Protective Effect of Ethanol Extract of *Vanda tessellata* Leaves on Methotrexate Induced Hepatotoxicity and Nephrotoxicity in Rats

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**Abstract**

Drugs and chemicals are considered as one of the major causes for hepatotoxicity and renal toxicity, inducing direct damage through multiple pathways including oxidative stress. The present study was aimed to investigate the protective effect of ethanol extract of *Vanda tessellata* leaves in methotrexate induced hepatotoxicity and nephrotoxicity rats. The ethanol extract of *Vanda tessellata* leaves were prepared and its protective effect were evaluated for methotrexate induced liver and kidney damaged in rats. The administration of ethanol extract of *Vanda tessellata* (150 and 300 mg/kg) significant (p<0.05) decrease in serum SGOT, SGPT, ALP, ACP and total bilirubin compared to control group rats treated MTX. Treating with ethanol extract of *Vanda tessellata* showed significant decrease (p<0.05) in concentration of serum urea, Creatinine, Uric acid, Total protein and BUN compared to MTX treated groups. The findings of biochemical parameters illustrated that the ethanol extract of *Vanda tessellata* leaves showed protective properties against MTX induced hepatotoxicity and nephrotoxicity in rats.

**Keywords:** *Vanda tessellata*, Methotrexate, Hepatoprotective, Nephroprotective

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1 Introduction

Methotrexate (MTX) is a stable derivative of aminopterin and it is a first folic acid antagonist. MTX is commonly used for the healing of numerous malignancies, multiple sclerosis, dermatomyositis, sarcoidosis, psoriasis, rheumatoid arthritis and various inflammatory diseases. The MTX kill the cancer cell, but it can also affect the normal tissues. Hence the prolonged use of this drug causes toxicity to different organs of body. The major chronic side effects of MTX administration are nephrotoxicity and hepatotoxicity.

Clinically, hepatotoxicity and nephrotoxicity stays one of the noteworthy restrictions on its utilization in the doses desired. The MTX are associated with oxidative stress and produces reactive oxygen species, along with reduced antioxidant defense mechanism promote the development and progression of hepatotoxicity and nephrotoxicity. The different studies mentioned that the MTX-induced hepatotoxicity and nephrotoxicity are originated by the inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and inducible nitric oxide synthase (iNOS)

*Vanda tessellata* Roxb. (Family: Orchidaceae) an epiphytic perennial herb, scandent by the stout and having simple or branching aerial roots distributed throughout Bangladesh, Indian subcontinent to Indochina. It is a medicinal epiphytic perennial. It is locally known as Rasna, has been used in folk medicine for its multifarious medicinal properties (Fig. 1). The leaves juice is used for the treatment of certain inflammatory conditions. It is also instilled into the ear as a remedy for Otitis and the paste as febrifuge. 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, liver toxicity, kidney dysfunction, bronchitis and dyspepsia by traditional healers. It is also used to treat hiccough, piles and boils on the scalp. Roots is utilized in stiffness and allied disorders. It is an incredible panacea for oligomenorrhea, amenorrhea and dysmenorrheal, as it increases uterine contractions. It is additionally solution for auxiliary syphilis...
and scorpion sting. Unani specialists hold it to be diuretic and tonic to the liver.

*Vanda tessellata* has not been evaluated in depth for its pharmacological properties, in spite of its traditional use in numerous medical conditions. The plant has an alkaloid, glycoside, tannins, β-sitosterol, γ-sitosterol and a long chain aliphatic compound, fatty oils, resins and colouring matters. Till date, no scientific study was done on its hepatoprotective and nephroprotective activity of extract of *Vanda tessellate* leaves. Therefore, the present study was designed to investigate the hepatoprotective and nephroprotective activity of ethanol extract of *Vanda tessellata* leaves, toxicity caused by methotrexate in experimental animals.

![Image of Vanda tessellate plant](image-url)

**Fig 1: Image of Vanda tessellate plant**

### 2 Material and Methods

#### 2.1 Plant material

The fresh leaves of *Vanda tessellata* were collected and authenticated by an authority in plant taxonomy. The leaves were shade dried and made coarsely powdered for extraction purpose.

#### 2.2 Extract preparation

The powdered leaves of *Vanda tessellata* were extracted with ethanol (95%) through soxhlet extraction method. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. After that ethanol extract of leaves was kept in air tight container for further study.

#### 2.3 Animals

The animals were carried for experiment from the authorised animal house of Bhopal Nobles College of Pharmacy, Undaipur (RJ), India. All Wistar albino rats were healthy and 150 gm to 220 gm of body weight. The animals were kept in air conditioning environment and temperature was maintained to 25±2 °C with conventional laboratory food and fresh drinking water. The bedding of animals was changed every 3rd day.

#### 2.4 Methodology

The rats were randomly divided into four groups (6 rats per group) as follows:

- **Group 1:** Control group receive normal saline until termination of the experiment.
- **Group 2:** Methotrexate (MTX) group, will give a single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.
- **Groups 3:** Received extract at the dose 150 mg/kg once daily for 28 consecutive days and single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.
- **Groups 4:** Received extract at the dose 300 mg/kg once daily for 28 consecutive days and single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.

After 28 days, all the animals were fasted for 18 h and blood samples will collect from the retro-orbital plexus with the last oral administration 1 h before. The serum samples were obtained analysed for hepatic and renal function.

#### 2.5 Protective effect of extract

Rats were randomly divided into four groups (6 rats per group) as follows:

- **Group 1:** Control group receive normal saline until termination of the experiment.
- **Group 2:** Methotrexate (MTX) group, will give a single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.
- **Groups 3:** Received extract at the dose 150 mg/kg once daily for 28 consecutive days and single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.
- **Groups 4:** Received extract at the dose 300 mg/kg once daily for 28 consecutive days and single injection of MTX (20 mg/kg, i.p.) on the 1st, 7th, 14th, 21st and 28th days.

After 28 days, all the animals were fasted for 18 h and blood samples will collect from the retro-orbital plexus with the last oral administration 1 h before. The serum samples were obtained analysed for hepatic and renal function.

#### 2.6 Biochemical determination

Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum bilirubin were determined for hepatic function.

The blood urea, creatinine, uric acid, total protein were analyzed for kidney function.

#### 2.7 Statistical analysis

The results were expressed as the Mean±SEM for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnet’s t-test. Results were considered to be statistically significant at p<0.05.

### 3 Results and Discussions

#### 3.1 Hepatoprotective effect of extract

Hepatoprotective efficacy of ethanol extracts at the dose of 150 and 300 mg/kg body weight were determined in MTX induced hepatotoxicity model. The rats treated with MTX, developed significantly liver damage, were observed from the alteration in the activities of serum enzyme namely SGOT, SGPT, ALP, ACP and total bilirubin in serum (Table 1).

The SGOT, SGPT, ALP, ACP and total bilirubin values were significantly increased in the MTX treated rats as compared with the normal control group (P<0.05). The rise in liver function test indicates the necrosis of liver caused by MTX. Treatment with ethanol extracts of *Vanda tessellata* at the dose of 150 and 300 mg/kg body weight decreased the activity of SGOT, SGPT, ALP, ACP and total bilirubin compared to MTX treated groups (P<0.05). The findings exhibited that the extract offer protection against toxin as evidenced by remarkable reduction in all serum enzyme (P<0.05), and illustrated that ethanol extract has strong
protective action against MTX induced liver damage.

Results of the study exhibited that animals treated with MTX showed marked liver injury as indicated by significant increase in liver transaminases, SGOT, SGPT, ALP and ACP. These cytosolic enzymes are the best indicator of liver necrosis. Increase in their activities in the serum indicates a leakage in cell membrane, which in turn, is associated with hepatocyte death.

These biochemical changes were significantly reduced by ethanol extracts of Vanda tessellata leaves suggesting that Vanda tessellata leaves could effectively counteract MTX - induced liver cell injury.

3.2 Nephroprotective effect of extract

In MTX treated groups of animals the concentration of serum urea, Creatinine, Uric acid, Total protein and BUN were considerably increased than the normal animals which indicates severe nephrotoxicity. Treating with ethanol extract of Vanda tessellata showed significant decrease (p<0.05) in concentration of serum urea, Creatinine, Uric acid, Total protein and BUN compared to MTX treated groups. It expressed the ethanol extract of Vanda tessellate leaves possessed renal protective properties(Table 2).

### Table 1: Effect of ethanol extract of Vanda tessellata on liver function test for different parameters in animals treated with MTX

| Treatment                      | SGOT (AST) (U/L) | SGPT (ALT) (U/L) | ALP (U/L) | ACP (U/L) | Bilirubin (mg/100 ml of blood) |
|--------------------------------|------------------|------------------|-----------|-----------|-------------------------------|
|                                |                  |                  |           |           | Direct (mg/dl) | Total (mg/dl) |
| Normal rats                    | 81.22±2.65       | 73.25±2.33       | 115.61±4.12 | 121.72±3.51 | 0.19±0.03 | 0.35±0.01 |
| Control rats (MTX 20 mg/kg)    | 201.63±5.71*     | 192.58±3.28*     | 302.17±3.56* | 273.28±2.84* | 2.14±0.02* | 4.01±0.07* |
| Extract (150 mg/kg) + MTX (20 mg/kg) | 101.26±3.48*     | 93.44±6.51*      | 165.11±4.36* | 173.29±3.68* | 0.52±0.08* | 0.61±0.06* |
| Extract (300 mg/kg) + MTX (20 mg/kg) | 84.73±2.56*     | 78.57±4.47*      | 121.48±3.72* | 131.52±5.24* | 0.23±0.02* | 0.42±0.05* |

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal group, **P<0.05 when compared with MTX treated group considered as statistically significant.

### Table 2: Effect of ethanol extract of Vanda tessellata on Kidney function test in animals treated with MTX

| Treatment                      | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | BUN (mg/dl) | Total protein (gm/dl) |
|--------------------------------|--------------|-------------------|--------------------|-------------|-----------------------|
| Normal rats                    | 26.29±0.84   | 6.71±0.43         | 0.43±0.25          | 18.41±0.72  | 5.17±0.16              |
| Control rats (MTX 20 mg/kg)    | 151.44±0.73* | 12.15±0.62*       | 3.29±0.31*         | 98.63±0.83* | 25.89±0.13*            |
| Extract (150 mg/kg) + MTX (20 mg/kg) | 74.07±0.36* | 8.22±0.41*       | 0.71±0.67*         | 32.34±0.31* | 11.87±0.19*            |
| Extract (300 mg/kg) + MTX (20 mg/kg) | 30.39±0.18* | 5.56±0.79*       | 0.47±0.51*         | 19.21±0.17* | 6.76±0.16*             |

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal group, **P<0.05 when compared with MTX treated group considered as statistically significant.

### 4 Conclusion

The present study concluded that pretreatment with ethanol extract of Vanda tessellata leaves improved the MTX-induced hepatic and kidney damage. Hence, administration of Vanda tessellata might reduce the side effects of methotrexate without compromising its efficacy. Further studies assessing the potential usefulness of Vanda tessellata treatment in MTX-induced toxicities on other organs and organ systems are required which may provide an effective way to improve their therapeutic efficacy.

### 5 Conflict of interest

We declare that we have no conflict of interest.

### 6 Author’s contributions

AC, YSS, MC, AP and PAR performed whole experimental procedures. All authors read and approved the final manuscript.

### 7 References

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