Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Bioactive Compound of *Ocimum sanctum* Carvacrol Supplementation Attenuates Fluoride Toxicity in Sodium Fluoride Intoxicated Rats: A Study with Respect to Clinical Aspect

Kondeti Ramudu Shanmugam[1], Mavulapati Siva[1,2], Sahukari Ravi[2], Bhasha Shanmugam[2], Kesireddy Sathyavelu Reddy[2]

[1]Department of Zoology, TRR Government Degree College, Kandukur, Prakasam, [2]Division of Molecular Biology and Ethanopharmacology, Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

Submitted: 02-09-2018 Revised: 04-10-2018 Published: 26-04-2019

ABSTRACT

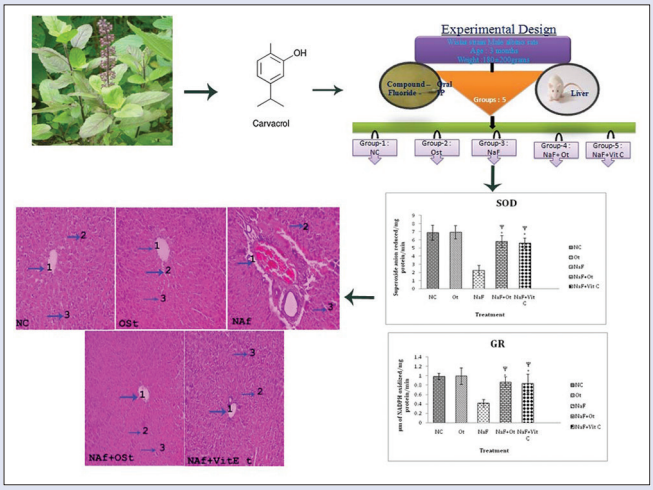
Background: *Ocimum sanctum* (OS) Linn. commonly known as Holy Basil or Tulsi is an Ayurvedic herb of India. The culinary, medicinal, and industrial importance of this plant led to explore its chemical and pharmacological properties. **Objective:** The present study was carried out to know the anti-oxidant activity of carvacrol bioactive compound of OS in sodium fluoride (NaF) rats and free-radical scavenging activities of OS. **Materials and Methods:** Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH), GSH-s-transferase (GST), malonaldehyde (MDA), alanine aminotransferase (AAT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), calcium (Ca), and phosphorus (P_4) levels are estimated in all experimental groups. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), and hydroxyl radical activities are analyzed in the ethanolic extract of OS. **Results:** SOD, CAT, GPx, GR, GSH, activities, and Ca levels depleted and GST, MDA, AAT, AST, ALKP, and P_4 levels elevated in NaF intoxicated rats. Whereas carvacrol supplementation normalized all the antioxidant enzymes and hepatic markers in NaF toxicity rats. DPPH, H_2O_2, and hydroxyl radical of OS showed potent free-radical scavenging activities. In addition, histopathological studies also prove that carvacrol protected the liver tissue from fluoride toxicity in rats. **Conclusion:** The present study revealed that carvacrol of OS modulated the antioxidant enzymes and hepatic stress markers in NaF rats. Our research study will be helpful in the development of new active principle and nutraceuticals in the area of drug resistance and therapeutic compounds against disease vectors.

Key words: Antioxidant enzymes, carvacrol, free radical scavenging activity, *Ocimum sanctum*, rats

SUMMARY

- To summarize, the current investigation clearly confirmed that carvacrol bioactive compound of *Ocimum sanctum* protects the hepatic tissue from sodium fluoride toxicity.

Abbreviations used: OS: *Ocimum sanctum*; NaF: Sodium fluoride; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GR: Glutathionereductase; GSH: Glutathione; GST: Glutathione-s-transferase; MDA: Malonaldehyde; AAT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALKP: Alkaline phosphatase; Ca: Calcium; P_4: Phosphorus; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; H_2O_2: Hydrogen peroxide; TLC: Thin-layer chromatography; HPLC: High-performance liquid chromatography; EDTA: Ethylenediaminetetra acetic acid; $FeCl_3$: Ferric chloride; TCA: Trichloroacetic acid; TBA: Tert-Butyl alcohol; NaOH: Sodium hydroxide; BHA: Butylated hydroxyanisole; SPSS: Statistical package for the social sciences; GC-MS: Gas chromatography–Mass spectrometry; TBARS: Thiobarbituric acid-reactive substances.



Correspondence:

Dr. Kondeti Ramudu Shanmugam,
Department of Zoology, TRR Government Degree College, Kandukur, Prakasam - 523 105, Andhra Pradesh, India.
E-mail: drshanmugam2016@gmail.com

DOI: 10.4103/pm.pm_436_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Medicinal plants play a central role in traditional medicines and are precursors for the modern pharmaceuticals and allopathic medicines.[1] The scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many human health problems, including diabetes, cancer, and infectious diseases.[2,3] The medicinal value of plants lies in the bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds that produce a definite physiological action against diseases.

Cite this article as: Shanmugam KR, Siva M, Ravi S, Shanmugam B, Reddy KS. Bioactive compound of *Ocimum sanctum* carvacrol supplementation attenuates fluoride toxicity in sodium fluoride intoxicated rats: A study with respect to clinical aspect. Phcog Mag 2019;15:S144-9.

Ocimum sanctum (OS) is known as Holy Basil in English or Tulasi in Hindi, belongs to the family Lamiaceae and is considered as a sacred plant in Hindu culture.[4] OS Linn. has been widely known for its medicinal value for thousands of years.[5] Folklore claims that OS have antiseptic, antimicrobial, anti-bactericidal, anti-inflammatory, anti-oxidative, antiulcer, antidiarrheal, and chemopreventive properties.[6,7]

Carvacrol ($C_6H_3[OH][C_3H_7]$) is a natural component found in OS. Carvacrol, is a monoterpenoid phenol. It has antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, anti-inflammatory, angiogenic, and antiparasitic properties.

Fluorosis is a global disease. Humans take fluoride from water, food, and air, which can develop into dental, skeletal, and nonskeletal fluorosis. The clinical manifestations of fluorosis are mainly teeth and bone damage, but organs and soft tissues such as the liver, kidney, nervous system, reproductive system, blood vessels, and muscles may also be damaged by this disease.

The objective of this study was to investigate the antioxidant, hepatoprotective activity, of carvacrol in NaF intoxicated rats. This is the first report on the effect of carvacrol of OS in fluorosis rats.

MATERIALS AND METHODS

Selection and collection of plant material

Leaves of OS were collected from Tirupati, Andhra Pradesh, India and identified by the Dr. Madhav Chetty, Department of Botany, S. V. University, Tirupati. Voucher specimen of OS No 1183 was deposited in the Botany department.

Extraction of bioactive compound

The bioactive compound carvacrol of OS was extracted by different methods. OS was grinded and add ethanol. Filter the mixture and dry it, by using a rotoevaporator, the mixture is then washed with hexane. This, in turn, gets washed again, with ethyl acetate. At this point, the mixture is dried again, this time using a rotoevaporator and freeze-drying to ensure complete dryness. Once the mixture is completely dry, the mixture is again treated with hexane and ethyl acetate. At this point, thin-layer chromatography (TLC) is performed. After the TLC, the compound is dissolved in 99% ethanol and is further purified using TLC.

The compound is soaked in ethyl acetate, once more. After filtering and drying, it is subjected to high-performance liquid chromatography (HPLC). It is this last process, HPLC that enables the identification of many compounds. Of which, carvacrol is isolated, but check the carvacrol compound with standard compound and used for the study [Figure 1].

Chemicals and reagents

All chemicals used were of analytical grade and were purchased from Sigma–Aldrich, Merck and all chemicals and reagents were used without further purification.

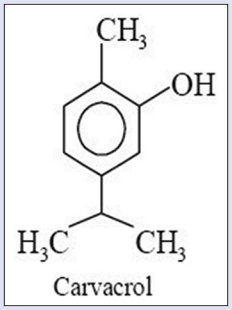


Figure 1: Structure of Carvacrol bioactive compound of *Ocimum sanctum*

Preparation of extracts

Leaves of the plant were air-dried for several days. The dried plant materials were ground to a coarse powder using a dry mill. For the extraction procedure, 500–800 g of powdered plant material was soaked in 95% ethanol for 72 h. The solvent was then removed by rotary evaporation. The dried extract was used for the study of free radical scavenging activities.

Grouping of animals

The rats were divided into five groups of six rats in each group and treated as follows:

- Group I – Normal Control (NC): Six rats were received saline through orogastric tube for 60 days
- Group II – Ocimum Bioactive compound (Carvacrol) Control (OSt): Six rats received OS bioactive compound Carvacrol (25 mg/kgBW) for 60 days
- Group III – NaF treatment (Na F t): Six rats received the sodium fluoride (NaF) (10 mg/kgBW) dissolved in drinking water for 60 days
- Group IV – NaF + Ocimum Bioactive compound (Carvacrol) treatment (NaF + OSt): Six rats received NaF (10 mg/kgBW) dissolved in drinking water and ocimum bioactive compound carvacrol (25 mg/kgBW) for 60 days
- Group V – Na F + Vitamin C treatment (NaF + Vitamin C): six rats received NaF and Vitamin C for 60 days.

After completion of 60 days treatment and after 24 h of the last treatment the animals were sacrificed by cervical dislocation and the liver tissue was excised at 4°C. The tissues are washed with ice cold saline and immediately stored in a deep freezer at −80°C for further biochemical analysis. Blood samples are also collected, serum was separated and stored for biochemical estimations.

Biochemical estimations

The selected antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH), GSH–s– transferase (GST), and malonaldehyde (MDA), levels were monitored by the methods of Misra and Fridovich,[8] Aebi,[9] Flohe and Gunzler,[10] Carlberg and Mannervik,[11] Akerboom and Sies,[12] Habig *et al.*,[13] and Ohkawa *et al.*,[14] respectively. Alanine aminotransferase (AAT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) activities and Calcium (Ca), phosphorus (P_4) levels are measured using standard kits.

The experiments were carried out in accordance with guidelines Approved by the institutional Animal Ethics Committee (ResolutionNo. 10/08/a/CPCSCA/IAEC/SVU/09-10/ZOOL/KRS/Dt. 25.09.2009) in its resolution number (9/IAEC/SVU/2001/dt. 4.03.2002).

Estimation of free radical scavenging activity

The hydroxyl radical scavenging activity of OS was determined by the method of Halliwell *et al.*[15] The reaction mixture consisted of 1 mM Ethylenediaminetetra acetic acid (EDTA), 10 mM Ferric chloride, 10 mM H_2O_2, 10 mM deoxyribose, 1 ml of different dilutions of the extract (100–500 µg/ml), 50 mM phosphate buffer (pH 7.4), and 0.1 ml of ascorbic acid in sequence. The mixture was incubated at 37°C for 1 h. A volume of 1 ml portion of the incubated mixture was mixed with 1 ml of 10% Trichloroacetic acid and 1 ml of 0.5% Tert-Butyl alcohol (TBA) (in 0.025 M Sodium hydroxide containing 0.025% butylated hydroxyanisole) to develop the pink chromogen measured at 532 nm.

The ability of OS to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch *et al.*[16] A solution of H_2O_2 (2 mM) was prepared in phosphate buffer (0.1 mM, pH 7.4) at 20°C. An aliquot of the extracts was dissolved in phosphate buffer (1 ml; 0.1 mM, pH 7.4) at

various concentrations (100–500 µg/ml) and mixed with 600 µl of H_2O_2. Ascorbic acid was used as the reference standard. The concentration of H_2O_2 was measured by reading the absorbance at 230 nm after 10 min against a blank solution containing phosphate buffer without H_2O_2. The extracts were capable of scavenging H_2O_2 in a concentration-dependent manner.

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of OS. Different concentrations of each ethanolic extract were added and an equal volume of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm.[17]

Statistical analysis

The data are expressed as mean values with their standard deviation Readings of the five different groups were compared using one-way ANOVA analysis with Dunnett's multiple comparison test. Statistical analysis was performed using the Statistical package for the social sciences (SPSS) (Version 13.5; SPSS Inc., Chicago, IL, USA). Using M. S. Office, excel software the data have been analyzed for the significance of the main effects (factors), and treatments along with their interactions. Differences were considered statistically significant at $P < 0.001$.

RESULTS

Free radical scavenging effect

In DPPH test, the ability of a compound to act as a donor for hydrogen atoms or electrons was measured spectrophotometrically. The scavenging activities of DPPH exerted by OS ethanolic extract and each extract as well as ascorbic acid are summarized in Figure 2. The ethanolic extract at the concentration of 100, 200, 300, 400, and 500 µg/mL exhibited 54.2%, 65.5%, 72.4%, 76.58%, and 86.9% inhibition, respectively, where standard drug ascorbic acid at the same concentration exhibited 63.07%, 68.37%, 78.46,% 87.73%, and 90.89% inhibition, respectively. In the present investigation, OS at different doses demonstrated significant DPPH scavenging activity indicating their abilities to act as radical scavengers.

The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA at low pH form a pink chromogen. Any hydroxyl radical scavenger added to the reaction would compete with deoxyribose for the availability of hydroxyl radicals, thus reducing the amount of MDA formation. We herein tested the scavenging activity of each extract along with positive control ascorbic acid. OS at concentration 100, 200, 300, 400, and 500 µg/mL inhibited the production of hydroxyl radicals by 40.2%, 62.19%, 70.46%, 81.24%, and 83.68%, while at same concentration, ascorbic acid showed 57.47%, 63.02%, 71.53%, 89.71%, and 83.23%, respectively [Figure 2]. In the current study, OS at different doses demonstrated significant hydroxyl radical scavenging activity.

The ethanolic extract at the concentration of 100, 200, 300, 400, and 500 µg/mL exhibited 31.2%, 52.46%, 62.84%, 71.4% and 82.6% inhibition, respectively, where standard drug α-tocopherol at the same concentration exhibited 23.07%, 68.37%, 72.46%, 76.73%, and 90.89% of H_2O_2 inhibition, respectively. The composition of H_2O_2 into water may occur according to the antioxidant compounds as the antioxidant component present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O. Our results showed that the H_2O_2 scavenging activity of OS decreases than that of α-tocopherol.

Effect of carvacrol on hepatic antioxidant enzymes in fluorosis rats

In the present investigation, SOD, CAT, GPx, GR, activities, GSH levels are depleted in fluoride intoxicated rats. However, with carvacrol supplementation in NaF rats, we observed the significant elevation of these anti-oxidant enzymes. However, GST, MDA levels significantly increased in fluoride intoxicated rats, but carvacrol treatment decreased GST and MDA levels in NaF rats [Figure 3].

Effect of carvacrol of on serum markers and fluorosis markers in fluorosis rats

In the current study, we observed that AAT, AST, ALKP activities, and P_4 levels are elevated and Ca levels depleted in NaF intoxicated rats. This

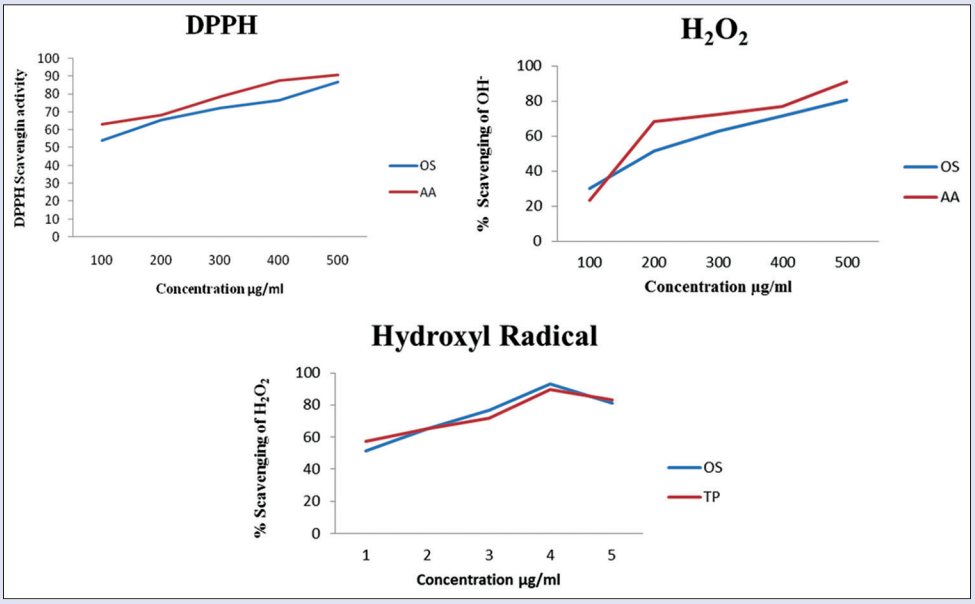


Figure 2: Free radical scavenging activity (Hydrogen peroxide, Hydroxy Radicals and 2,2-Diphenyl-1-picrylhydrazyl) of ethanolic extract of *Ocimum sanctum* with ascorbic acid and α tocopherol as standards

indicates severe liver damage in fluorosis condition. Carvacrol treatment for 60 days significantly modulated all the liver markers in fluorosis rats [Table 1].

Effect of carvacrol on liver tissue in fluorosis rats

In the current study, we observed the degeneration of central vein, hepatocytes, sinusoids, and blood coagulation in NaF intoxicated rats. However, with carvacrol treatment regeneration of central vein, hepatocytes, and sinusoids are observed in NaF rats. Thus, carvacrol supplementation protects the liver tissue from NaF toxicity in rats [Figure 4].

DISCUSSION

The present study was carried out to know the free radical scavenging effect of OS and antioxidant activity of the bioactive compound of OS Carvacrol in NaF intoxicated rats.

DPPH assay method is based on the ability of 1,1-diphenyl-2-picrylhydrazyl, a stable free radical, to decolorize in the presence of antioxidants.[18] DPPH has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. Antioxidants react with DPPH and convert it to 1-1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potential of OS. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants.[19] The study reported the free-radical scavenging activity of ethanolic extract of OS.[20] The standard antioxidant ascorbic acid has showed rich DPPH radical scavenging activity at lesser concentrations than the ethanolic extract of OS [Figure 2].

H_2O_2 is endogenously produced in the cell, regulates various important physiological processes.[21] Nevertheless, it is overabundance in a cell causes cellular damage because of its interaction with cellular metal ions generates highly reactive hydroxyl radicals. H_2O_2 changed into the water in the presence of electron donor by cellular enzymes and external antioxidants. The ethanolic extract of OS converted the H_2O_2 into the water on concentration-dependent manner. The increased concentrations of the extract have shown an increase in H_2O_2 scavenging activity [Figure 2].

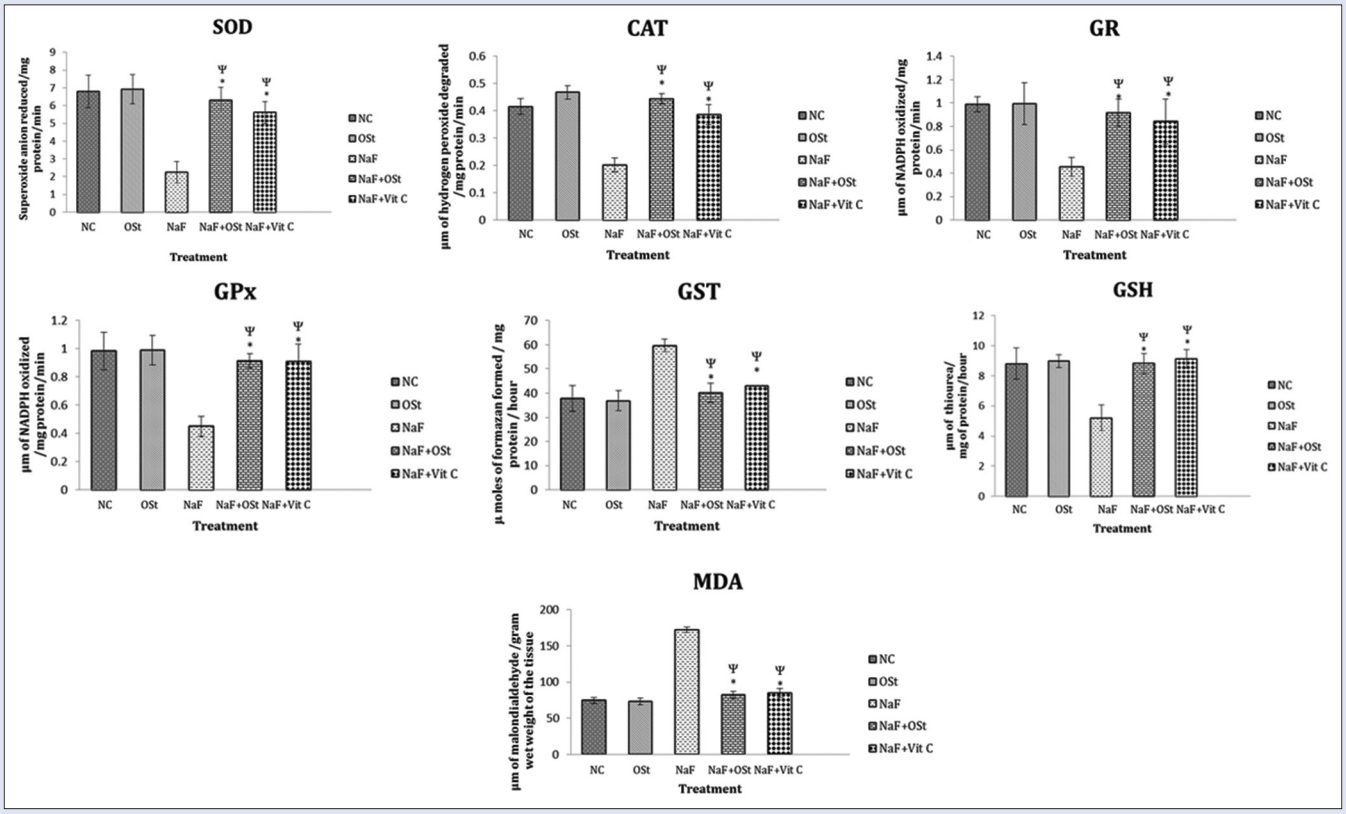


Figure 3: Effect of Carvacrol on superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione-s-transferase, glutathione and malonaldehyde levels in the liver tissues of Normal and Fluorosis rats. Data are expressed as means ± standard deviation ($n = 6$). *The values are significant compared to the following: control (*$P < 0.001$), Fluorosis (*$P < 0.01$) (Dunnett's multiple comparison tests)

Table 1: Effect of carvocral on liver markers and fluorosis markers in sodium fluoride rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Ca (mg/dL)	PP (mg/dL)
Group I (NC)	586.82 (±12.48)	94.12 (±1.68)	414 (±8.26)	11.31 (±0.62)	14.24 (±0.24)
Group II (OSt)	545 (±11.26)	117.535 (±2.14)	385 (±10.24)	11.46 (±0.22)	12.46 (±0.16)
Group III (NaFt)	606.442* (±6.48)	101.62* (±8.68)	614* (±11.268)	8.2* (±0.0.72)	16.82* (±0.036)
Group IV (NaF + Ot)	572.267* (±4.62)	87.48* (±4.52)	380* (±8.42)	10.84* (±0.082)	13.14* (±0.032)
Group V (NaF+Vitamin C)	66.731* (±3.14)	84.189* (±4.12)	151* (±08.14)	11.22* (±0.12)	13.86* (±0.064)

All the values are means±SD of six individual observations. *Significant at $P<0.001$ with respect to normal control. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; Ca: Calcium; PP: Phosphorus; SD: Standard deviation

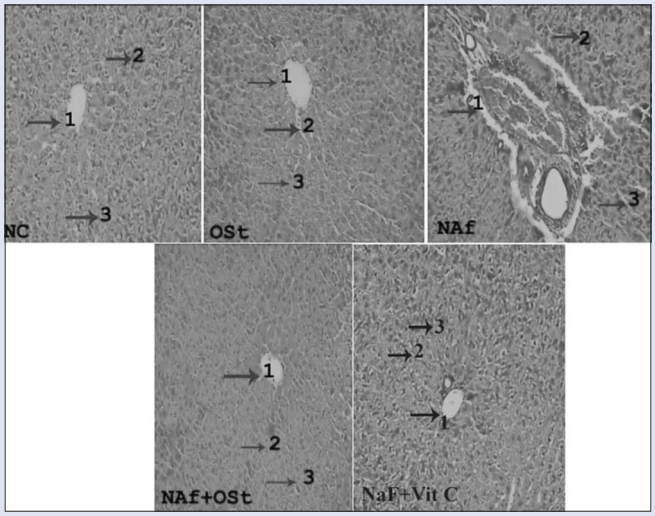


Figure 4: Effect of Carvacrol on Liver tissue in Fluorosis rats. (1) Normal Control (Nc). (i) Normal central vein, (ii) Normal hepatocytes, (iii) Normal sinusoids. (2) Carvocral treatment (OSt): (i) Nomal cenntral vein, (ii) Normal hepatocytes, (iii) Normal sinusoids. (3) Sodium fluoride (i) Degeneration of central vein, (ii) Degeneration of hepatocytes, (iii) Degeneration of sinusoids. (4) Sodium fluoride + Carvocral treatment (sodium fluoride + OSt) showed (i) Regeneration of central vein, (ii) Regeneration of hepatocytes, (iii) Regeneration of sinusoids. (5) Sodium fluoride + Vitamin C treatment (sodium fluoride + Vitamin C) (i) Regeneration of central vein, (ii) Regeneration of hepatocytes, (iii) Regeneration of sinusoids

Overall comparison of radical scavenging properties of ethanolic extract with standard ascorbic acid and α tocopherol showed that the OS has shown lower activity than standard, though it has a huge number of antioxidant compounds (found in GC–MS analysis). This difference could be due to the presence of individual antioxidant compounds in the extract which are lower at given concentrations, whereas ascorbic acid and α-tocopherol as standard and completely individual compound; hence, it ultimately showed rich antioxidant activity at tested concentrations than ethanolic extract.

The antioxidant property of active compounds in the ethanolic extract of OS was confirmed by DPPH radical, Hydroxyl, and H_2O_2 assays. The importance of these tests is likely to evaluate the antioxidant activity of OS.[22]

In the present investigation, the antioxidant enzymes SOD, CAT, GPx, GR, and GSH are depleted in NaF intoxicated rats. This shows that NaF supplementation for 60 days causes depletion of these antioxidant enzymes. This may be due to the oxidative stress and free radical production in NaF rats. However with Carvacrol of Ocimum treatment in NaF rats for 60 days reversed these antioxidant enzymes. Carvacrol may be responsible for the upregulation of antioxidant enzymes in NaF rats. Carvacrol treatment significantly recuperated the activities of antioxidant enzymes in NaF intoxicated rats [Figure 3].

Carvacrol supplementation may decrease the production of free radicals in fluorosis rats; this may cause up-regulation of antioxidant enzymes. Carvacrol treatment also decreased oxidative damage in NaF intoxicated rats. Hence, all the hepatic antioxidant enzymes are normalized with carvacrol treatment in NaF rats.

Oxidative stress is recognized to be associated with more than 100 diseases such as diabetes, hepatitis, and other diseases. There is a strong correlation between TBARS as a marker of lipid peroxidation and products that reflect oxidative damage to DNA. MDA and GST levels elevated in NAF rats. Oxidative stress in fluorosis rats may be responsible for upregulation of MDA and GST. However, carvacrol treatment, MDA and GST levels depleted in NaF rats [Figure 3].

Liver damage induced by NaF generally reflects disturbances of liver cell metabolism which lead to changes in the activities of many enzymes. AAT, AST, and ALKP are sensitive markers of liver toxicity and their elevated levels are indicative of cellular leakages and loss of functional integrity of cell membrane in the liver. In the current study, we reported significant elevation in AAT, AST, and ALKP activities in NaF intoxicated rats. This shows that during fluorosis condition hepatic cellular dysfunction may result in the upregulation of the liver markers. Whereas carvacrol supplementation depleted serum markers in NaF rats. This show the hepatoprotective activity of carvacrol. Carvacrol could minimize the production of toxic free radicals and enhance the activities of their scavengers, diminishing hepatocellular injury in NaF rats [Table 1].

Ca and P_4 are fluorosis markers. In our study, we reported that Ca levels decreased and P_4 levels increased in NaF intoxicated rats. This may be due to alterations in the metabolism of Ca and P_4. Whereas carvacrol treatment for 60 days, these fluorosis markers are came back to near to normal levels.

In the present study, we reported that fluorosis rat liver showed the degeneration of central vein, hepatocytes, sinusoids, and blood coagulation. However with carvacrol treatment regeneration of central vein, hepatocytes, and sinusoids are observed in NaF rats. This shows the hepatoprotective effect of carvacrol [Figure 4].

The antioxidant and free radical scavenging activities of OS might be due to the presence of phenolic compounds in extracts which is confirmed by phytochemical analysis. Furthermore, it can be concluded that the antioxidant activity of plant extracts is not limited to phenolics. Activity may also due to other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins.[23]

To summarize, the current investigation clearly confirmed that carvacrol bioactive compound of OS protects the hepatic tissue from NaF toxicity. In this study, we reported that antioxidant enzymes and liver markers are modulated with carvacrol treatment in NaF-induced fluorosis rats. This may be due to the free radical scavenging activity of Ocimum. Our gas chromatography–mass spectrometry analysis also confirmed the presence of many bioactive compounds in Ocimum and these compounds may have pharmacological activities. To the best of our knowledge, this study is the first report on the effect of Carvacrol of Ocimum in fluorosis rats.

Acknowledgment

The authors are grateful to the University Grants Commission, (UGC), New Delhi, India for the sanction of Major Research Project UGC MR-Major-Zol 2013-36027 dated 15/10/2015.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sindhia VR, Bairwa R. Plant review: *Butea monosperma*. Int J Pathol Clin Res 2010;2:90-4.
2. Gupta SS. Prospects and perspectives on natural plant products in medicine. Indian J Pharmacol 1994;26:1-2.
3. Shanmugam KR, Mallikarjuna K, Kesireddy N, Sathyavelu Reddy K. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol 2011;49:893-7.

4. Khare CP. Indian Medicinal Plants. An Illustrated Dictionary. Berlin: Springer-Verlag; 2007.
5. Soni A, Sosa S. phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharmacogn Phytochem 2013;2:22-9.
6. Prakash J, Gupta SK. Chemopreventive activity of *Ocimum sanctum* seed oil. J Ethnopharmacol 2000;72:29-34.
7. Siva M, Shanmugam KR, Shanmugam B, Venkata SG, Ravi S, Sathyavelu RK, *et al. Ocimum sanctum*: A review on the pharmacological properties. Int J Basic Clin Pharmacol 2016;5:558-65.
8. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.
9. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
10. Flohe L, Gunzler WA. Assays of glutathione peroxidise. Methods Enzymol 1984;105: 114.
11. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985;113:484-90.
12. Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 1981;77:373-82.
13. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
15. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 1987;165:215-9.
16. Ruch RJ, Chug SU, Klaunig JE. Spin trapping of superoxide and hydroxyl radicals. Methods Enzymol 1984;105:198-209.
17. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13:8-17.
18. Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother Res 2007;21:615-21.
19. Jao CH, Ko WC. 1, 1-Dipheny1-2 picrylhydrazyl (DPPH) radical scavenging by protein hydrolysates from tuna cooking juice. Fish Sci 2002;68:430-5.
20. Kath RK, Gupta RK. Antioxidant activity of hydroalcoholic leaf extract of *Ocimum sanctum* in animal models of peptic ulcer. Indian J Physiol Pharmacol 2006;50:391-6.
21. D'Autréaux B, Toledano MB. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 2007;8:813-24.
22. Abu Bakar M, Mohamed M, Rahmat A, Fry J. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem 2009;113:479-83.
23. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem 2003;83:547-50.