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EVALUATION OF INVIVO ANTIOXIDANT ACTIVITY OF *VANDA TESSELLATA* ROXB. IN ALBINO WISTAR RATS

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ABSTRACT

Vanda tessellata Roxb. (Family: Orchidaceae) plants have been used in the indigenous medicine such as Ayurveda and local traditional medical practices. To investigate the antioxidant activity of the petroleum ether extract of *Vanda tessellata Roxb.* (PVT) was investigated in rats with carbon tetrachloride (CCl_4) induced erythrocyte damage. Simultaneous administration of the PVT (200 and 400 mg/kg body weight/day i.p) with carbon tetrachloride (1ml/kg of body weight) to rats for alternate days of two weeks protected the loss of functional integrity and membrane lipid alteration in red blood cells induced by oxidative stress. PVT inhibited the accumulation of lipid peroxidation products in the plasma as well as maintained the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. The PVT further had the ability to decrease the membrane fluidity induced by carbon tetrachloride. It can therefore be suggested that the PVT possess an erythrocyte protective activity against drug induced oxidative stress. These findings also provide a rationale for further studies on isolation of active principles and its pharmacological evaluation.

Keywords: Antioxidant enzymes, Carbon tetrachloride, Lipid peroxidation. *Vanda tessellata*, PVT, Erythrocyte Damage.

INTRODUCTION

Serious attention is now paid to the cytotoxicity of active oxygen/free radicals as the cause of various pathological conditions. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration [1]. It is widely accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias [2].

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Free radicals such as hydroxy radicals, superoxide anion radicals and singlet oxygens are agents that attack the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation [3]. It is thought that, if the in vivo activity of enzymes or scavengers is not high enough to inhibit these radicals, various diseases such as arteriosclerosis, liver disease, diabetes, inflammation, renal failure or accelerated aging may result [4].

Lipid peroxidation is also strongly associated with aging and carcinogenesis [5]. However, living systems are protected from active oxygen species by enzymes such as superoxide dismutase, glutathione peroxidase and catalase. These living systems have also been reported to receive non-enzymatic protection by endogenous antioxidants such as α-tocopherol, ascorbic acid, β-carotene, and uric acid [6].

Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions. There is a worldwide trend toward the use of natural antioxidants. The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest [7].

Vanda tessellata Roxb. (Family: Orchidaceae) plants have been used in the indigenous medicine such as Ayurveda and local traditional medical practices. The leaf juice is used for the treatment of certain inflammatory conditions. It is also instilled into the ear as a remedy for otitis. The leaves in the form of a paste are applied to the body to bring down fever. The *leaves* are used in rheumatism, nervous problems, bronchitis, dyspepsia and fever. Unani practitioners hold it to be laxative and tonic to the liver. It is also used to treat hiccough, piles, boils on the scalp, etc. This plant *leaves* is reported to contain an alkyl perulate and beta sistosterol -D- glucoside. The dried whole herb also contains long chain alkanes and alkanol sistosterol, resin, saponin, tannins, fatty acids, colouring agents, etc. Medicinal orchids, in general, are not subjected to detailed pharmacological studies. Scientific studies on medicinal orchids can lead to the development of invaluable drugs to certain medical conditions [8].Therefore, to justify the traditional claims the present study was undertaken to find out if petroleum ether extract of *Vanda tessellata Roxb.* leaves demonstrates the antioxidant activity against CCl_4 induced rats models of erythrocyte damage using lipid peroxidation and the antioxidants superoxide dismutase (SOD) and catalase as biomarkers. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant collection

The leaves of *Vanda tessellata* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of August 2011 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

Leaves of *Vanda tessellata* were shade dried, and the dried leaves were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using Petroleum ether. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10%w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.

Animals used

Adult albino rats (Wistar strain) of either sex with weighing 150 - 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water *ad libitum*. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from Institutional ethical committee has been taken to carry out and complete this study.

Acute Toxicity Study

The acute toxicity of petroleum ether extract of *Vanda tessellata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [9].

Experimental design

Body weight of animals was recorded and then they were divided into 6 groups of 6 rats each Propylene glycol (PG) was used as a carrier of PVT extracts (200 and 400 mg/kg body weight/day) as well as for carbon tetrachloride (1 ml/kg body weight), administered intraperitoneally alternate days for 14 days. The following experimental groups were used: Group I, II, III, IV, V and VI were received distilled water + PG (Normal control), PVT 200mg/kg + PG (Herb control), PVT 400mg/kg + PG (Herb control), Carbon tetrachloride in PG, PVT 200mg/kg + carbon tetrachloride in PG, PVT 400mg/kg + carbon tetrachloride, in PG respectively. On the 15^{th} day rats were kept fasting for 12 hours and sacrificed by cervical dislocation. Blood was collected from the jugular vein into tubes containing heparin, centrifuged at 3000 rpm for 15 min and the resulting buffy coat removed. The packed cells were washed three times with physiological saline (0.9% NaCl), lysed by suspending them in cold distilled water, and then centrifuged at 7000 rpm for 30 min. The resulting pellet contained the erythrocyte membrane and the supernatant represented the haemolysate.

Biochemical estimation

Plasma resulting from the initial centrifugation was used for measuring lipid peroxidation following the method of Gutteridge and Wilkins [10] while the haemolysate was used for the estimation of superoxide dismutase [11] and catalase [12] activities. Lipids from the erythrocyte membrane were extracted using the method of Folch et al [13]. The concentration of cholesterol and phospholipids were determined using previously established methods [14,15]. The cholesterol/phospholipid ratio was then calculated.

Statistical analysis

The data were expressed as mean ± standard error

mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

The results of preliminary phytochemical screening of the petroleum ether extract of *Vanda tessellata Roxb.* revealed that presence of alkaloids, flavonoids, glycosides, steroid, tannins, saponins, terpeniods and absence of steroids.Table-1 shows the effect of PVT on carbon tetrachloride induced oxidative stress. Treatments with the extracts significantly (P <0.01) prevented the accumulation of lipid peroxidation products in the plasma. Intoxication of the rats with carbon tetrachloride also led to significant increases in superoxide dismutase and catalase activities, while simultaneous administration of carbon tetrachloride with the PVT significantly (P <0.01) decreased these activities.

Intoxication with carbon tetrachloride causes an increase in membrane cholesterol, a decrease in membrane phospholipid and a subsequent increase in the cholesterol to phospholipid ratio. At the doses of PVT 200 & 400 significantly (P<0.01) decreases the cholesterol, phospholipids and cholesterol/phospholipid ratio also (Table 2).

Table 1. The effects of *PVT* on lipid peroxidation products and primary antioxidant enzymes of the erythrocytes of carbon tetrachloride -intoxicated rats

Group	Design of treatments	Lipid peroxidation x 10^{-6} (units)	Enzyme activities (Units/mg protein)	
			Superoxide dismutase	Catalase
I	Control, PG	0.24 ± 0.01	195.41 ± 3.2	1.7 ± 0.2
II	PVT 200mg/kg + PG	0.24 ± 0.07	194.74 ± 4.7	1.6 ± 0.2
III	PVT 400mg/kg + PG	0.22 ± 0.02	185.43 ± 3.3	1.6 ± 0.4
IV	CCl_4 + PG	0.44 ± 0.04[a]	260.47 ± 2.4[a]	4.4 ± 0.3[a]
V	CCl_4 + PVT 200mg/kg	0.34 ± 0.07[*b]	228.33 ± 2.4[*b]	3.22± 0.4[*b]
VI	CCl_4 + PVT 400mg/kg	0.32 ± 0.06[**b]	210.25 ± 1.2[**b]	2.33 ± 0.2[**b]

PG=Propylene glycol; Carbon tetrachloride= CCl_4; PVT=Petroleum ether extract of *Vanda tessellata Roxb.*
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. **a** means Comparison between Group IV Vs Group I, II & III. **b** means Comparison between Group V and VI Vs Group I, II, III & IV.
*p<0.05;** p<0.01; ns-non significant.

Table 2. Effect of *PVT* on erythrocyte membrane lipids and cholesterol/phospholipid ratio of carbon tetrachloride -intoxicated rats

Group	Design of treatments	Cholesterol (mg/100µl)	Phospholipid (mg/100µl)	Cholesterol /Phospholipid
I	Control, PG	0.60 ± 0.02	1.04 ± 0.04	0.62 ± 0.04
II	PVT 200mg/kg + PG	0.61 ± 0.04	1.15 ± 0.02	0.64 ± 0.02
III	PVT 400mg/kg + PG	0.62 ± 0.02	1.04 ± 0.02	0.56 ± 0.01
IV	CCl_4 + PG	0.82 ± 0.04[a]	0.82 ± 0.04[a]	0.94 ± 0.04[a]
V	CCl_4 + PVT 200mg/kg	0.71 ± 0.01[*b]	0.94 ± 0.05[*b]	0. 72 ± 0.04[*b]
VI	CCl_4 + PVT 400mg/kg,	0.62 ± 0.02[**b]	0.95 ±0.01[**b]	0.81 ± 0.02[**b]

PG=Propylene glycol; Carbon tetrachloride= CCl_4; PVT=Petroleum ether extract of *Vanda tessellata Roxb.*
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test. **a** means Comparison between Group IV Vs Group I, II & III. **b** means Comparison between Group V and VI Vs Group I, II, III& IV. ; *p<0.05;** p<0.01; ns-non significant

DISCUSSION AND CONCLUSION

The results obtained in this study indicate the rigidity of the membranes. Administration of PVT prevented changes in membrane lipids as well as those in membrane fluidity. It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases [16]. Under normal physiological conditions low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed due to cell damage. Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase normally challenge oxidative stress. In this study, carbon tetrachloride damage to erythrocytes was confirmed by the increases in lipid peroxidation products, superoxide dismutase and catalase activities, and decreases in membrane fluidity. The

increased superoxide dismutase activity resulted in the accumulation of hydrogen peroxide, which stimulated increases in catalase activity. Pre-treatment of experimental animals with the PVT exhibited an improved free radical scavenging resulting in decreased activities of superoxide dismutase and catalase, and the concentration of lipid peroxidation products towards normal.

The cumulative effect of carbon tetrachloride resulted in increases in erythrocyte membrane peroxidation, which may also lead to hemolytic changes. It has been shown that micro-viscosity of a membrane increases markedly with increases in cholesterol to phospholipid ratio thus leading to cellular rigidity [17]. Intoxication of experimental animals with carbon tetrachloride altered membrane structure and function as shown by the increases in cholesterol and subsequent decreases in phospholipid concentrations, hence increased cholesterol to phospholipid ratio. Cooper et al [18] reported that alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system. Thus *Vanda tessellata* plays a role in peroxidation by inhibiting the free radical attack on bio-membranes.

Since reactive oxygen species are involved in stress and pathogenesis of cancer, diabetes mellitus, atherosclerosis, and dementia, the use of this plant may be beneficial in preventing initiation or progress of such disorders. Efforts are in progress in our laboratory to isolate and purify the active principle involved in the antioxidative efficacy of this medicinal plant.

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