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HEPATOPROTECTIVE ACTIVITY OF AQUOES EXTRACT OF *OCIMUM TENUIFLORUM*

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ABSTRACT

The aim of our study was to evaluate the hepatoprotective activity of Tulsi (*Ocimum tenuflorium*) leaves on paracetamol-induced hepatotoxicity in albino rats as compared with silymarin. evaluate whether the combination of Tulsi (*Ocimum tenuflorium*) and silymarin had a synergistic or an additive hepatoprotective activity.Drug-induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for one in 600 to one in 3500 of all hospital admissions. Developing countries are reliant on medicinal plants as their main source of treatment for diseases.Black Tulsi (*Ocimum tenuflorium*), is a well known medicinal plant, which grows wild as well as in households and temples in India. It has been traditionally regarded as possessing rejuvenating, tonic and vitalizing properties that contribute to longevity and a healthy life.[7] Leaves of *Ocimum tenuflorium* possess expectorant, diaphoretic, antiseptic, spasmolytic, stimulant and anticatarrhal properties and are used as cold and cough remedies.

INTRODUCTION

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for one in 600 to one in 3500 of all hospital admissions.[1] Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental illnesses. Herbal treatments are the most popular form of traditional medicine. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients.[2] However, no scientific data regarding the identity and effectiveness of these herbal products were available, except in the treatise of Ayurveda and Unani medicine.[3] The World Health Organization (WHO) has laid emphasis on promoting the use of traditional medicine for health care.[2] Hence, we see a focus on research on traditional and herbal medicine, especially in developing countries, with individual as well as collaborative efforts by national research

organizations.[4] There is an acute necessity of reliable hepatoprotective drugs in modern medical practice. Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials.[5] More than 700 mono- and polyherbal formulations from over a hundred different plants are available for use.[6] Sacred or Holy Basil, i.e. Black Tulsi (*Ocimum tenuflorium*), is a well known medicinal plant, which grows wild as well as in households and temples in India. It has been traditionally regarded as possessing rejuvenating, tonic and vitalizing properties that contribute to longevity and a healthy life.[7] Leaves of *Ocimum tenuflorium* possess expectorant, diaphoretic, antiseptic, spasmolytic, stimulant and anticatarrhal properties and are used as cold and cough remedies, for fever, pain,[8] gastrointestinal disorders (like dyspepsia, vomiting), worm infestations, skin diseases, snakebite and scorpion sting.[9] Significant hepatoprotective activity of *Ocimum tenuflorium* was reported earlier against paracetamol, carbon tetrachloride and anti-tubercular drug-induced hepatoxicity in albino rats.[9–11] We wanted to build on these findings in relation to paracetamol-induced hepatotoxicity and observe for a synergistic or an additive effect with the combination of *Ocimum tenuflorium* and a standard hepatoprotectant.

Thus, the aim of our study was to:

1. Evaluate the hepatoprotective activity of Tulsi (*Ocimum tenuflorium*) leaves on paracetamol-induced hepatotoxicity in albino rats as compared with silymarin.
2. Evaluate whether the combination of Tulsi (*Ocimum tenuflorium*) and silymarin had a synergistic or an additive hepatoprotective activity.

Experimental Animals:

Healthy albino rats (*Rattus norvegicus*) of Wistar strain (both male and female), weighing 100–200 g each (obtained from sanzyme private limited,hyderabad) were given the standard diet with water *ad libitum* during the entire period of the experiment as per the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facilities.[12]

Drugs:

All drug suspensions were prepared for the different groups with 3% (W/V) aqueous suspension of gum acacia as vehicle.

Test drug

Ocimum tenuflorium alcoholic leaf extract (OSE). This was prepared as follows:One kilogram of fresh *Ocimum tenuflorium* leaves, identified by Mr. gopal dixit, PhD in Botany,Osmania university , was collected and washed thoroughly with cold water, dried in the shade at room temperature and, thereafter, crushed in an electrical mixer-grinder. Hundred grams of this air-dried powder of the leaves was soaked in 90% ethyl alcohol and was allowed to stand for 15 min in a tightly covered container. The soaked powder was then transferred to a percolator, where it was firmly packed in and allowed to macerate for 24 h at room temperature, followed by slow percolation. The procedure was repeated over the next 24 h, with sufficient amounts of 90% alcohol until no further extraction was possible. Alcohol was evaporated to a soft extract and the residue was transferred to a vacuum desiccator, thus, obtaining the dried leaf alcoholic extract of *Ocimum tenuflorium*.[13-14]We got5 g of a dark black ish-black and sticky extract (5% dry weight of powdered leaves). The OSE suspension was used in doses of 200 mg/kg BW and 100 mg/kg BW for the respective groups as per previous studies in other models of hepatotoxicity.[9,10]

Standard hepatoprotective

Silymarin (SILY) powder (obtained from Micro Labs Ltd., Bangalore, India) was used to make the suspension in doses of 100 mg/kg BW and 50 mg/kg BW for the respective groups following the method of Mankani *et al*. and Mansour *et al*.[15-17]

Hepatotoxin

Paracetamol (PCM) powder (I.P.) (obtained from micro labs , Hyderabad , India) was used to make the suspension in a dose of 2 g/kg BW for the respective groups.

Methods

The experiment was carried out on 30 healthy albino rats for 10 days. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week.

Grouping and Treatment Schedule

The rats were randomly divided into five groups of six animals each after weighing, recording and numbering. Each group received treatment as follows:
Group A :-Normal control 5 ml Vehicle /kg BW/ Per day
Group B: -paracetmol (2 gm/kg) 5 ml/kg BW. / Per day
Group C:-200mg test drug in 5 ml/kg BW. / Per day
Group D:-100mg silymarin in 5 ml/kg BW. / Per day
Group E:-200mg test drug +50mg silymarin in 5 ml/kg BW. / Per day
Dosing and Administration **of Drugs**

The drug suspensions and the vehicle were administered per orally by an intragastric feeding tube at a uniform volume of 5 ml/kg BW.

Induction of Hepatic Injury: A single dose of paracetamol 2 g/kg BW/day was given to groups B, C, D and E on the eighth day of the experiment. It was administered after overnight fasting of the animals, i.e. the diet was restricted 12 h prior to the administration of paracetamol. However, free access to water was permitted.[18-19]

Laboratory Assessments

On the 10^{th} day, blood was collected from the hearts of the animals under light ether anesthesia. The blood was kept undisturbed for 30 min and the clot was dispersed with a glass rod. The samples were centrifuged for 15–20 min at 2000 rpm to separate the serum and then sent for liver function tests (LFT), namely total serum protein, albumin globulin ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[20-24]

Histopathological Examination

The rats were then sacrificed (on the 10^{th} day) under deep ether anesthesia and the liver samples were excised and washed with normal saline. A record of each liver was made, regarding size and shape, color and presence or absence of any nodule. Then, the livers were fixed immediately in 10% formalin solution. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin and examined microscopically for histopathological changes.[25-28]

Statistical Analysis:

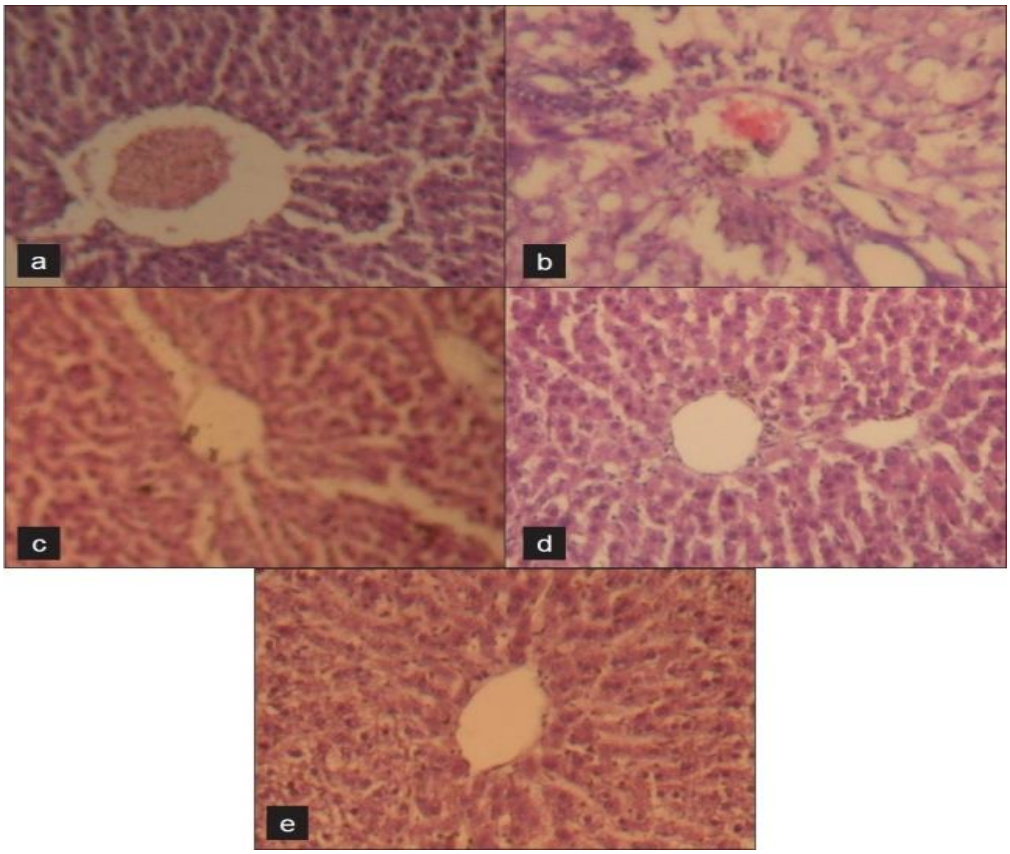
The results, obtained from the LFT were presented as mean and standard error of mean (SEM) for each group (mean ± SEM). All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability.

Table 1: Statistical Analysis

Groups	Total protein (g/dL)	Albumin globulin ratio	Serum (IU/L)AST	Serum ALT (IU/L)
A-Vehicle control	7.1±0.12	1.48±0.06	38±0.11	31±0.21
B-Paracetmol(PCM)	4.9±0. 20	0.1±0.86	748±3.52	510±18.36
C-TEST 200mg	5.0±0.10*	1.01±0.56*	271±28.19*	83±8.56*
D-.SILY100mg	6.86±0.16	1.28±0.06	96±3.01	45±5.06
ETEST 100mg PCM+SILY50mg	6.05±0.11	1.3±0.66	227±21.11*	72±6.85*

Table No 1 Results are expressed as mean :SEM(N=6)*P<0.01compared with the PCM group,*p <0.01 compared with the silymarin group; P <0.01 obtained is highly significant; AST-aspartate amino trasferase,ALT-alanine amino transferase,ALP-alkaline phosphatase,PCM-paracetamol,SILY-silymarin

Fig No 1 Photomicrographs of rat liver (hematoxylin and eosin) under low power (×100), (A) shows normal hepatic architecture; (B) shows hepatic necrosis; (C, D and E) show varying degrees of hepaticregeneration



A comparison was made with the experimental control (paracetamol) group and with the standard (silymarin). We took a *P*-value of <0.01 (highly significant) as our desired level of significance. Effects of the alcoholic leaf extract of *Ocimum tenuflorium* on total protein, albumin globulin ratio, , aspartate aminotransferase and alanine aminotransferase in paracetamolinduced hepatotoxicity in albino rats (10th day of the experiment) [29-31]

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