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Effects of *Thuja Occidentalis* Extract on Histo-Pathological Parameters in Rabbits Treated with and Without Carbon Tetrachloride

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ABSTRACT

The aim of this research work was to evaluate the effect of low dose of *Thuja occidentalis* for three months on rabbit. To achieve this aim histopathology of heart, liver, stomach and kidney tissues of control groups as well as *Thuja occidentalis* treated groups with and without carbon tetrachloride was carried out on rabbits. Liver function test of the group injected CCl4 was also done. Our study demonstrated that minor harmful effects were found in liver and kidney tissues injected carbon tetrachloride but no major toxicity was observed in any of the examined tissues due to potent anti-oxidant potential of active constituents present in *Thuja occidentalis*.

Keywords: *Thuja occidentalis*, Histopathology, Liver Function Test, Carbon tetrachloride, Hepato-protective

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INTRODUCTION

Thuja occidentalis is a monoecious coniferous plant, belongs to family Cupressaceae. It has numerous pharmacological and therapeutic effects like anti-oxidant, anti-microbial, hepato-protective, anti-cancer, anti-diabetic, anti-ulcer, anti-viral, anti-pyretic, antitussive, astringent, diuretic, emmenagogue, expectorant, emollient, anti-wart, anti-psychotic and stomachic, particularly due to its volatile oil constituents[1, 2a & b, 3, 4]. *T. occidentalis* extract contains α-pinene, β-pinene, α-thujone, β-thujone, fenchone, borneol, acetic acid, formic acid, iso-valeric acid, terpineol, sabinene, camphene, limonene, myrcene, γ-terpinene, terpinolene, camphor, valerianic acid, β-sitosterol, quercitrin, rhodoxanthin, tannic acid, resins, mucilage, vitamin C, fats, proteins, fibers, carbohydrates, calcium, coumarin, p-coumarin, umbelliferone, flavonoids, kaempferol, kaempferol-3-O-rhamnoside, mearnsitrin, myricetin, myricitrin, procyanidin, prodelphinidin catechin and gallocatechine, as reported by Anderson *et al.* 2009, Bouie *et al.* 1999, Hafez *et al.* 2004, Offergeld *et al.* 1992, Jahan *et al.* 2006 [3, 5, 6, 7,8,]. At present *T. occidentalis* is widely used in homeopathy system of medicine and evidence-based phytotherapy.

MATERIAL AND METHOD

Plant material

T. occidentalis mother tincture (Willmer Schwabe, Germany; Lot no. 7100710) was purchased from homeopathic drug suppliers. The extract obtained was stored in cool, dry place for further studies.

Chemicals & Reagents

All the chemicals and reagents used were of analytical grade and purchased from Merck (Germany).

Experimental Animals

The male rabbits (1.5 kg body weight) were purchased from Animal House of Dow University of Health Sciences (DUHS), Karachi and kept in animal house for a period of 15 days to acclimate. Male rabbits were fed with their normal diet and water. Their weights were checked on random basis. The drug was administered at the interval of 24 hours for a period of 3 months. Carbon tetrachloride was injected 6 hours before taking blood for carrying out liver function test to group III & IV by cardiac puncture and sacrificing[9]. Animal studies were carried out according to Ethical Principles and Guidelines for Experiments on Animals formulated jointly by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences.

Animal grouping and drug dosing for histo-pathological examination

Four groups were made namely group I (positive control), group II (male test group without

CCl_4), group III (Negative control) and group IV (male test group with CCl_4):

Group 1 (Positive control): Six animals were kept as male positive control. Water and food was provided to the animals during the whole period of experiment.

Group 2 (without CCl_4 group - male test group): Six animals were administered 0.025gm of Test drug extract, water and food was provided to the animals during the whole period of experiment.

Group 3 (Negative control): Six animals were kept as male negative control. Water and food was provided to the animals during the whole period of experiment.

Group 4 (with CCl_4 group – male test group): Six animals were administered 0.025gm of Test drug extract, water and food was provided to the animals during the whole period of experiment.

Liver Function Tests (LFTs)

Serum samples were obtained by centrifugation of blood at 1300 x g for 15 min. The Menarini Classic Chemistry Analyzer was used to determine liver function tests[10].

Histopathological Analysis

The liver, kidney, heart and stomach tissues were dehydrated separately with ethanol of graded concentrations. The tissues were passed through xylene solution to clear the ethanol and facilitate molten paraffin wax infiltration (55°C). After that, they were treated with paraffin wax and cast into blocks; sections of 5µm thickness were cut with microtome. These were later placed on clean glass slide. The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded using an Olympus Research Microscope (model BX51)[11, 12].

Statistical analysis

Results of the study were presented as a mean plus or minus Standard Error of Mean (Mean ± SEM). Differences between control and treatment groups were analyzed by student t-test[13].

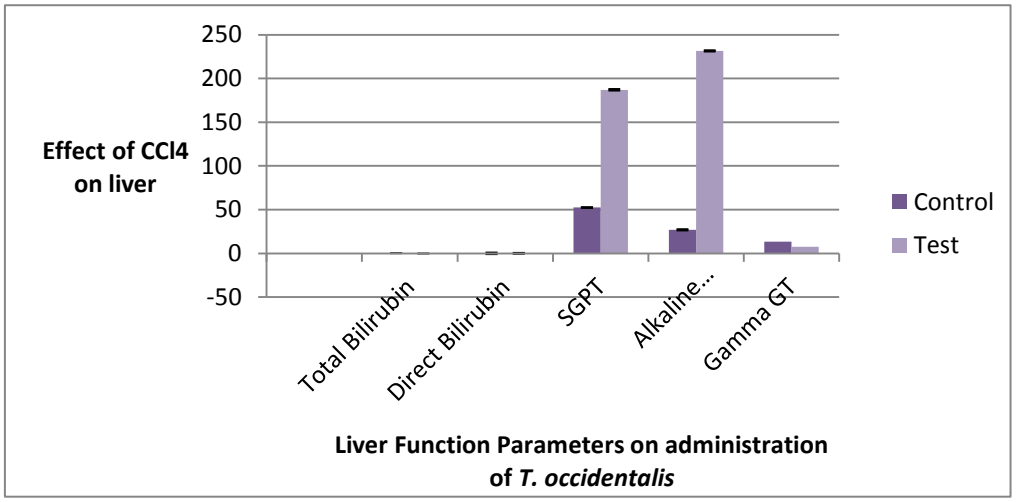
RESULTS AND DISCUSSION

T. occidentalis extract is nowadays used mostly in homeopathy as a mother tincture or a dilution for the treatment of warts, cancer, bronchial catarrh, amenorrhea, and rheumatism.

Our results in Liver Function Test of rabbits (*T. occidentalis* + CCl_4) revealed lowered total bilirubin (0.075±0.0083) and Gamma GT (7.5±0.836) levels whereas direct bilirubin was slightly raised (0.08±0.0063) and SGPT (187±0.632) and alkaline phosphatase (231.5±0.836) levels were found significantly raised (Table 1; Graph 1).

Table 1: Shows the effect on Carbon tetrachloride on Liver Enzymes of Rabbits with and without *T. occidentalis* extract

Liver Function Test Parameters	Control (M±SEM)	Test (M±SEM)
Total Bilirubin	0.33±0.0063	0.075±0.0083
Direct Bilirubin	0.07±0.0063	0.08±0.0063
SGPT	52.5±0.836	187±0.632
Alkaline Phosphatase	27±0.632	231.5±0.836
Gamma GT	13.33±0.7302	7.5±0.836



Graph 1: Shows the effect of *T. occidentalis* extract on liver function parameters of rabbit in comparison with the control. *T. occidentalis* extract was administered in the dose of 25mg for 3 months. Blood for liver function test was taken 6 hours after the IM injection of carbon tetrachloride prior to dissection.

In male group rabbits treated with *T. occidentalis* extract (without injection of carbon tetrachloride six hours before dissection), no significant pathology was observed in heart and stomach tissues. Mild portal inflammation and mild peri-portal fibrosis was found in liver tissues, while in kidney tissues, patchy chronic non-specific pyelonephritis was seen as compared to positive and negative control groups (Figures 1, 3, 5, 7).

In male test group rabbits treated with *T. occidentalis* extract (with injection of carbon tetrachloride six hours before dissection), slight pathological changes were observed in its heart and stomach tissues. Mild chronic active portal inflammation with peri-portal fibrosis was found in liver tissues. Portal tracts are mildly expanded with lymphocytic infiltrate and minimal fibrosis. Foci of piecemeal necrosis (interface hepatitis) are seen. Sinusoidal congestion is noted. Patchy lymphocytic infiltrate is seen in the tubule-interstitial compartment. Chronic non-specific pyelonephritis was found in kidney tissues as compared with positive and negative control groups (Figures 2, 4, 6, 8).

Ahmad *et al*. (2013) reported that low dose of *T. occidentalis* administered for a period of three months showed maintained complete blood count and total proteins, decreased platelet count, cardiac enzymes, HDL, LDL and blood glucose levels and raised direct bilirubin due to the presence of its active constituents (mentioned in introduction of this paper) that might have exerted protective effect on heart and stomach[14]. Slight toxicity was observed in liver and kidney tissues of carbon tetrachloride treated animals. Dubey & Batra (2008) also reported the hepato-protective effects of *T. occidentalis extract* in rats[2a]. Our findings are on *T. occidentalis* extract also shows hepato-protective effects against carbon tetrachloride induced liver damage.

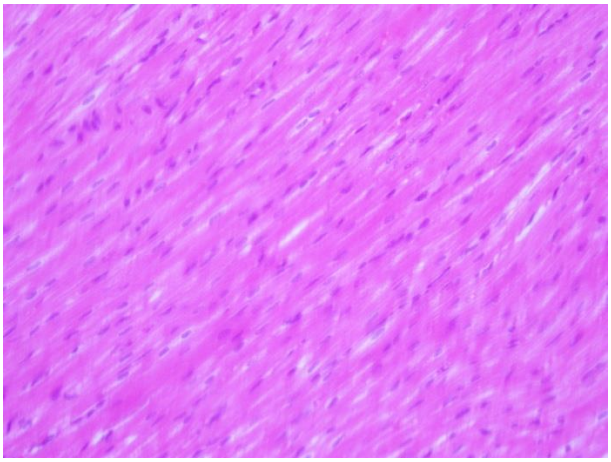


Figure 1: *T. occidentalis* treated (heart)

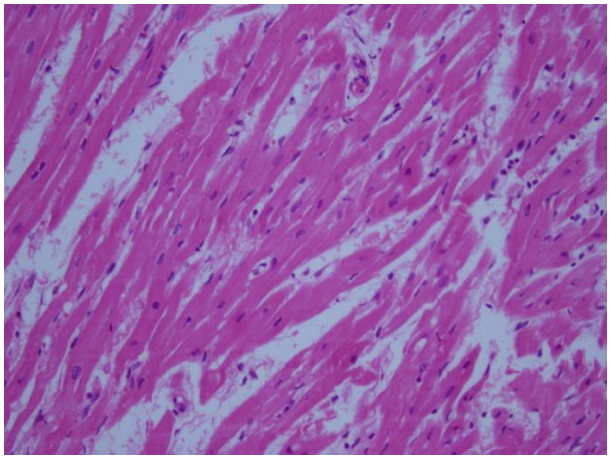


Figure 2: *T. occidentalis* treated (heart) injected CCl_4 6 hours before dissection

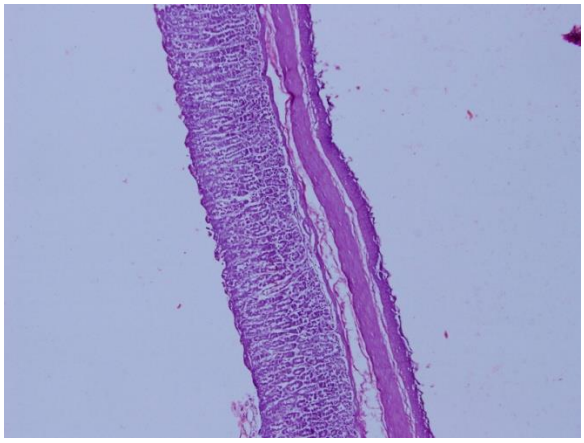


Figure 3: *T. occidentalis* treated (stomach)

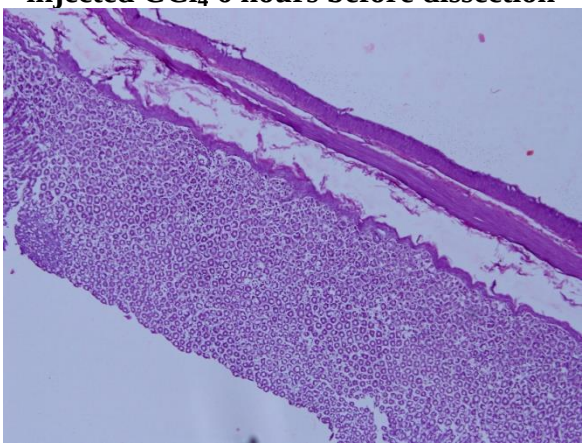


Figure 4: *T. occidentalis* treated (stomach) injected CCl_4 6 hours before dissection.

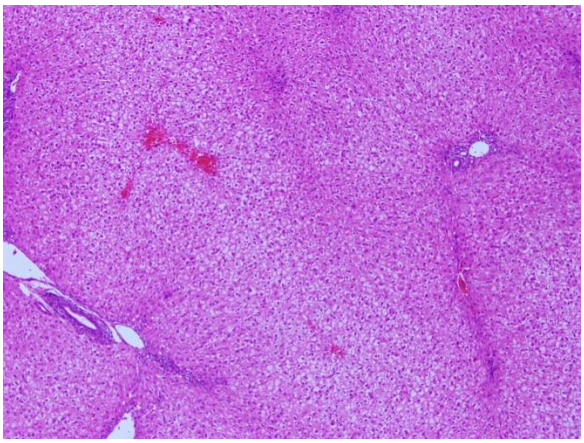


Figure 5: *T. occidentalis* treated (liver)

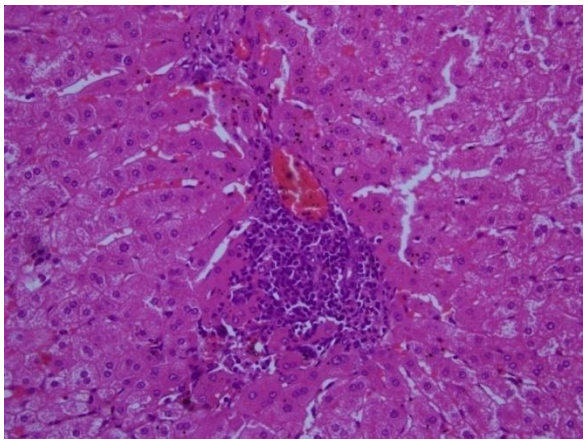


Figure 6: *T. occidentalis* treated liver injected CCl_4 6 hours before dissection

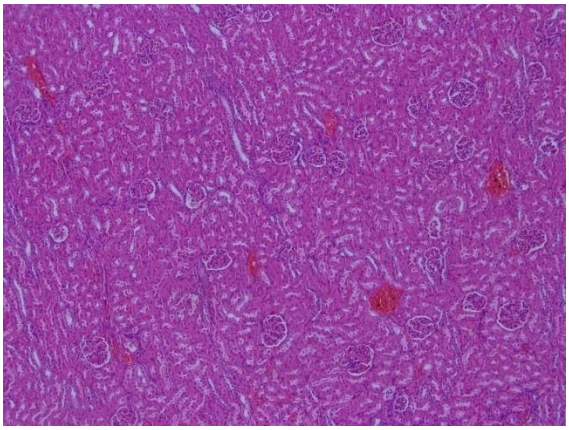


Figure 7: *T. occidentalis* treated (kidney)

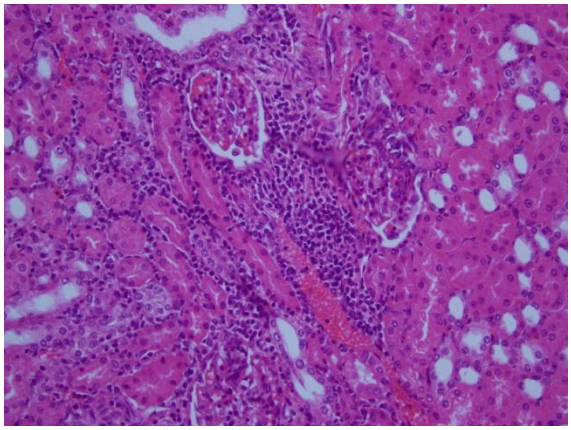
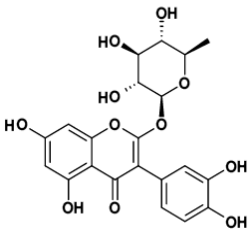
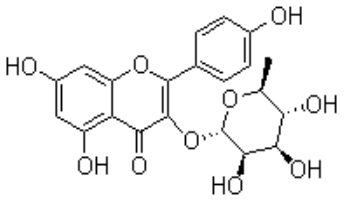


Figure 8: *T. occidentalis* treated kidneys injected CCl_4 6 hours before dissection

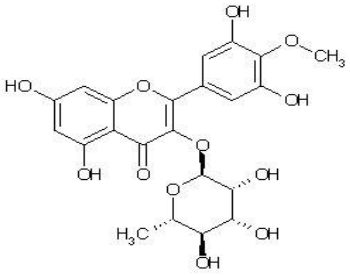
It is also reported that flavonoids are having hepato-protective, antioxidant, cancer, cardiovascular, antibacterial activities. Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds. Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase and to modify intracellular signaling pathways in immune cells[2a, 15]. Chemical analysis reports on *T. occidentalis* extract showed that it is rich in flavonoids for example (Figure 9).



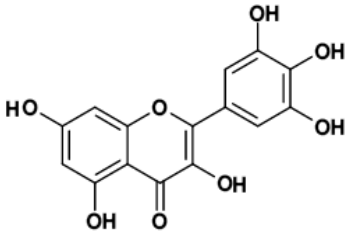
Quercitrin



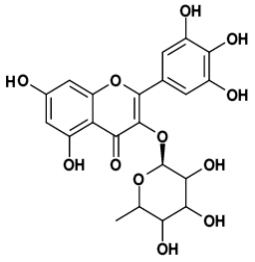
Kaemferol-3-O-rhamnoside



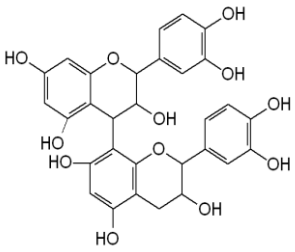
Mearnsitrin



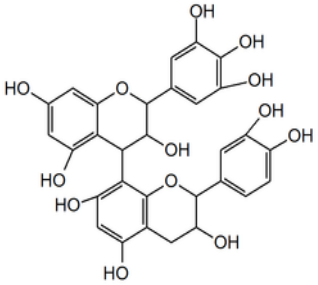
Myricetin



Myricitrin



Procyanidin



Prodelphinidin

Figure 9: Flavonoids rich constituent s of *T. occidentalis* extract

Other compounds like volatile oils, sterols, coumarins, tannins, vitamin C etc. are also played important role in protection/immunization of animal/human bodies.

CONCLUSION

The protective effects of *T. occidentalis* are due to its chemical contents that may be the basis of its therapeutic efficacy and beneficial effects in majority of the diseased states. More experimental and clinical trials are required to validate the safety profile and low toxicity level of *T. occidentalis*.

CONFLICT OF INTEREST

The authors have no conflict of interest either financial, commercial or in any other form.

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