having 50% ± 5% relative humidity with 12 h light and dark cycle. The rats were acclimatized to the laboratory conditions for one week prior to experimentation and provided with standard rat pellet diet (Rayans biotech Pvt Ltd., Hyderabad, India) and water *ad libitum*. The use and care of animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA), Government of India.

2.4. Total Phenolic Content

Total phenolic content of the extracts was determined by using Folin-Ciocalteu reagent method [5]. Folin-Ciocalteu colorimetry is based on a chemical reduction of the reagent with a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue colour with absorption maximum at 765 nm. By using standard Gallic acid calibration curve, measured the concentration of phenolic content in Gallic acid total equivalents using units mg/gm.

2.5. Superoxide Radical Scavenging Activity

Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich method, 1969 [6] which depends on light induced superoxide generation by riboflavin and the corresponding reduction of Nitroblue tetrazolium was measured spectrophotometrically at 560 nm.

2.6. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe²⁺/

EDTA/H₂O₂ system (fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS) [7].

2.7. DPPH Radical Scavenging Activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al.*, 2003 [8]. In DPPH assay, method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine.

2.8. CCl₄ Induced Intoxicated Rat Model

The animals were divided into 15 groups, each consisting of 6 animals. The standard and test group animals were treated with 100 mg/kg dose of Silymarin and 250, 500, 750 mg/kg dose of alcoholic extracts of selected plant drugs for 5 days. On the 6th day, 1 h after treatment with standard and test doses, the animals were intoxicated with CCl₄ in liquid paraffin (1:1 v/v, 1 ml of CCl₄/kg, p.o.). Serum was separated by centrifugation at 37°C and used for estimation of various biochemical parameters. Biochemical parameters like serum enzymes, Aspartate transaminase (AST) and Alanine transaminase (ALT) were estimated by Reitman and Frankel, 1957 method [9], Serum alkaline phosphatase (ALP) by King and Armstrong, 1980 method [10], Serum Total bilirubin (T.Bil) by Jendrassik and Grof, 1938 method [11] by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy).

2.9. Statistical Analysis

Results of the biochemical estimations are reported as Mean ± S.E.M. Significance was tested by One-Way analysis of variance (ANOVA) followed by Dunnett's post-hoc test using Graphpad Prism-5 software.

3. RESULTS

The selected four species of *Ocimum* genus showed variability in phenolic content in the following manner: *O. americanum* (70.0 µg/gm) > *O. sanctum* (56.2 µg/gm) > *O. gratissimum* (52.0 µg/gm) > *O. basilicum* (38.0 µg/gm). The alcoholic extracts of selected plants and Ascorbic acid showed antioxidant activity with IC₅₀ value on DPPH radical in the following manner: Ascorbic acid (60.24 ± 0.5 µg/ml) > *O. sanctum* (85.5 ± 0.7 µg/ml) > *O. americanum* (175.25 ± 1.5 µg/ml) > *O. basilicum* (220.30 ± 1.6 µg/ml) > *O. gratissimum* (230.35 ± 2.1 µg/ml), respectively (**Table 1**).

Similarly the IC₅₀ values of superoxide radical and

Table 1. Total Phenolic content of alcoholic extracts of *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum*.

Name of the alcoholic extract	GAE(Gallic acid equivalent) mg/gm
<i>O. sanctum</i>	56.2 ± 1.2
<i>O. americanum</i>	70.0 ± 2.2
<i>O. basilicum</i>	38.0 ± 2.1
<i>O. gratissimum</i>	52.0 ± 2.3

Each value represents as mean ± S.E.M (*n* = 3).

hydroxyl radicals are 80.24 ± 0.4 µg/ml (Ascorbic acid) > 225.23 ± 1.5 (*O. sanctum*) > 300.90 ± 2.1 µg/ml (*O. americanum*) > 350.33 ± 1.4 (*O. basilicum*) > 360.35 ± 1.3 µg/ml (*O. gratissimum*) and 190.20 ± 0.5 µg/ml (Ascorbic acid) > 140 ± 1.3 µg/ml (*O. sanctum*) > 200.90 ± 2.2 µg/ml (*O. americanum*) > 285.31 ± 1.2 µg/ml (*O. basilicum*) > 400.50 ± 1.3 µg/ml (*O. gratissimum*) respectively (**Table 2**).

Based on ALT levels, the percentage protection offered by the selected plant drugs at a dose of 750 mg/kg against CCl₄ is as follows: *O. sanctum* (83.72%), *O. americanum* (73.71%), *O. basilicum* (62.48%) and *O. gratissimum* (58.69%) respectively. The standard drug Silymarin produced 87.04% protection (**Table 3**).

4. DISCUSSION

The genus *Ocimum* consists of more than 30 species and the plants belonging to the genus contains numerous phytochemicals including phenolics. The selected four plant extracts *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum* contains phenolics ranging from 38.00 to 70.00 mg/gm gallic acid equivalents (GAE). Mostly the phenolics are possessing antioxidant activity and the plant extracts also showed good antioxidant activity. Among the four extracts *O. sanctum* showed better antioxidant activity.

Hepatotoxicity produced by CCl₄ seems to be mediated by reactive metabolite trichloro methyl free radical (.CCl₃) formed by the hemolytic cleavage of CCl₄ or even by more reactive species trichloro methyl peroxy free radical (Cl₃COO.) formed by the reaction of .CCl₃ with O₂ caused membrane damage of hepatocytes and resulted in centrilobular necrosis. The four plant extracts showed dose dependent hepatoprotective activity against CCl₄ induced hepatotoxicity and maximum percent protection was observed at a dose of 750 mg/kg. Among the four extracts *Ocimum sanctum* showed better activity and the results are comparable with that of the standard drug Silymarin. The mechanism of action may be due to its free radical scavenging (antioxidant) activity. Further the study revealed that it is not the quantity but the quality of phenolic compounds is important for showing good

Table 2. *In vitro* 50% inhibition concentration (IC₅₀) of alcoholic extracts of *O. sanctum*, *O. americanum*, *O. basilicum*, *O. gratissimum* and Ascorbic acid on free radicals scavenging activity.

Extracts/Compounds	Quantity of various extracts (µg)		
	Free radicals, reactive oxygen species		
	Superoxide Radical	Hydroxyl Radical	DPPH Radical
Alc. extract of <i>O. sanctum</i>	225.23 ± 1.5	140.0 ± 0.5	85.5 ± 0.7
Alc. extract of <i>O. americanum</i>	300.90 ± 2.1	200.90 ± 2.2	175.25 ± 1.5
Alc. extract of <i>O. basilicum</i>	350.33 ± 1.4	285.31 ± 1.2	220.30 ± 1.6
Alc. extract of <i>O. gratissimum</i>	360.35 ± 1.3	400.50 ± 1.3	230.35 ± 2.1
Ascorbic acid	80.24 ± 0.4	190.20 ± 0.5	60.24 ± 0.5

Each value represents as mean ± S.E.M (*n* = 3).

hepatoprotective activity and the same was proved by the good antioxidant activity of *Ocimum sanctum*.

5. CONCLUSIONS

The selected four species showed variability in phenolic content ranging from 38.00 to 70.00 mg/gm GAE. The selected plant extracts showed free radical scavenging activity in a dose dependent manner. Among the selected plants, *Ocimum sanctum* showed better free radical scavenging activity and the results are comparable with that of the standard drug Ascorbic acid. The four plant extracts showed hepatoprotective activity against CCl₄ induced intoxication. The study revealed the hepatoprotective activity of *O. americanum* for the first time and the results are comparable with that of *Ocimum sanctum*.

Based on ALT levels among the four extracts, *Ocimum sanctum* showed better activity (83.78 percent production) and the results are comparable with that of the standard drug Silymarin (87.04 percent protection). As per the superoxide and hydroxyl radical scavenging activity, the selected extracts showed good correlation between IC₅₀ value and percentage of hepatoprotection. Overall the study revealed the selected four plants possessing hepatoprotective activity and showed scopes for biomass utility.

6. ACKNOWLEDGEMENTS

The present work was carried out at Andhra University, Visakhapatnam, India in Minority International Research Training (MHIRT) programme-2010 awarded (NIH/NCMHD/MHIRT# 9T37 MD 001532) to Alcorn State University for their support and encouragement. The

Table 3. Effect of alcoholic extracts of *Ocimum* species on Serum parameters in rats intoxicated with CCl₄.

Treatment	Dose (p.o.)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	T.BIL (mg/dL)
Vehicle control	1 ml/kg	87.87 ± 2.6	45.33 ± 1.5	135.45 ± 4.9	0.10 ± 0.01
CCl ₄	1 ml/kg	247.48 ± 4.8	197.81 ± 5.0	363.51 ± 6.2	0.22 ± 0.01
Silymarin	50 mg/kg	112.03 ± 0.9 ^a (84.46)	65.09 ± 3.2 ^a (87.04)	196.42 ± 2.2 ^a (73.27)	0.13 ± 0.01 ^a (75.00)
	250 mg/kg	164.52 ± 2.3 ^a (51.98)	120.76 ± 2.2 ^a (50.53)	243.23 ± 2.8 ^a (52.74)	0.16 ± 0.01 ^a (50.00)
Alc. ext of <i>O. sanctum</i>	500 mg/kg	126.42 ± 1.7 ^a (75.85)	76.26 ± 3.6 ^a (79.72)	221.81 ± 2.8 ^a (62.13)	0.15 ± 0.01 ^a (58.33)
	750 mg/kg	110.18 ± 1.4 ^a (86.02)	70.06 ± 1.2 ^a (83.78)	175.46 ± 1.4 ^a (82.46)	0.13 ± 0.02 ^a (75.00)
	250 mg/kg	171.30 ± 2.3 ^a (47.73)	130.68 ± 2.4 ^a (44.03)	260.48 ± 1.8 ^a (45.18)	0.17 ± 0.01 ^a (41.67)
Alc. ext of <i>O. americanum</i>	500 mg/kg	144.10 ± 2.9 ^a (64.77)	101.16 ± 3.2 ^a (63.39)	250.25 ± 4.5 ^a (49.66)	0.16 ± 0.02 ^a (50.00)
	750 mg/kg	131.19 ± 1.0 ^a (72.86)	85.41 ± 1.7 ^a (73.71)	197.33 ± 1.0 ^a (72.87)	0.14 ± 0.02 ^a (66.67)
	250 mg/kg	185.08 ± 1.6 ^a (39.10)	141.42 ± 1.5 ^a (36.98)	276.42 ± 3.0 ^a (38.19)	0.18 ± 0.01 ^a (33.33)
Alc. ext of <i>O. basilicum</i>	500 mg/kg	155.79 ± 2.3 ^a (57.45)	112.80 ± 1.0 ^a (55.75)	259.09 ± 4.5 ^a (45.79)	0.16 ± 0.02 ^a (50.00)
	750 mg/kg	136.13 ± 1.0 ^a (69.76)	102.54 ± 1.3 ^a (62.48)	210.42 ± 1.1 ^a (67.13)	0.15 ± 0.02 ^a (58.33)
	250 mg/kg	189.01 ± 2.1 ^a (36.63)	150.24 ± 1.7 ^a (31.20)	281.93 ± 2.4 ^a (35.77)	0.18 ± 0.01 ^a (33.33)
Alc. ext of <i>O. gratissimum</i>	500 mg/kg	152.94 ± 3.3 ^a (59.23)	117.29 ± 2.6 ^a (52.81)	261.13 ± 6.7 ^a (44.89)	0.17 ± 0.01 ^a (41.67)
	750 mg/kg	148.40 ± 1.2 ^a (62.08)	108.32 ± 1.2 ^a (58.69)	240.10 ± 1.0 ^a (54.11)	0.14 ± 0.01 ^a (66.67)

^a*P* < 0.0001. All groups were compared with CCl₄ group. Values are mean ± S.E.M., *n* = 6 animals per group. Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 × (values of CCl₄ control—values of sample)/(values of CCl₄ control—values of control). *O. sanctum*—*Ocimum sanctum*; *O. americanum*—*Ocimum americanum*; *O. basilicum*—*Ocimum basilicum*; *O. gratissimum*—*Ocimum gratissimum*.

authors are also thankful to National Institute of Health (NIH), Maryland, USA for their financial assistance.

REFERENCES

- [1] Girish, S.A., Sudhir, G.W. and Avinash, K.D. (2004) Evaluation hepatoprotective effect of amalkadi ghrita against carbon tetrachloride-induced hepatic damage in rats. *Journal of Ethnopharmacology*, **90**, 229-232. <http://dx.doi.org/10.1016/j.jep.2003.09.037>
- [2] Sreelatha, S., Padma P.R. and Umadevi, M. (2009) Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced Hepatotoxicity in rats. *Food and Chemical Toxicology* **47**, 702-708. <http://dx.doi.org/10.1016/j.fct.2008.12.022>
- [3] Geesin, J.C., Gordon, J.S. and Bergand, R.A. (1990) Retinoids affect collagen synthesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts. *Archives of Biochemistry and Biophysics*, **278**, 350-355. [http://dx.doi.org/10.1016/0003-9861\(90\)90270-9](http://dx.doi.org/10.1016/0003-9861(90)90270-9)
- [4] Thresiamma, K.C. and Kuttan, R. (1996) Inhibition of liver fibrosis by ellagic acid. *Indian Journal of Physiology and Pharmacology*, **40**, 363-366.
- [5] Singleton, V. L. and Rossi, J. A. (1965) Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144-158.
- [6] McCord J.M. and Fridovich, I. (1969) Superoxide dismutase: An enzymic function for erythrocyte hemocuprien. *Journal of Biological Chemistry*, **244**, 6049-6055. [http://dx.doi.org/10.1016/0378-5173\(90\)90201-E](http://dx.doi.org/10.1016/0378-5173(90)90201-E)
- [7] Elizabeth Kunchandy. and Rao, M.N.A. (1990) Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, **58**, 237-240. [http://dx.doi.org/10.1016/S0378-8741\(03\)00043-6](http://dx.doi.org/10.1016/S0378-8741(03)00043-6)
- [8] Braca, A., Fico, G., Morelli, I., De Simone, F., Tome, F. and De Tommasi, N. (2003) Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *Journal of Ethnopharmacology*, **86**, 63-67.
- [9] Reitman, S. and Frankel, S. (1957) Determination of serum glutamate oxaloacetate and glutamate pyruvic acid transaminase. *American Journal of Clinical Pathology*, **28**, 56-63.
- [10] King, E.J. and Armstrong, A.R. (1934) Determination of serum and bile phosphatase activity. *Canadian Medical Association Journal*, **31**, 56-63.
- [11] Jendrassik, L. and Gróf, P. (1938) Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins. *Biochemische Zeitschrift*, **297**, 82-89.