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HEPATOPROTECTIVE ACTIVITY OF PET-ETHER EXTRACT OF *VANDA TESSELLATA* ROXB

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ABSTRACT

Petroleum-ether extract of *Vanda tessellata* Roxb leaf given orally at doses of 100, 200 and 400 mg/kg exhibited significant dose dependent hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Hepatotoxicity and its prevention were assessed by serum markers viz. cholesterol, triglycerides, alanine amino transferase and alkaline phosphatase.

Keywords: Hepatoprotective activity, Petroleum ether extract, CCl_4

INTRODUCTION

Vanda tessellata Roxb is a species of orchid occurring from the Indian subcontinent to Indo china. It is a medicinal epiphytic perennial; stem 30-60 cm long, stout, scandent by the stout, simple or branching aerial roots. Leaves succulent, 15-20 cm, long, linear, recurved, complicate. Flowers in 6-10 flowered racemes, reaching with the peduncle 15-25 cm long. Sepals yellow, tessellated with brown lines and with white margins. Petals yellow with brown lines and white margins, shorter than the sepals.

Vanda tessellata plants have been used in *Ayurveda* and local traditional medical practices[1].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[2]. The roots were used in rheumatism, nervous problems, bronchitis and dyspepsia[3]. It is also used to treat hiccough, piles and boils on the scalp. *V. tessellata* has not been evaluated in depth for its pharmacological properties, in spite of its traditional use in numerous medical conditions[4]. It is also a remedy for secondary syphilis and scorpion-sting. The roots possess significant anti-inflammatory activity. The plant has an alkaloid, glycoside, tannins, β-sitosterol, γ-sitosterol and a long chain aliphatic compound, fatty oils, resins and colouring matters. Roots contain tetracosyl ferrulate and β-sitosterol-D-glucoside[5]. It also enters the composition of several medicated oils for external application in rheumatism and diseases of the nervous system. Roots were reported to possess antibacterial and anti-tubercular properties[6]. The steroidal fraction obtained from *V. tessellata* possessed significant anti inflammatory activity against acute inflammation induced by carrageenan, serotonin and formaldehyde[7].

The methanol extract of this plant root also showed remarkable anti-inflammatory activity against carrageenan – induced oedema in rodents[8]. Lawler reported that several *Ayurvedic* preparations containing this plant (root or whole plant) were used as aphrodisiac[9]. However, no study was done on its hepatoprotective activity of petroleum-ether extract of *V. tessellata*. Therefore, the present study has been designed to investigate the petroleum-ether extract of *V. tessellata Roxb* for its hepatoprotective activity.

Silymarin is the drug of choice for liver cirrhosis and alcoholic liver diseases which is supported by number of studies[10]. Silymarin is mixture of flavonolignans from the fruits of *Silybum marianum* that has been known since ages and recommended in traditional European and Asian medicine mainly for liver disorders[11]. Hence in the present study silymarin was used as control to compare the efficacy of petroleum-ether extract of *V. tessellata Roxb* against CCl_4 – induced hepatotoxicity.

MATERIALS AND METHODS

The leaves of *V. tessellata Roxb* were procured from the local market and authenticated by botanist. A voucher specimen is deposited in the department of Pharmacology, Prince Salman Bin Abdul Aziz University.

Preparation of extract: *V. tessellata* leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w.

Animals: Wistar rats (150-175 g) were procured from the institutional animal house. The animals had free access to the standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions, and they were maintained at a room temperature of 25 ± 1°C; a relative humidity of 45-55% and in 12:12 light/dark cycles. All the experiments were conducted in strict compliance according to the ethical principles and the guidelines which were provided by CPCSEA. The study protocol was approved by the institutional animal ethical committee.

Acute toxicity study: Acute oral toxicity studies were performed according to OECD guidelines[12]. Female Wistar rats were used to determine the LD_{50} of petroleum-ether extract of *V. tessellata Roxb*. Tween-80 1% v/v was used as vehicle to suspend the petroleum-ether extract. The petroleum-ether extract was administered in a dose of 2g/kg orally to a group of 3 rats. The animals were observed for changes in autonomic or behavioral responses for 6 hrs.The animals were kept under observation for 14 days to detect any mortality. The petroleum-ether extract were found to be non-toxic up to dose of 2g/kg body weight.

Induction of hepatic injury: Hepatic injury was induced in rats by subcutaneous administration of a single dose of 0.3 ml/kg CCl_4 mixed with equal volume of olive oil on the 7th day, 2h after the last treatment[13]. Animals were grouped as:

Group I:	Control group, treated with vehicle (2.0 ml, p.o.) daily for 7 days, followed by olive oil treatment (0.3 ml s.c.) on day 7.
Group II:	Treated with vehicle (2.0 ml, p.o) daily for 7 days, followed by CCl_4 on day 7
Group III:	Treated with silymarin (25 mg, p.o.) daily for 7 days, followed by CCl_4 on day 7
Group IV,V,VI:	Treated with petroleum-ether extract of *Vanda tessellata Roxb* suspended in propylene glycol at doses of 100, 200, 400 mg/kg daily for 7 days followed by CCl_4 on day 7 respectively.

On day 9, 48h after CCl_4 administration, blood sample of each animal was taken from abdominal aorta under Pentobarbitone anaesthesia (35 mg/kg i.p) and serum cholesterol, [14] triglycerides,[15] alanine amino (ALT),[16] and alkaline phosphatase (SAP),[17] were evaluated.

Statistical analysis

All values are expressed as mean± SD. The results were calculated and subjected to analysis of variance (ANOVA) followed by student t – test.

Table 1: *Effect of V. tessellata on CCl4 – induced liver damage in rats*

Groups	Treatment (mg/kg) p.o. for 7 days	Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)	ALT (mg/dl)	SAP (U/I)
Group I [(a)]	Vehicle	41.10±1.21	89.69±1.00	54.34±1.65	69.24±1.42
Group II	CCl_4 (0.3ml s.c.)	101.76±3.27[b]	76.40±1.03[b]	451.00±8.89[b]	148.29±6.22[b]
Group III	Silymarin/25	34.57±1.08***	89.60±1.36***	109.7±2.71***	78.64±1.20***
Group IV	*V. tessellata* 100mg/kg	98.46±1.73	88.10±1.11**	243.52±6.95***	118.74±2.23*
Group V	*V. tessellata* 200mg/kg	79.61±1.09**	87.46±1.22***	162.12±3.67***	97.41±1.23***
Group VI	*V. tessellata* 400mg/kg	66.95±1.39***	86.99±0.87***	137.03±1.80***	82.30±1.04***

N=6; Values are expressed as mean±S.D; ***P≤0.001, **P≤0.01,*P≤0.05 in comparison to CCl_4 – treated group; [a] On day 7, in place of CCl_4 challenge, the animals were administered olive oil 0.3 ml s.c.; [b] P≤0.001 in comparison to propylene glycol – treated group

RESULTS AND DISCUSSION

It is well known that carbon tetrachloride is converted by Cytochrome P – 450 mixed function oxygenises in smooth endoplasmic reticulum of liver into toxic metabolite, mainly trichloromethyl radical (CCl_3). This free radical in the presence of oxygen may causes peroxidation of lipids on target cell resulting in extensive damage.[18]

Administration of CCl_4 (0.3 ml s.c.) to rats produced hepatotoxicity showed by significant increase in the serum level of ALT and SAP as well as altered lipid profile in comparison to control group as shown in the Table 1.

Petroleum ether extract of *Vanda tessellata Roxb* given at doses of 100, 200, 400 mg/kg not only prevented the rise in serum level of ALT and alkaline phosphatase but also improved serum lipid profile in a dose dependent manner.

Silymarin, a well known hepatoprotective drug showed results comparable to that reported in the results.[19]

The extract of Petroleum-ether extract of *Vanda tessellata Roxb* confers significant protection against CCl_4 induced liver injury and probable mechanism is by maintenance of structural integrity of hepatocyte cell membrane and may be due to the ability to suppress the oxidative degradation of DNA[20].

The results indicate that the Petroleum-ether extract of *V. tessellata Roxb* has significant hepatoprotective activity in albino rats with CCl_4 liver injury.

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