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## ORIGINAL ARTICLE

### IN VIVO ANTIOXIDANT ACTIVITY OF ORTHOSIPHON STAMINEUS IN STREPTOZOTOCIN INDUCED IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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### Abstract

**Objective of the study:** The present study aims to investigate in vivo antioxidant activity of *Orthosiphon stamineus*. Plant extracts were prepared using successive solvent extraction process using petroleum ether, chloroform and ethanol. **Methods:** Type 2 diabetic rats were treated with extracts at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg for 21 days and tested for changes in thiobarbituric acid reactive substances (TBARS), conjugated dienes, catalase and peroxidase levels. **Results:** Significant increase in TBARS and conjugated dienes and reduction in catalase and peroxidase were observed. All the extracts could significantly decrease TBARS and conjugated dienes; significantly increase catalase and peroxidase levels. Dose dependent and potent antioxidant effect was observed with ethanolic and petroleum ether extracts. The results of this study highlighted the antioxidant potential of *O. stamineus* and can be useful for protecting oxidative damage of pancreatic cells. **Conclusion:** The study highlighted that the mechanisms can prevent damage of other organs and is beneficial for prevention of diabetic associated complications.

**Key words:** *Orthosiphon stamineus*, Type 2 diabetes, in vivo antioxidant activity, TBARS, Conjugated dienes, Catalase, Peroxidase.

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## INTRODUCTION

Type 2 Diabetes mellitus is becoming the prime cause of morbidity and mortality in the world population. Type 2 diabetes is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. Reactive oxygen species are the by products in type 2 diabetes, generated during protein glycation and as a consequence of advanced glycation end products receptor binding; they impair insulin signalling pathways and induce cytotoxicity in pancreatic beta cells.(1) This had drawn importance of antioxidants role in treatment of diabetes and its associated complications. Herbal remedies contain large amounts of antioxidants such as flavonoids, polyphenols, vitamin C and E, Carotinoids.(2,3) Research studies reported that antioxidants plays important role in treatment of diabetes and its associated complications. Orthosiphon stamineus is one of the promising plants for treatment of various diseases and disorders. O.stamineus was reported for in vitro antioxidant (4,5,6), treatment of renal stones and gout(7,8,9); anti-inflammatory and analgesic activities (10), antimicrobial (11,12), hepatoprotective (13,14,15), hypoglycemic and antihyperlipidemic properties.(16,17) In addition, it is rich in phenolic compounds.(18) There were no reports for its in vivo antioxidant activities in type 2 diabetic rats. Hence we have undertaken this study to evaluate its petroleum ether, chloroform and methanolic fractions for their in vivo antioxidant activities in streptozotocin induced type 2 diabetic rats.

## Materials and methods

Collection of plant material: The roots of Orthosiphon stamineus were collected washed with water and shade dried. They were pulverized using a ball mill until a coarse powder is obtained. Authentication of plant was carried out by our taxonomist and plant specimen is preserved in our herbarium (KLU46048).

Preparation of plant extracts: Roots powder was subjected to successive solvent extraction

in Soxhlet apparatus for 72 hours with the solvents in the order of increasing polarity. We have used petroleum ether, chloroform and ethanol for extraction. Then the collected concentrates were filtered and the filtrates were evaporated to dryness using vacuum evaporator under reduced pressure. The obtained extracts were freeze dried and preserved in a vacuum desiccator.

In vivo antioxidant activity; All the three extracts prepared were tested for in vivo antioxidant activity. Male Sprague-Dawley rats were used for this research study. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee. Animals were divided into various groups of six each and were fed with standard diet and water ad libitum. They were kept in clean and dry cages and maintained in well-ventilated animal house with 12 h light-12 h dark cycle. Type 2 diabetes was induced in overnight fasted rats by a single intraperitoneal (IP) injection of 65 mg/kg streptozotocin (STZ) was followed after 15 minutes with IP administration of 230 mg/kg nicotinamide.(19) STZ was dissolved in sodium citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycaemia was confirmed by the elevated glucose levels in plasma after 72 h. The animals having blood glucose concentration of more than 180 mg/dl were selected and used for the study. Animals were divided into five groups consisting of six rats in each group. Treatments were given as below

Animals were divided into five groups of six rats each.

Group I: normal control rats received the vehicle (1% Gum acacia suspension).

Group II: Diabetic control rats received the vehicle (1% gum acacia suspension)

Group III: Diabetic rats were administered extract (250 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Group IV: Diabetic rats were administered extract (500 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Group V: Diabetic rats were administered extract (1000 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Each extract was tested according to the above protocol and doses for 21 days. Only petroleum ether extract was administered using Tween 80 as additional surfactant to enable the extract miscible in the prepared suspension.

All the rats were fasted for 16 hrs before experimentation, but allowed free access to water. Twenty-four hours after the last treatment animals were sacrificed. Liver and kidneys were dissected out and stored at  $-20^{\circ}\text{C}$  for the quantification of the levels of the products of free radicals conjugated diene (CD) (20) and thiobarbituric acid reactive substances (TBARS) (21) and for the assessment of the activities of the antioxidant enzymes catalase (CAT)(22) and peroxidase.(23)

### Statistical analysis

Data is expressed as mean  $\pm$  standard error of mean. Statistical analysis was done using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnet's t-test. P values  $<0.05$  were considered significant.

## RESULTS AND DISCUSSION

Orthosiphon atamineus roots were extracted successfully with petroleum ether, chloroform and ethanol. The yield was 8.6%, 12.1% and 14.9%/Kg of the dried leaves respectively.

The results of the in vivo antioxidant effect of petroleum ether, chloroform and ethanolic extracts of *O. stamineus* on type 2 diabetic rats are presented in tables 1,2,3,4,5 & 6. Levels of TBARS and conjugated dienes were increased significantly and catalase and peroxidase levels were decreased significantly in diabetic rats. The petroleum ether and ethanolic extracts produced dose dependent decrease in the MDA levels (TBARS) in the serum. The serum MDA levels of the groups treated with 250 mg/kg of extract was significantly ( $p<0.05$ ) lower when compared to diabetic control group. The extracts

produced dose dependent decrease in serum levels of MDA and maximum effect ( $p<0.01$ ) were observed in 1000 mg/kg treated rats. Conjugated diene levels were reduced significantly in extract treated rats and maximum effect was observed when treated with 1000 mg/kg dose. Significant decrease ( $p<0.05$ ) in the serum level of catalase dismutase levels were observed in 250 mg/kg dose treated rats. Dose dependent reduction in serum catalase dismutase was observed and was significantly ( $p<0.01$ ) higher in rats treated with 1000 mg/kg dose of both extracts when compared with normal control group. Significant increase ( $p<0.05$ ) in the serum level of catalase activity was observed in 250 mg/kg dose treated rats with both the extracts. Dose dependent serum catalase activity was observed and was significantly ( $p<0.01$ ) higher in rats treated with 1000 mg/kg dose when compared with control group. Significant increase ( $p<0.05$ ) in the serum peroxidase levels was observed in 250 mg/kg dose treated rats with both the extracts. Dose dependent rise in peroxidase levels was observed and was significantly ( $p<0.01$ ) higher in rats treated with 1000 mg/kg dose when compared with control group. These effects are prominently observed in both liver and kidney tissues.

The chloroform extract produced a significant decrease in the MDA levels (TBARS) in the serum when treated with high dose ( $p<0.05$ ). Conjugated diene levels were reduced significantly in extract treated rats when treated with 1000 mg/kg dose ( $p<0.05$ ). The extract could not produce dose dependent decrease in serum levels of MDA, conjugated dienes and significant effect when compared with normal control group rats. Significant decrease ( $p<0.05$ ) in the serum level of catalase dismutase levels was observed in 1000 mg/kg dose treated rats ( $p<0.05$ ). Dose dependent reduction in serum catalase dismutase was not observed in rats treated with various doses of extracts when compared with control group. Significant increase ( $p<0.05$ ) in the serum level of catalase activity was observed in 1000 mg/kg dose treated rats. Dose dependent serum catalase activity was not observed in rats

treated with various doses of extracts when compared with control group. Significant increase ( $p < 0.05$ ) in the serum peroxidase levels was observed in 1000 mg/kg dose treated rats. Dose dependent rise in peroxidase levels not observed in rats treated with various doses of extracts when compared with control group.

Thiobarbituric acid reactive substances (TBARS) and conjugated dienes are the indicators of oxidative injury and lipid peroxidation. The results shown by the petroleum ether and ethanolic extracts indicated protective role of oxidative damage in both kidney and liver tissues.(25) Diabetic complications such as atherogenesis, coronary heart failure, and nephropathy display causal relationship with oxidative stress and lipid peroxidation. The extracts could reduce the levels of TBARS and conjugated dienes in both kidney and liver tissues. Catalase (CAT) considered as most important  $H_2O_2$  removing enzyme and also a key component of antioxidative defense system.  $H_2O_2$  is the product of conversion of oxygen radical ( $O_2^-$ ) by superoxide dismutase.  $H_2O_2$ , a secondary type of reactive oxygen species are still toxic. It is also more stable than  $O_2^-$  and it can pass through cell membranes more easily than  $O_2^-$ .  $H_2O_2$  can also react with  $O_2^-$  to form the  $OH^-$  ion, which is more toxic than  $H_2O_2$  and  $O_2^-$ .

.(26) CAT is needed for the conversion of  $H_2O_2$  to a non-toxic substance (water), to protect the cell from the negative effects of ROS. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage.(27) Hence the extracts improved significantly CAT and peroxidase levels in kidney and liver tissues and these effects are important for prevention of detrimental effects of reactive oxygen species. Among the extracts ethanolic extract produced effect has no significant difference with normal rats values ( $P < 0.05$ ). In this study, *O.stamineus* possessed in vivo antioxidant activity. It will be beneficial for preventing oxidative stress induced free radical damage in diabetic condition. This mechanism will be useful for further deterioration of pancreatic cells. In addition, the extracts can be beneficial for prevention of free radical mediated diabetic associated complications such as diabetic nephropathy, retinopathy and cardiomyopathy. Formation of reactive oxygen species is a direct consequence of hyperglycemia.(28) This plays an important role in development and progression of diabetic vascular complications including nephropathy.(29) Hence this study had highlighted the possible beneficial effects of *O.stamineus* roots.

Parameters	Normal Control	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed /15 min/mg tissue)	3.44±0.09	5.08±0.29	4.64±0.24*	4.12±0.19**	3.87±0.21**
CD (nM/mg tissue)	318.60±8.51	512.90±19.58	465.09±18.12*	411.29±11.56*	379.63±16.25**
CAT (tissue/mg)	7.04±0.10	5.02±0.18	5.68±0.12*	5.92±0.14*	6.31±0.11**
Peroxidase (mM of $H_2O_2$ consumption/ gm of tissue)	9.98±0.16	5.93±0.27	6.39±0.16*	6.95±0.09*	7.41±0.15**

**Table 1:** Effect of petroleum ether extract of *O.stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated kidney tissue after 21 days of experimental study.

Parameters	Normal Control	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	2.19± 0.14	3.72± 0.14	3.27± 0.11*	2.82± 0.10**	2.64± 0.16**
CD (nM/mg tissue)	205.14± 9.088	415.37± 21.58	379.25± 24.01*	318.02± 18.64**	265.75± 23.15**
CAT (tissue/mg)	9.48± 0.29	4.24± 0.38	6.88± 0.62*	8.60± 1.04**	9.95± 1.55**
Peroxidase (mM of H <sub>2</sub> O <sub>2</sub> consumption/gm of tissue)	15.09± 0.44	8.05± 1.62	10.68± 1.22*	12.99± 1.15**	14.36± 1.19**

\* $p < 0.05$  when compared with diabetic control group. \*\* $p < 0.01$  when compared with diabetic control group

**Table 2:** Effect of petroleum ether extract of *O. stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidise in isolated liver tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	5.08±0.29	4.95±0.26	4.81±0.18	4.41±0.12*
CD (nM/mg tissue)	512.90±19.58	495.21±14.75	481.35±24.08	450.62±18.41*
CAT (tissue/mg)	5.02±0.18	5.08±0.14	5.25±0.16	5.41±0.14*
Peroxidase (mM of H <sub>2</sub> O <sub>2</sub> consumption/gm of tissue)	5.93±0.27	6.04±0.11	6.29±0.24	6.48±0.16*

**Table 3:** Effect of chloroform extract of *O. stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidise in isolated kidney tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	3.72±0.14	3.64±0.08*	3.55±0.16	3.32±0.09*
CD (nM/mg tissue)	415.37±21.58	402.14±21.54	392.18±19.90	345.29±17.10*
CAT (tissue/mg)	4.24±0.38	4.60±0.25	4.97±0.62	5.88±0.28 *
Peroxidase (mM of H <sub>2</sub> O <sub>2</sub> consumption/gm of tissue)	8.05±1.62	8.92±0.92	9.61±1.04	10.79±0.84*

\* $p < 0.05$  when compared with diabetic control group.

**Table 4:** Effect of chloroform extract of *O.stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidise in isolated liver tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	5.08±0.29	4.52±0.21*	3.99±0.08**	3.35±0.14**
CD (nM/mg tissue)	512.90±19.58	462.39±14.28*	405.62±19.35**	337.83±17.42**
CAT (tissue/mg)	5.02±0.18	5.69±0.18*	6.05±0.12**	7.12±0.23**
Peroxidase (mM of H <sub>2</sub> O <sub>2</sub> consumption/gm of tissue)	5.93±0.27	6.37±0.14*	6.77±0.28**	7.08±0.16**

\* $p < 0.05$  when compared with diabetic control group. \*\* $p < 0.01$  when compared with diabetic control group.

**Table 5:** Effect of ethanolic extract of *O.stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidise in isolated kidney tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>O. Stamineus</i> (250 mg/kg)	<i>O. Stamineus</i> (500 mg/kg)	<i>O. Stamineus</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	3.72±0.14	3.24±0.07*	2.68±0.05**	2.14±0.14**
CD (nM/mg tissue)	415.37±21.58	380.14±19.04*	330.63±16.39**	294.55±21.44**
CAT (tissue/mg)	4.24±0.38	6.41±0.37*	8.02±0.94**	9.63±1.14**
Peroxidase (mM of H <sub>2</sub> O <sub>2</sub> consumption/gm of tissue)	8.05±1.62	10.24±0.87*	12.95±1.15**	14.04±0.99**

\* $p < 0.05$  when compared with diabetic control group. \*\* $p < 0.01$  when compared with diabetic control group.

**Table 6:** Effect of ethanolic extract of *O.stamineus* on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated liver tissue after four weeks of experimental study.

## CONCLUSION

*O.stamineus* ethanolic extract and petroleum ether extracts produced significant lowering of TBARS and conjugated dienes and dose dependent effect was observed. They could also increase the levels of catalase and peroxidase. Ethanolic extract could produce potent effect in type 2 diabetic rats.

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