ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 8, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO ANTIOXIDANT POTENTIAL AND HEPATOPROTECTIVE ACTIVITY OF TAXUS WALLICHIANA

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Received: 02 September 2018, Revised and Accepted: 27 April 2018

ABSTRACT

Objectives: *Taxus wallichiana,* the member of family Taxaceae is used for the cure of various ailments by a large ethnic population native to the forests in Kashmir valley. The aim of this study is to evaluate antioxidant potential and hepatoprotective action of *T. wallichiana* as claimed by the ethnic population segment.

Methods: Antioxidant potential of different *T. wallichiana* extracts was evaluated by estimating the total phenolics, 1,1-diphenyl -2-picrylhydrazyl (DPPH), reducing power, microsomal lipid peroxidation (LPO), and hydroxyl radical scavenging activity through standard protocols. Hepatoprotective activity of *T. wallichiana* was determined by evaluation of liver marker enzyme (aspartate transaminase, alanine transaminase, and alkaline phosphatase) levels using standard protocols and by histological analysis of rat liver tissues of different animal groups against CCl_4 induced liver damage.

Results: Phenolic content expressed as gallic acid equivalents (GAE/g) was found maximum in case of ethanolic extract (153.34 mg GAE/g), followed by extracts of methanol (149.24 mg GAE/g), hexane (134 mg GAE/g), ethyl acetate (128.9 mg GAE/g), chloroform (121.37 mg GAE/g), and aqueous (113.00 mg GAE/g). At concentration of 700 µg/ml, DPPH radical scavenging activity of methanol extract was 88.29% with IC_{50} (212.00 µg/ml), aqueous (82.47%) with IC_{50} (258.29 µg/ml), and ethyl acetate (79.57%) with IC_{50} (301.80%µg/ml). The reducing power of the extracts increased in a concentration dependent manner. At concentration of 700 µg/ml, 79%, 68%, and 56% inhibition was observed with extracts of methanol, aqueous, and ethyl acetate on microsomal LPO with IC_{50} values of 126.09, 168.83, and151.96 µg/ml, respectively. Superoxide radical scavenging activity of *T. wallichiana* extracts increased in a dose-dependent manner with IC_{50} values 170.30 µg/ml (methanol), 257.00 µg/ml (aqueous), and 297.55 µg/ml ethyl acetate extract. *T. wallichiana* extracts exhibited antioxidant effects on calf thymus DNA damage. The results further depict the hepatoprotective action of *T. wallichiana* extract at doses of 100 and 300 mg/kg and were comparable with that of standard treatment comprising 50 mg/kg-day of Vitamin C, a known hepatoprotective agent. The data were substantiated with histopathological studies of rat liver sections.

Conclusion: These results comprehensively depict that *T. wallichiana* extracts are endowed with the free radical sequestering potential and be employed as potential antioxidant and hepatoprotective arsenal against many oxidative stress linked diseases.

Keywords: *Taxus wallichiana*, Radical scavenging, 1,1-Diphenyl -2-picrylhydrazyl, DNA protection, Hepatoprotective, Histopathology.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i8.22345

INTRODUCTION

Taxus wallichiana is a member of family *Taxaceae* found in temperate Himalayas at an altitude of 1800–3300 m and geographically distributed in Europe, North America, North India, Pakistan, China, and Japan [1]. It is a medium-sized evergreen tree attaining a height of 10–28 m. Its leaves are flat, dark green, and spirally arranged [2]. In Asia, it is distributed along Afghanistan through the Himalayas to the Philippines and is widely found in India and Pakistan. The Himalayan yew has high ethnobotanical importance and medicinal value [3]. *T. wallichiana* has an incredible history of its usage in the traditional system of medicine. The native people living close to forests possess substantial degree of traditional acumen on plant utilization. Himalayan medicinal plants form important constituents of alternative medicinal systems such as Amchi, Ayurveda, Han Chinese, Unani, and other traditional medicine systems that are prevalent in this region. Existing literature on *T. wallichiana* shows its analgesic, antipyretic, anti-inflammatory, immunomodulatory, antiallergic, anticonvulsant, anticociceptive, antiosteoporotic, antibacterial, antifungal, antiplatelet, and antispasmodic activities, and vasorelaxing effect [2,4-7]. In India, its leaves and bark are used in Unani drug "*Zarnab*" prescribed as a sedative, aphrodisiac and as cure for bronchitis, asthma, epilepsy, snake bite, and scorpion stings [3]. Its leaves and bark are used in treating rheumatism and its extracts are used in hair oils. It is consumed as decoctions, herbal tea, and juice for treating cold, cough, respiratory infections, indigestion, and epilepsy. It is used locally on the infected wounds and burns [6,8].

Biological oxidative stress caused due to reduced production of antioxidants in the body and the prominent elevation in the levels of oxidants/free radicals is implicated in varied human pathologies notably cancer, diabetes, inflammation, neurodegenerative diseases, and atherosclerosis diseases. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species have seriously damaging effects on biological macromolecules such as lipids, proteins, DNA, and carbohydrates that constitute the cellular components [9-13]. As a prominent example, free radicals damage liver leading to acute and chronic hepatic injury [14,15]. Hepatotoxic effect of carbon tetra chloride (CCl_4), a potent free radical is the result of its biotransformation to trichloromethyl free radical (CCl_3) or trichloroperoxyl radical (CCl_3O_2) by the mixed-function cytochrome P_{450} oxygenase system of the endoplasmic reticulum that causes oxidative stress and membrane damage characterized by atrophy of parenchyma, necrosis and degeneration [16-19]. This study focuses on evaluating the potential of various extracts of *T. Wallichiana* to scavenge oxidants/free radicals such as hydroxyl radical, superoxide radical, and 1,1-diphenyl -2-picrylhydrazyl (DPPH); besides, determining their reducing power and ability to inhibit lipid peroxidation (LPO).

In addition, the study also focuses on assessing the hepatoprotective action of *T. wallichiana* active extract(s) in Wistar rat model. Thus, this study will prove beneficial to testify the traditional therapeutic claims pertaining to *T. wallichiana* and provides the basis for the future research to use this medicinal plant as a source of effective drugs against oxidative stress related diseases.

METHODS

The leaves of *T. wallichiana* were collected from Gulmarg area of Jammu and Kashmir (India) during the month of July 2014, identified at the Centre of Plant Taxonomy (COPT), Department of Botany, University of Kashmir, and authenticated by Akhtar Hussain Malik (curator). A reference specimen has been retained in the herbarium under reference number 2380-KASH.

The identified plant material was shade dried under room temperature at 30±2°C. The dried plant material was ground into powder using mortar and pestle and passed through a sieve of 0.3mm aperture size. The powder obtained was successively extracted in different solvents using Soxhlet extractor (60–80°C). The extracts were then concentrated with the help of rotary evaporator under reduced pressure and the solid extracts were stored under refrigeration for further use.

Phytochemical screening of the crude extracts

Phytochemical screening was performed using various standard procedures.

Determination of total phenol content (TPC)

The phenolic content was determined by Folin–Ciocalteu reagent method as described by McDonald *et al*. [20] with slight change. 0.5 ml of extract was mixed with 0.1 ml of Folin–Ciocalteu reagent (0.5 N) and incubated at room temperature for 5 min. Then, 2.5 ml Na_2CO_3 solution was added and the mixture was incubated for 30 min before the absorbance was recorded at 760 nm. The TPC is expressed in terms of gallic acid equivalents (GAE) (mg/g of extracted compound).

DPPH free radical scavenging activity

DPPH assay was performed using the modified method of Kim *et al*. [21]. The free radical formed from DPPH was allowed to react with 100–700 µg/ml of the extracts and incubated for 30 min before absorbance was read at 517 nm. The percentage inhibitory activity was calculated by the following formula:

$$\%\text{Inhibition}=\left(A_0\right)-\left(A_1\right)\times 100$$

Where (A_0) is the absorbance without extract and (A_1) is the absorbance with plant extract.

Reducing power assay

The assay was conducted according to the method of Oyaizu [22]. Various concentrations of *T. wallichiana* extract added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$]. The reaction mixture was incubated for 20 min at 50°C. Then, the reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid (TCA). The reaction mixture was centrifuged at 3000 rpm for 10 min and the upper layer of the solution (2.5 ml) collected, followed by the mixing with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm.

$$\text{Reduction}(\%)=[1-(1-A_C/A_S)]\times 100$$

Where, (A_C) is absorbance of standard at maximum concentration tested and (A_S) is absorbance of sample.

Hydroxyl radical scavenging

Hydroxyl free radical, resulting from Fenton reaction was determined by degradation of deoxyribose that results in formation of thiobarbituric acid reactive species (TBARS) [23]. Reaction mixture containing 25 mM deoxyribose, 10 mM Ferric chloride, 100 mM ascorbic acid, 2.8 mM H_2O_2 in 10 mM KH_2PO_4 (pH 7.4), and various concentrations of *T. wallichiana* extracts. The reaction mixture was incubated for 1 h at 37°C, followed by addition of 1 ml of 1% TBA and 1 ml of 3% TCA. Then heated the reaction mixture at 100°C for 20 min before measuring absorbance at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation.

$$\%\text{Inhibition}=(A_C-A_O/A_C)\times 100$$

Where A_C is the absorbance in the presence of control and A_0 is the absorbance in the presence of the extract.

Superoxide radical scavenging activity

The assay based on the degree of competence of different extracts of *T. Wallichiana* to inhibit formazan formation through sequestering the superoxide radicals produced in Riboflavin-light-NBT system [24]. The reaction mixture contained 50 mM phosphate buffer (pH7.6), 20 µg riboflavin, 12 mM EDTA, and NBT 0.1 mg/3 ml, supplemented in order. Started the reaction with illuminating the reaction mixture of various concentrations of extract/standard for 90 s. Instantly after illumination, the absorbance was read at 590 nm. Butylated hydroxytoluene (BHT) was used as positive control. The percentage inhibition calculated using the following equation:

$$\%\text{Inhibition}=(1-A_S/A_C)\times 100$$

Where *Ac* is the absorbance of the control and *As* is the absorbance in the presence of extracts.

LPO assay

The LPO assay was carried out by the method described earlier [25]. Liver microsomes were incubated for 5 min with and without of plant extracts (50 µ–300) µg. Then, 100 µM $FeSO_4$ and 50 µM H_2O_2 were added and incubated in 0.15 M NaCl (pH 7) for 20 min at 37°C. Incubation for control was performed with blank received vehicle only and for induced with vehicle cum liver microsomes but devoid of plant extract. Reaction was stopped by adding TCA-TBA reagent (5% w/v) and the extent of LPO was determined as malondialdehyde (MDA) formed per mg of protein at 532 nm absorbance.

$$\%\text{inhibition}=1-(\text{Induced}-\text{Treated}/\text{Induced}-\text{Control})\times 100$$

Hepatoprotective activity of *T. wallichiana* extracts

In CCl_4 treated rats, the biochemical parameters reflect the change in the levels of various liver enzymes markers (aspartate transaminase [AST], alanine transaminase [ALT], and alkaline phosphatase [ALP]) in serum directly referring to alterations in the hepatic structural integrity [26]. In this study, we have evaluated the hepatoprotective potential of methanolic extract of *T. wallichiana* against CCl_4 intoxication. Animals were divided into five groups, each of six animals. The level of liver marker enzymes (ALT, AST, and ALP) of all groups of animals was evaluated. The result was substantiated with histopathological study of liver sections of different groups of Wistar rats.

Experimental design

Wistar rats weighing 200–230 g were divided into five groups, each group consisting of six animal **(**n=6). These groups were arranged in the following order:

Group I: Received olive oil vehicle only at 5 ml/kg-day.
Group II: Received CCl_4 in olive oil vehicle only.
Group III: Received Vitamin C (50 mg/kg-day).
Group IV: Received 1.0 ml of plant extract (100 mg/kg/body wt) orally for 19 days.
Group V: Received 1.0 ml of plant extract (300 mg/kg/body wt) orally for 19 days.

On the 19 days, animals from Groups II to V were injected intraperitoneally with CCl_4 in olive oil vehicle at a dosage of 1 ml/kg body weight. The rats were sacrificed 48 h after CCl_4 administration and before which blood samples were taken from retro-orbital

plexus liver tissues were taken and preserved in 10% methanol for histopathological analysis.

Histopathological examination and grading

After proper processing liver tissues of Wistar rats were embedded in paraffin using an embedding machine. Paraffin blocks were cut using a rotary ultramicrotome, spread onto glass slides and then dried overnight. Slides were monitored under a light microscope after being stained with hematoxylin and eosin dyes and mounted, and then histopathological grading was performed.

Statistical analysis

Determination of TPC, DPPH radical, superoxide radical, hydrogen peroxide, hydroxyl radical, and LPO assays was conducted in triplicates. The value for each sample was calculated as mean±standard deviation.

RESULTS

Qualitative phytochemical investigation

The results of qualitative phytochemical investigation of the crude extracts of *T. wallichiana* are shown in Table 1. In the methanol extract, maximum amount of flavonoids, terpenoids, and phenols were present. Cardiac glycosides and steroids were present in moderate amounts in methanolic and aqueous extract of the plant. Tannins and saponins were absent in all the extracts, although aqueous extract showed fairly good amount of saponins.

Total phenolics

Phenols being important antioxidants due to the presence of phenolic groups readily reduce the free radicals put hence stop to otherwise fast flowing cascade of reactions, which are etiologically implicated in many disorders.

Various crude extracts of the plant *T. wallichiana* were screened for phenolic content and it was found that highest phenolic content was recorded for methanolic extract of *T. wallichiana* (153.34 mg GAE/g), followed by methanolic extract (149.24 mg GAE/g), hexane (134.0 mg GAE/g), ethyl acetate (128.9 mg GAE/g), chloroform (121.37 mg GAE/g), and least was recorded for hexane (113.0 mg GAE/g).

DPPH radical scavenging activity of *T. wallichiana* extracts

The DPPH radical scavenging method is the most reliable method for determining the antioxidant potential of extracts (Fig. 1). TPC manifest its trend strongly in DPPH radical scavenging assay. Seven concentrations (100–700 µg/ml) of plant extracts were evaluated and percentage inhibition was recorded in dose-dependent manner. The highest DPPH activity was observed in methanolic extract (91.25%) followed by aqueous (87.64%), ethanol (85.23%), and ethyl acetate (83.27%) at highest concentration (700µg/ml), whereas BHT was used as standard showed (94.62%) at the same concentration. The IC_{50} values of three extracts were recorded as 212.00, 258.29, and 301.80 µg/ml, respectively, which was inversely related to the antioxidant capacity (Table 2).

Reducing power

The reducing power was increasing in a dose-dependent manner. As shown in Fig. 2, a higher absorbance value indicates a stronger reducing power of the samples. The reducing power activity of methanolic extract at 100–700 µg/ml was increased from 0.225 nm to 0.745 nm, respectively. The reducing power activity of the aqueous and ethyl acetate extracts also increased in a concentration dependent manner. At the higher concentration of the extracts (700 µg/ml), we observed absorbance of 0.601 nm and 0.518 nm, respectively.

Superoxide radical scavenging activity of *T. wallichiana* extracts

Superoxide radicals produced from riboflavin-light-NBT system can be measured by their ability to reduce NBT. The minimizing trend in absorbance at 590 nm in the presence of plant extracts and the reference standard BHT show their abilities to sequester superoxide radicals. Superoxide radical scavenging increased in concentration dependent manner (Fig. 3). At the higher concentration of the plant extract (600 µg/ml), we observed 83.50%, 75.62%, and 71.01% inhibition with methanol, aqueous, and ethyl acetate extract, respectively. The IC_{50} values (Table 2) of the plant extracts on superoxide scavenging activity were 170.30 µg/ml, 257.00 µg/ml, and 297.55 µg/ml, respectively. BHT a known antioxidant showed 89.57% inhibition at the concentration of 600 µg/ml.

Microsomal LPO of *T. wallichiana* extracts

The anti-LPO activity of different extracts of *T. wallichiana* was evaluated using rat liver microsomes. Addition of $FeSO_4$ and H_2O_2 to the microsomal suspension increased TBARS. When the microsomes had been pre-treated with extracts, the production of TBARS was inhibited markedly in a dose-dependent manner. At concentrations 50-300 µg/ml, the extracts exhibit different degrees of anti-LPO activity. The percentage inhibition for ethyl acetate extract ranges from 23% to 56% at the concentrations of 50–300 µg/ml, aqueous extract inhibition rate was found to be 30–68% and for methanolic extract the percentage inhibition varies from 35% to 79% at the same concentrations, respectively (Fig. 4). The half inhibitory concentration (IC_{50}) of the extracts in this system was 126.09 µg/ml (methanol), 168.83 µg/ml (aqueous extract), and 151.96 µg/ml with ethyl acetate extract (Table 2). BHT a known antioxidant used

Table 1: Phytochemical analysis of various extracts of *T. wallichiana*

Parameters	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Aqueous extract
Steroids	++	+++	−	+	++
Terpenoids	++	+	++	++	+
Tannins	−	−	+	+	+
Flavonoids	++	+++	++	+++	+++
Saponins	−	+	−	−	++
Phenols	−	+	−	+	++
Volatile oils	−	−	−	−	−
Cardiac glycosides	+	+	−	+	+
Alkaloids	−	−	−	−	−

+: Present, −: Absent. *T. wallichiana: Taxus wallichiana*

Table 2: IC_{50} values (µg/ml) of *T. wallichiana* extracts

Extracts	DPPH	Superoxide radical	LPO	Hydroxyl radical
Methanolic	212.00±2.29	170.30±2.25	126.09±3.07	82.34±1.98
Aqueous	258.29±3.24	257.00±3.70	168.83±2.67	175.33±2.72
Ethyl acetate	301.80±4.42	297.55±4.12	151.96±2.38	199.05±3.65

The data were presented as means±SD of three parallel measures and evaluated by one-way ANOVA. SD: Standard deviation, DPPH: 1,1-diphenyl -2-picrylhydrazyl, LPO: Lipid peroxidation, *T. wallichiana: Taxus wallichiana*

Table 3: Effect of methanolic extract of *T. Wallichiana* on biochemical parameters in CCl_4 induced toxicity in Wistar rat model

Group	ALT IU/l	AST IU/L	LDH IU/L
Normal	23.55±3.4	28.61±5.02	64.38±3.62
Positive Control	129.42±10.76	126.53±11.2	236.50±9.60
Vitamin C (50 mg/kg)	60.40±2.10	48.79±4.2	165.64±7.2
T. wallichiana extract (100 mg/kg)	86.62±4.90	91.63±5.7	203.47±8.02
T. wallichiana extract (300 mg/kg)	69.55±5.20	63.35±6.2	168.50±6.67

The data are presented as mean±SD for six animals (n=6) in each observation and evaluated by one-way ANOVA followed by Bonferroni's t-test. ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, SD: Standard deviation, *T. wallichiana: Taxus wallichiana*

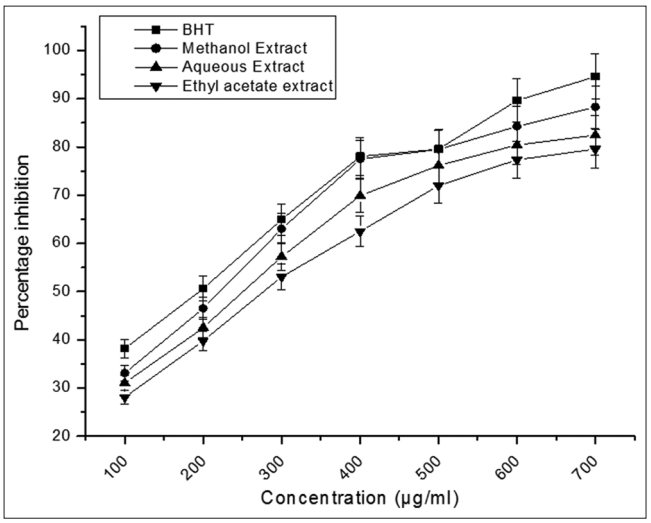

Fig. 1: Effect of different extracts of *Taxus wallichiana* and known antioxidant butylated hydroxytoluene on 1,1-diphenyl -2-picrylhydrazyl radical scavenging activity, measured at 517 nm, with each result representing mean±standard deviation of 3 separate experiments

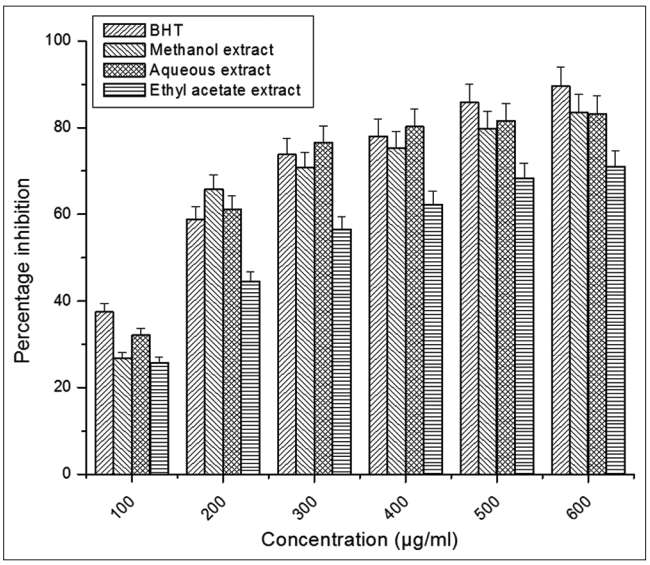

Fig. 3: The effect of different extracts of *Taxus wallichiana* and known antioxidant butylated hydroxytoluene on superoxide radical activity, measured at 590 nm, with each result representing mean±standard deviation of 3 separate experiments

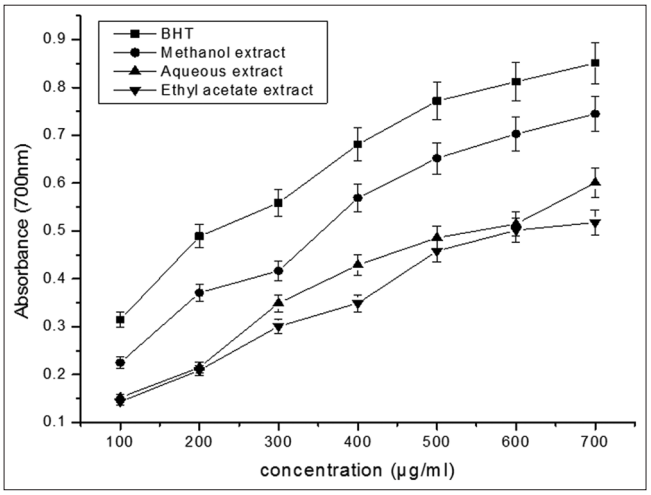

Fig. 2: The effect of different extracts of Taxus wallichiana and known antioxidant butylated hydroxytoluene on reducing power activity, measured at 700 nm, with each result representing mean±standard deviation of 3 separate experiments

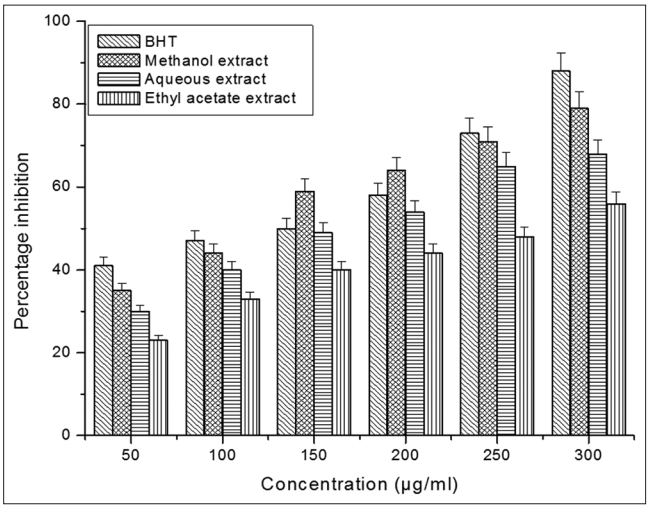

Fig. 4: The effect of different extracts of Taxus wallichiana and known antioxidant butylated hydroxytoluene on lipid peroxidation levels measured at 532 nm, with each result representing mean±standard deviation of 3 separate experiments

in the study inhibited the TBARS formation up to 88.00% at the concentration of 300 µg/ml.

Hydroxyl radical scavenging activity of *T. wallichiana* extracts

A significant decrease in concentration of hydroxyl radical was observed due to *T. wallichiana* extracts (Fig. 5). All the extracts exhibited significant activity, above 70% in a concentration dependent manner with maximal inhibition of 89% with methanol extract, 78% with aqueous extract, and 72% with ethyl acetate extract at 300 µg/ml with

IC50 value of 82.34 µg/ml (methanol), 175.33 µg/ml (aqueous), and 199.05 µg/ml with ethyl acetate extract (Table 2). BHT used as standard inhibits the hydroxyl radical up to 96% with the same concentration.

Hepatoprotective potential and histopathological study

CCl_4 administration resulted significant elevation of ALT, AST, and lactate dehydrogenase (LDH) (Table 3), and this probably occurs through induction of hepatic damage in control animals as compared to normal animals [27], while standard drug Vitamin C treatment reduced ALT, AST, and LDH concentration in animals of standard group and those were almost equivalent to normal. In the present study, CCl_4 elevated levels of ALT, AST, and LDH in positive control animals which may be due to reduced function of liver due to toxicity. Treatment with methanolic extract of *T. wallichiana* significantly reduced concentration of above serum parameters in animals of therapeutic groups which could be due to protection given by *T. wallichiana* methanolic extract.

Histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of *T. wallichiana*. Breakage of carbon tetrachloride leads to the formation of free radicals, resulting in steatosis, centrilobular necrosis, and cytoplasmic vacuolation as observed in toxic control group. However, these pathological changes were moderately prevented by methanolic extract groups and standard group. Normal hepatic integrity similar to normal group was seen in the Vitamin C-treated group. While groups treated with methanolic extract at100 and 300 mg/kg body weight showed almost normal hepatic architecture with less infiltration of fat and absence of necrosis (Fig. 6). It is evident that the *T. wallichiana* caused regeneration of liver parenchyma cells and treated hepatic cell damage due to CCl_4 toxicity.

DISCUSSION

Plant-derived medications are used as the basis of many of the modern pharmaceuticals that we use today for the treatment of our various ailments [28]. *T. wallichiana* is one of the medicinally important plants used ethnopharmacologically for the treatment of various disorders. The curative benefit of medicinal plants is usually attributed to their antioxidant properties. Antioxidants are considered body's first line of defense oxidative stress induced damage ingestion of dietary antioxidants has been implicated in suppressing the free radical production or scavenging free radicals and may thus prevent harmful effect of these free radicals. Several phytochemicals such as tannins, phenols, and flavanols are considered as good antioxidants. Phytochemical analysis exhibited that *T. wallichiana* constitute antioxidant compounds such as flavonoids, tannins, and phenols and showed considerable IC_{50} values in different extracts help in scavenging the free radicals and protect us from various diseases. They exercise their action either by cleansing the ROS or protecting the antioxidant machinery of the cell [29]. In the present study, *in vitro* antioxidant and hepatoprotective activity of different extracts of *T. wallichiana* were tested using DPPH, OH, superoxide radical scavenging, and LPO assays. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule [30]. The DPPH test is extensively used method to assess the free radical scavenging effect of plant extracts. This method is based on the reduction of DPPH solution in the presence of antioxidant resulting in the formation of non-radical DPPH-H by the reaction. The stable DPPH was reduced by all the extracts and thus changing the color from purple to yellow to varying degree depending on the presence of antioxidant compounds. The degree of discoloration indicates the scavenging potential of the extract. In the present study, among all the extracts tested, the highest capacity to neutralize DPPH radicals was found for the methanolic extracts. Similar results were obtained by Beknal *et al*. while evaluating the antioxidant potential of *Drynaria quercifolia* rhizome [31].

Phenolics are range of different secondary metabolites and have the great ability of sequestering of free radicals or chelation of metal ions.

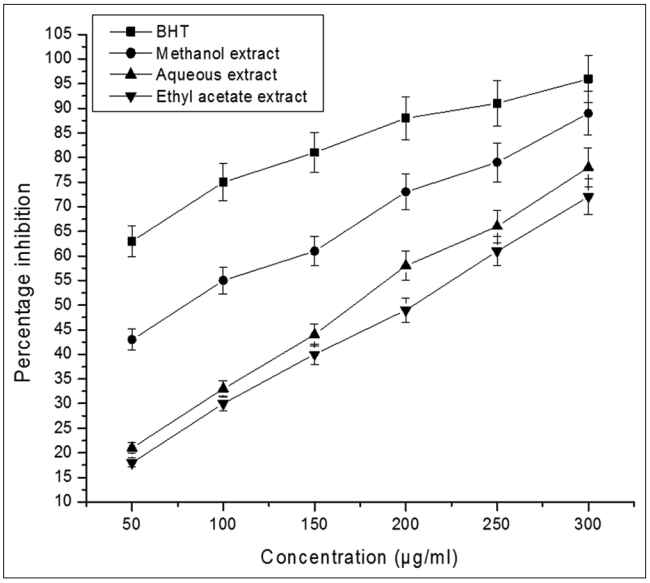

Fig. 5: The effect of different extracts of *Taxus wallichiana* and known antioxidant butylated hydroxytoluene on the hydroxyl activity, measured at 532 nm, with each result representing mean±standard deviation of 3 separate experiments

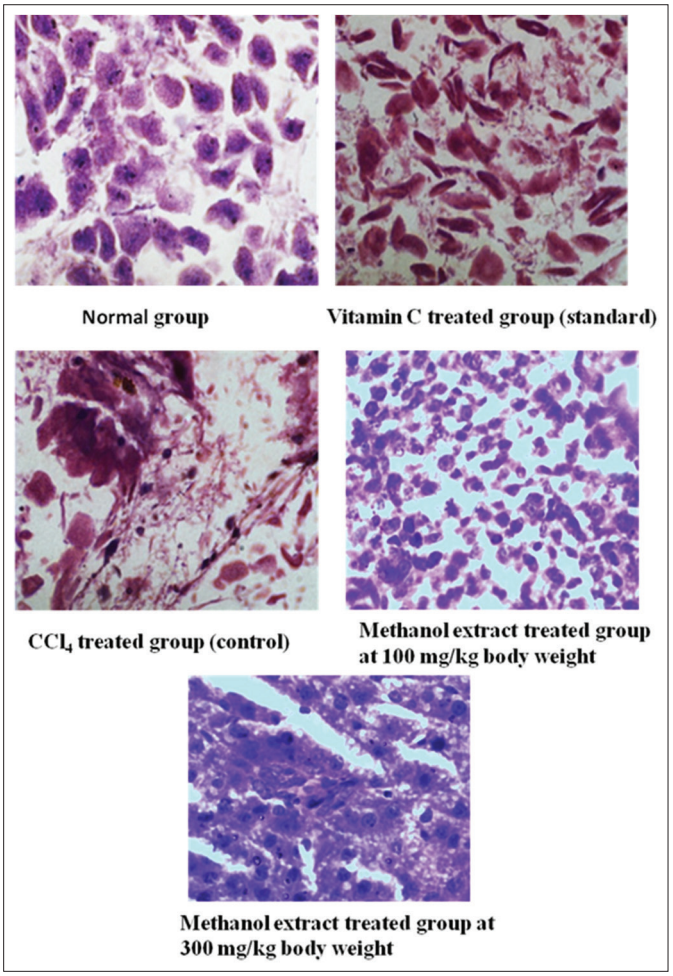

Fig. 6: Histopathological study confirming the hepatoprotective action of methanolic extract (100 mg/kg body weight and 300 mg/kg body weight) of Taxus wallichiana in CCl4 induced hepatotoxic rats (n=6 in each group) when compared to normal, control, and standard

It has been reported that the antioxidant activity of phenol is chiefly due to its redox properties, hydrogen donors, and singlet oxygen quenchers [32]. Hence, TPC was estimated through modified Folin–Ciocalteu method.

In reducing power assay, the readiness to donate electron by a bioactive compound is index of its reducing power and antioxidant activity [33]. Antioxidants cause the reduction of Fe^{3+} into $Fe^{2+,}$ then changing the solution into various shades from green to blue, depending on the reducing power of compounds [34,35]. The ferric reducing power activity of different extracts of *T. wallichiana* seems to be due to the presence of polyphenols which correlate to its TPC and phytochemical screening. Based on the measured absorbance, the concentration of phenolics was expressed as milligram per GAE. Methanolic extract with TPC (149.24±2.70 mg/GAE) increases the reducing power capacity of *T. wallichiana* in a dose-dependent manner. Hence, methanolic extract of *T. wallichiana* possibly acts as electron donors and could react with free radicals to alter them into more stable products and then cease the free radical chain reactions. Our findings are in tune with the results observed by Irshad *et al.* and were studying the antioxidant activity of *Cassia fistula* extracts [36].

Superoxide anion (O_2^{-}) is one of the most important agents from free radicals. It acts as a precursor of more ROS such as single oxygen and hydroxyl radicals that have the capacity of reacting with biological macromolecules and thereby incurring tissue damage, and also plays a pivotal role in peroxidation of lipids [37]. Methanol extract with IC_{50} value of 170.30±2.27 µg/ml showed strong superoxide radical scavenging activity than aqueous and ethyl acetate extract.

In the present study, we measured the potential of *T. wallichiana* extracts to inhibit LPO in rat liver microsomes induced by the Fe^{2+}/ ascorbate system. Different extracts protected against LPO induced by Fe^{2+} considerably reduced MDA content in a concentration dependent manner. Methanol extract had the greatest inhibiting activity (79.00%), with the lowest IC_{50} value 126.09 µg/ml. similar results were reported by Gul *et al.* while studying the antioxidant and anti-proliferative activities of *Abrus precatorius* leaf extract [38].

Hydroxyl radical is produced by a mixture of Fe^{3+}, H_2O_2, and ascorbic acid and is assessed by monitoring the degraded fragments of deoxyribose, through malondialdehyde (MDA) formation and DNA strand breaks in calf thymus DNA [39]. The plant extracts or drugs scavenging the hydroxyl radical, probably by either sequestering the radical or may chelate the Fe^{2+} ion, hence halting the Fenton's reaction. Plant extracts endowed with polyphenols are reported to quench oxygen-derived free radicals by lending a hydrogen atom or an electron to the free radical or by their chelating ability of the aromatic ring [40].

In our study, we tested different extracts of *T. wallichiana* and it was observed that extracts exhibit a dose-dependent hydroxyl radical scavenging activity and prevents the Calf thymus DNA damage. Our results are in tune with the results reported by Sundararajan and Koduru, while studying the antioxidant activity of *Limnophila heterophylla* [41]. Furthermore, similar results were obtained by Saiah *et al*. while analyzing the antioxidant activity of six Algerian medicinal plants [42].

The results obtained from estimation of biochemical parameters suggest that *T. wallichiana* at the doses 100 mg/kg and 300 mg/kg of methanolic extract possess significant hepatoprotective property in CCl_4 induced liver toxicity in rat model. This study indicated the dose-effect relationship of *T. wallichiana* extract. The histopathological studies supported the results of biochemical tests treated with 100 mg and 300 mg/kg doses of methanolic extract of *T. wallichiana* protecting the cellular integrity and architecture of the liver. The histopathological studies strongly suggest that 100 mg and 300 mg/kg doses of methanolic extract of *T. wallichiana* protect the cellular integrity and architecture of the liver. Similar results were obtained by Singh *et al.* while examining the antioxidant, and hepatoprotective activities of *Amorphophallus campanulatus* [43].

The DPPH, superoxide radical, hydrogen peroxide, hydroxyl radical, and LPO assays gave corresponding results for the antioxidant activity measured in methanol, aqueous, and ethyl acetate us extracts of *T. wallichiana*. These results refer to a correlation among different antioxidant assays, especially when the assays are compared with the DPPH, which is one of the most extensively used method for screening the antioxidant activity of plant extracts.

CONCLUSIONS

Overall, it could be concluded that *T. wallichiana* possesses a potent antioxidant activity. In our study, we also observed that the extracts have a very tangible role in protecting liver from free radical damage. The preliminary chemical examination of different extracts of *T. wallichiana* has shown the presence of a number of polyphenols which may be responsible for the hepatoprotective activity. Additional studies are needed to characterize the active compounds and to clarify the *in vivo* potential of this plant.

ACKNOWLEDGMENTS

Authors are thankful to faculty of the Department of Biochemistry, University of Kashmir for their timely help and insightful ideas. We are also grateful to curator, COPT, University of Kashmir.

AUTHOR'S CONTRIBUTION

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors have no personal or financial conflicts of interest associated with this work.

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