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PHYTOCHEMICAL PROFILE, ANTI-OXIDANT AND HEPATOPROTECTIVE ACTIVITY *OCIMUM TENUIFLORUM* LEAVES AGAINST CARBON TETRACHLORIDE–INDUCED HEPATOTOXICITY IN MICE

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Abstract
Ocimum tenuiflorum is a medicinal plant with therapeutic potential, belongs to the family Lamiaceae also known as, tulsi and is known for its anti-oxidant and hepatoprotective activity. I have been selected groups were divided into two different doses, test groups (200 and 400 mg/kg) of the crude extracts, the standard drug (silymarin 100 mg/kg), and the hepatotoxicant carbone tetrachloride was negative control. The result of anti-oxidant and hepatoprotective activity suggested that effects of *Ocimum tenuiflorum* leaves extract were tested on phytochemical, results showed the presence of alkaloid, saponins, steroid, phenolic compounds flavonoids, tannins as chemical constituents may have anti-oxidant and hepatoprotective activity, which is not evaluated till now.Amoung the results, shows The amount of steroids was estimated to be 11.85 mg/g and 57.69 mg/g respectively for methanol extracts respectively. The amount of alkaloids was estimated to be 121.69 mg/g and 12.30 mg/g respectively for methanol extracts respectively. The amount of phenolic compounds was estimated to be 71.32 mg/g for methanol extract. The amount of flavonoids was estimated to be 116.60 mg/g respectively for methanol extracts respectively. The IC50 concentration of methanol extract was noticed to be very close to standard suggest that the activity of methanol extract was very high. The 80% methanol extract decreased the absolute and relative weight of the liver of mice at the doses of 200 and 400 mg/kg ($p< 0.01$ and $p< 0.001$, respectively). It also suppressed the plasma levels of AST and ALT ($p< 0.001$) in the aforementioned doses. Among extracts, the methanol fraction showed maximum hepatoprotective activity in its dose of 400 mg/kg ($p< 0.001$, in all cases). *Ocimum tenuiflorum* is endowed with hepatoprotectiveactivity, probably mediated via its antioxidant and anti-inflammatory activity. Thus, *Ocimum tenuiflorum* can be taken as one candidate for the development of hepatoprotective agents because of its good safety profile.

***Keywords*:** *Ocimum tenuiflorum, 1, 1- diphenyl-2-picryl hydrazyl* (DPPH), diclofenac, alanine aminotransferase (ALT), aspartate aminotransferase (AST).

Introduction

Herbal Medicine

Significance of herbal medicine

During thousands of years of early human existence many natural materials were identified for combating human ailments. The earliest mention of the medicinal use of plants has been found in Rigveda. Medicinal plants have been used since ancient times as medicines for the treatment of diseases and still play a key role in world health. The chemical diversity of plants has made them one of the main sources for the isolation of bioactive organic compounds [1]. Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites. Plants are the best source of active secondary metabolites which are beneficial to mankind in treating many diseases. Many plant origin drugs have been reported with biological properties like Analgesic, Anti-inflammatory. India harbours about 15% out of the 20,000 medicinal plants of the world, of which 90% of them are found growing wild in different climatic conditions. The tribal and rural populations of India largely depend on medicinal plants for their health care as well as

for their livestock. This attracted the attention of several botanists that lead to an array of reports on ethnomedicine. Medicinal plants are the main sources of chemical substances with potential therapeutic effects.

Conventional and Traditional Medicines

Conventional or synthetic drugs used in the treatment of diseases are sometimes inadequate and can have serious adverse effects. There is a worldwide trend to search for traditional medicines. Siddha medicare is an ancient system of medicine popular amongst Tamil speaking world practiced for over several thousand years. So, there are several diseases and their cure methods are available on this planet, the need is to identify the correct source of the drug, which can give better results as compare to the active principles used to cure the diseases in current scenario. The newer studied active principle shall be with higher safety, efficacy and less in side effects. So, the plant sources are available more as compare to the other sources. There are several plants in which the analgesic and anti-inflammatory activity was reported earlier with the better curability and effectiveness. Here in this, work it is wishes to find out a better source for the analgesic and anti-inflammatory activity with comparison to other medicinal plants.

Antioxidant activity

Antioxidants are molecules that can delay or prevent an oxidative reaction [5] catalyzed by free radicals. Oxygen is an essential element for life to perform biological functions such as catabolism of fats, proteins and carbohydrates in order to generate energy for growth and other activities. However, a parallel role of oxygen as a toxic agent for living tissues has also been discovered. The biological combustion produces harmful intermediates called free-radicals. A free-radical is simply defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. It may be superoxide (O^{2-}), thiyl ($RS^{\cdot}$) or nitric oxide ($NO^{\cdot}$) in which the unpaired electron delocalized between both atoms. The $O2^{-}$, hydroxyl radicals (O.H) and other reactive oxygen species (ROS) such as H_2O_2 are continuously produced in vivo. Oxygen, though not dangerous by itself, is involved in the generation of various kinds of "reactive oxygen species" (ROS). Primarily ROS play an important role in the host defense mechanism against microorganisms, but the increased production of ROS is associated with the onset of a variety of diseases including cancers [6], inflammations [7], neuro-degeneration [8], Parkinson's disease [9], artherosclerosis [10] and per-mature aging [11].

Plant-derived antioxidants:

Plant-derived antioxidants were discovered from different sources of plant parts and plant origin. The antioxidant hypothesis claims that antioxidants can prevent oxidative damages, and reduce the risks of chronic disease [12]. The hypothesis is in line with efforts to discover natural antioxidants, especially plant-derived antioxidants. Plant-derived antioxidants are mainly obtained from diets, such as vitamin A, C, E and carotenoids [13]. Plant polyphenols or phenolic compounds are now widely studied by researchers due to complexity of their chemical nature and the ubiquitous occurrence in plant materials [14]. Dried Hibiscus sabdariffa flower that is drunk as red tea is a rich source of polyphenols, especially anthocyanin and also vitamin C [15]. Coriander (*Coriandrum sativum*) is rich with well known antioxidants such as quercetin, caffeic acid, cineole, geraniol, borneol, eucalyptol, cinnamic acid, ferrulic acid and rutin [16]. Chili pepper (*Capsicum annuum*) is rich in ascorbic acid, beta-carotene, caffeic acid, capsaicin, hesperidin, kaempferol and quercetin.

Hepatoprotective Activity:

Liver is the largest key organ in the human body that deals in maintaining individual health with metabolism, absorption, secretion, storage, and detoxifying functions in the body, constituting around 2.5% of an adult‟s body weight [17]. It receives 75% of the cardiac blood through hepatic portal vein and hepatic artery. The hepatic portal vein brings the absorbed nutrients from the gastrointestinal tract to the liver which takes up, stores and distributes nutrients and vitamins. It plays a central role to maintain blood glucose level and regulates the circulating blood lipids by secretion of very low density lipoproteins and also synthesized the plasma protein. It is associated with detoxification from the exogenous and endogenous challenges like xenobiotics therefore it is at risk to damage from drugs and other substances such as viral infection and alcoholism. Due to excessive exposure of hazardous chemicals unwanted free radicals are generated, that they overcome the natural defensive system, leading to hepatic injury and develop into jaundice, cirrhosis and fatty liver.

Patterns of hepatic injury

Chemical substances make a wide scope of clinical and pathological damage. Biochemical marker enzyme such as alanine transferase, aspartate amino transferase, alkaline phosphatase and bilirubin are usually used to signify liver wort injury. Disintegration and intracellular accumulation of hepatocytes damage may produce distinct cell enlargement with unevenly clustered cytoplasm presenting giant clear spaces. Accumulation of adipose tissue drops inside the hepatic cell is identified as steatosis

Drug/chemical-induced hepatic injury Hepatic injury

Liver is the general indication of drug toxicity [19] and reports for 50% more cases of acute liver failure. Hepatotoxicity is the biggest problem for the development of drug and is the foremost cause for drug-withdrawal method from the market place. The drug-induced liver diseases are categorized as type A, predictable (inherent and dose-dependent) and type B, unpredictable (low incidence and may or may not be dose dependent) called as idiosyncratic. In susceptible individuals, when chemical agents produce non-predictable toxicity with no warning injury occurs. Liver cell injury caused by various toxic substances like the antibiotic, chemotherapeutic factors, carbon tetrachloride (CCl_4), thioacetamide, anti-tubercular drugs,

excessive intoxicant use of alcohol and microorganisms etc. Isoniazid, Rifampicin and Pyrazinamide are the first line drug of tuberculosis treatment and all are metabolized in the liver. The single use of drug may response in the rapid growth of resistance or failure of treatment. So the first line drugs are used in combination, or with 6 other practice of medicine. The best known toxic drug effect is hepatotoxicity varies by 2% and the severity of toxicity is increased by 28% when these drugs are used in combination [20].

Medicinal Plants as Hepatoprotective therapeutics

In Indian systems of medicine, integer of herbs and herbal formulations are recommended. These formulations are time tested, with good efficacy and efficiency. Amongst the herbs only few herbs has special intention for search for hepatoprotective medicine, while other remains with the text of reference books. Some of drugs are *Boerhaviadiffusa, Picrorhizakurroa, Phyllanthus niruri, Phyllanthus emblica, Aloe vera*etc[21].

Introduction to plant (*Ocimum tenuiflorum*)

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.

Ocimum tenuiflorum, also known as ***Ocimum sanctum***, ***holy basil***, or ***tulasi***, is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

Botanical Classification:

Kingdom: Planta
Divison: Magnoliaphyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: Sanctum

Other Names

English name - holy basil/sacred basil
Hindi name - tulsi
Sanskrit name - tulasi
Gujarati name – tulsi

Uses

- Used for bronchial asthma; expectorant and bronchodilator effects.
- Used against respiratory ailments including bronchititis and tuberculosis.
- Used for rhinitis (inflammation of nasal mucus membrane).
- Can serve as a cure and prophylactic as well for the severe acute respiratory syndrome (SARS) – The root of the tulsi plant should be crushed and boiled with turmeric powder for a few minutes, after which it should be filtered. Consuming two spoonfuls of this potion twice daily will cure SARS and prevent contracting of the disease.
- Tulsi tea with honey is a good expectorant especially in cased where fever is involved.

Morphological charecters

Holy basil is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems. Leaves are green or purple; they are simple, petioled, with an ovate blade up to 5 cm (2 in) long, which usually has a slightly toothed margin; they are strongly scented and have a decussate phyllotaxy. The purplish flowers are placed in close whorls on elongated racemes [6].

Materials and Methods

Instrumentation

All the glassware use was of Borosilbrand. Water Bath and Thermostatic heating mantle was used for extraction of phyto-constituents. Elico double beam UV visible spectrophotometer with spectral manager version 2.2 was used for spectral analysis. Standard quartz cuvetts of 1cm cell was used for spectrophotometer sample holders.

Chemicals and Reagents

Ocimum tenuiflorum leaves were procured from local market, the haepatotoxicant carbon tetrachloride was purchased from S.D.Fine chemical s, Mumbai. The chemicals and reagents like DPPH, Ascorbic Acid was obtained as a gift from Aurobindo Pharm Ltd., Hyderabad. All other chemicals were procured of analytical grade.

Preparation of Reagents

DPPH solution Preparation: 40mg of DPPH was weighed accurately and dissolved in 50ml of methanol. It was kept in a magnetic stirrer to dissolve homogeneously. Then make the solution up to the mark in a 100mL volumetric flask.

Preparation of Ascorbic Acid Standard solutions: 10mg of Ascorbic Acid was accurately weighted and quantitatively transferred into a 10ml volumetric flask. Approximately 5ml Methanol were added and the solution and sonicated for 15 min. The flask was makeup to volume with mobile phase, and mixed. After filtration, an amount of the solution containing a concentration of 1000µg/ml of Ascorbic Acid was obtained. This was used as standard stock solution. Required dilutions were prepared from the standard stock solution.

Collection of plant material

The plant *Ocimum tenuiflorum* collected from natural habitat i.e. beside the house in Guntur, Andhra Pradesh. The leaves of the plant were separated, cleaned with sterile cotton and were shade dried. After the complete dry, plant parts were powdered separately and the powders were preserved in air tight container and were used when required.

Extraction procedure

The powdered material was weighed in a fixed quantity and was subjected to soxhelt extraction using Chloroform, Methanol and Water in successive mode respectively for 48h. The extraction was continued till colorless solvent appear in the thimble of soxhelt extractor. The solvent was then recovered using distillation apparatus and the concentrated extract was further evaporated using a rotator vacuum evaporator to get dry powder separately for each of the solvent. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of pharmacological activities.

Evaluation of Antioxidant Activity by DPPH free radical scavenging assay

For determination of DPPH free radical of the plant extracts, the fallowing procedure was fallowed.

- At first, 6 test tubes were taken to make aliquots of 6 concentration of each of the plant extract (5, 10, 50, 100 and 500 µg/mL) with the samples.
- Ascorbic acid was taken as standard and hence six different concentrations of the ascorbic acid also taken in six test tubes.
- 3mL of DPPH solution was added on each test tube by pipette.
- The room temperature was recorded and kept the test tubes for 30min to complete the reactions.
- DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank.
- After 30 minutes, the absorbances of each test tube were measured by a UV spectrophotometer at 517nm.
- IC_{50} were measured from % Inhibition vs. Concentration graphs.
- % DPPH inhibition was calculated using the fallowing formula

$$I = [(Ab - Ae) / Ab] \times 100$$

Where I is the percentage of inhibition,

Ab is the absorbance of the blank sample and Ae is the absorbance of the extract.

Anti-inflammatory activity

Albumin denaturation inhibition assay was performed for the determination of anti-inflammatory activity of leaf extracts of *Ocimum tenuiflorum*. The assay was performed based on the procedure described by Syed *et al*., 2018 [45] using diclofenac as standard. The test sample at selected concentration was dissolved in 1 mL of 2.5% Dimethyl formamide and diluted with 0.2 M phosphate buffer (pH 7.4). Test solution (1 mL) containing different concentration of drug was mixed with 1 mL of 1 mM albumin solution in 0.2 M phosphate buffer and incubated at 27 °C in water bath for 10 min. The turbidity was measured at 660 nm spectrophotometrically. Percentage inhibition of denaturation was calculated.

$$\text{Inhibition in \%} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$$

The results observed in the study was used for calculating the IC_{50} concentrations and the extracts showing less IC_{50} concentrations was proved to be hiving more activity.

In vivo hepatoprotective studies

Ocimum tenuiflorum results in acute hepatitis in rats which resembles human viral hepatitis following a single dose. Hence, the present study was undertaken to evaluate the in vivo hepatoprotective activity of different solvent extracts of *Ocimum tenuiflorum, carban tetrachloride* as hepatotoxicant.

Selection and maintenance of animals

Healthy adult male albino rats of Wistar strain weighing between 180-220 g were obtained from the animal house, Hindu College of Pharmacy, Guntur, India for the screening of hepatoprotective activity of the plant extracts. The animals were housed in polypropylene cages in adequately, well ventilated room and maintained under standard environmental conditions (22-28°C, 60-70% relative humidity, 12 h dark/light cycle). The animals were fed with standard rat feed pellets and water.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n = 3) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for a overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg / kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If

mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight.

Preparation of the drug for the experimental study

The extracts and the standard drugs were administered in the form of suspension in water with 1% sodium carboxymethyl cellulose as suspending agent.

Preparation of the standard

Silymarin, a known hepatoprotective agent was used as the reference for comparison at a dose of 25 mg/kg body weight in 0.3% CMC.

Experimental design

Animals were divided into 11groups comprising of 6 animals in each group. Each group received the following treatment.

Group I	Normal control (0.3% CMC)
Group II	Hepatotoxicant (carbon terachloride 600 mg\kg) by i.p route as single dose
Group III	Aqueous extract of carbon terachloride at a dose of 200 mg/kg body weight.
Group IV	Aqueous extract of carbon terachloride at a dose of 400 mg/kg body weight
Group V	Methanol extract of carbon terachloride at a dose of 200 mg/kg body weight
Group VI	Methanol extract of carbon terachloride at a dose of 400 mg/kg body weight
Group VII	Chloroform extract of carbon terachloride at a dose of 200 mg/kg body weight
Group VIII	Chloroform extract of carbon terachloride at a dose of 400 mg/kg body weight
Group IX	Silymarin positive control

All these treatments were given orally for 14 days. On the 14thday, after 1 h of sample administration, except normal control animals (G-1) all the animal in groups (G-2 to G-11) received single dose of 600 mg/kg intraperitoneal injection of carbon terachloride dissolved in saline. After 48 hours carbon terachloride administration all the animals were sacrificed by cervical decapitation under light ether anaesthesia. The liver and blood were collected from all these animals on the same day for biochemical and histopathological estimations.

Isolation of blood serum for biochemical studies

Blood was collected from jugular veins and centrifuged (3000 rpm for 10 min) to obtain serum. The serum was used for marker enzyme estimation. Assay of aspartate aminotransferase (AST or SGOT).

Estimation of aspartate aminotransferase (AST or SGOT)

Aspartate aminotransferase was estimated by the method of King.

Reagents

1. Phosphate buffer 0.1 M, pH 7.5.
2. Substrate: 1.33 g of DL-aspartic acid and 15 mg of 2-oxoglutarate were dissolved in 20.5 ml of 1 N sodium hydroxide and made up to 100 ml with buffer.
3. 0.02% 2, 4-dinitrophenyl hydrazine (DNPH): 20 mg of DNPH in 100 ml of 1 N hydrochloric acid.
4. 0.4 N Sodium hydroxide.
5. Standard: 11 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer. This contains 1 µm of pyruvate/ml.

Procedure

1.0 mL of buffered substrate was incubated at 37°C for 10 min. Then 0.2 mL of enzyme was added and mixture was incubated at 37°C for 1 hr. To the control tubes enzyme was added after the reaction and it was arrested by the addition of 1.0 ml of DNPH reagent. The tubes were kept at room temperature for 30 min. A set of standard pyruvate solution was also treated in a similar manner. Then 5.0 ml of sodium hydroxide was added. The colour developed was read at 540 nm. The enzyme activity is expressed as U/L.

Assay of alanine aminotransferase (ALT or SGPT)

Alanine aminotransferase was assayed by the method of King.

Reagents

1. Phosphate buffer 0.1 M, pH 7.5.
2. Substrate: 1.78 g of DL-alanine and 30 mg of 2-oxoglutarate were dissolved in 20 ml of buffer, 0.5 ml of 1 N sodium hydroxide was added and made up to 100 ml with phosphate buffer, pH 7.5.
3. 0.02% 2,4-dinitrophenyl hydrazine (DNPH): 20 mg of DNPH in 100 mL of 1 N hydrochloric acid
4. 0.4 N sodium hydroxide
5. Standard: 11 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer. This contained 1 µm of pyruvate/ml.

Procedure

1.0 mL of substrate was incubated at 37°C for 10 min. Then 0.2 mL of enzyme solution was added. The tubes were incubated at 37°C for 30 min. To the control tubes enzyme was added after arresting the reaction with 1.0 ml of DNPH reagent. The tubes were kept at room temperature for 20 min. Then 5.0 ml of 0.4 N sodium hydroxide was added and then the colour developed was read at 540 nm.The enzyme activity is expressed as U/L

Assay of total protein

Protein was estimated by the method of Lowry.

Reagents

1. Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide

Solution B: 0.5% copper sulphate in 1% sodium potassium tartrate.

50 ml of solution A was mixed with 1 ml of solution B just before use.

2. Folin's phenol reagent: One volume of Folin's reagent was diluted with two volumes of distilled water just before use.

3. Standard bovine serum albumin (BSA): 20 mg of BSA was dissolved in 100 ml of distilled water. Few drops of sodium hydroxide (alkali) were added to aid complete dissolution of BSA and to avoid frothing; it was allowed to stand overnight in a refrigerator.

Procedure

0.1 ml of 10% homogenate was diluted to 1 ml with water. From this diluted samples 0.1 ml was made up to 1 ml with water. Standards were taken and made up to 1 ml with water. 1 ml water was used as blank. To all tubes 4.5 ml of alkaline copper reagent was added and kept at room temperature for 10 min. Then 0.5 ml of Folin's phenol reagent was added and the colour developed was read after 20 min at 640 nm. Protein content is expressed as mg/dl of fresh or wet tissue.

Results

Extraction of Phyto-constituents from *Ocimum tenuiflorum*

Extraction and evaluation of pharmacological properties like Antioxidant Activity, Anti-Diabetic activity and Anti-inflammatory activity of different solvent extracts of leaves of *Ocimum tenuiflorum* was studies. *Ocimum tenuiflorum* is an Indian medicinal plant having significant medicinal properties. The plant parts *Ocimum tenuiflorum* were collected, Pre-washing, drying, grinding to obtain a homogenous. Care was taken such that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples.

The solvent used for the extraction is based on the polarity of the active constituent. Different solvent systems are available to extract the bioactive compound from natural products. Here the solvents having different polarities i.e. Chloroform, Methanol and Water were used as solvents for the extraction of medical active compounds form the leaves of *Ocimum tenuiflorum*. The soxhlet extraction method was fallowed for the extraction of phyto-constituents. Figure 2 showing the soxhlet extraction of medical compounds in the study.

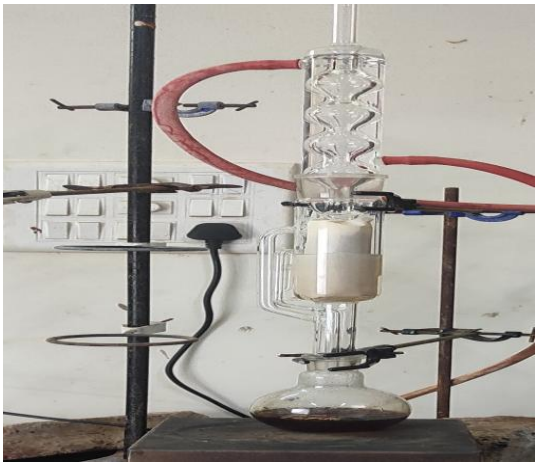

Figure 1: Soxhlet extraction of the whole plant of *Ocimum tenuiflorum*

The extraction was continued until the completed extraction was over and no possible extractable compounds present in the plant. After completion of series of extraction using the three solvents, the solvent was recovered and the extract was dried using rotator vacuum evaporator. The crude extract obtained was weighed and it was found that for 1kg of the plant contain19.7 gr of compounds extract with chloroform, 175.6 gr in methanol and 132.7 gr are extracted using water solvent.

Phytochemical screening

Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design. The most important of these bioactive constituents of

plants are alkaloids, tannins, flavonoids and phenolic compounds. The present study deals with the screening based on phytochemical tests of *Ocimum tenuiflorum* for identifying their chemical constituents.The phytochemical screening results were given in table 1 and figure 4 presents the screening photos in the study.

Table 1: Preliminary phytochemicals screening results of *Ocimum tenuiflorum*

S. No	Screening Tests	Chloroform	Methanol	Water
1	Steroids	Positive	Positive	Negative
2	Triterpenoids	Negative	Positive	Negative
3	Saponins	Negative	Positive	Positive
4	Steroidal saponin	Negative	Positive	Negative
5	Triterpenoid saponin	Negative	Positive	Positive
6	Alkaloids	Negative	Positive	Positive
7	Carbohydrates	Negative	Positive	Positive
8	Flavonoids	Positive	Positive	Negative
9	Phenols	Negative	Positive	Negative
10	Amino acids	Negative	Negative	Positive
12	Fixed oils and fats	Negative	Negative	Negative

Quantitative estimation of phytochemicals

The quantitative determination of the secondary metabolites reveals there concentrations in different solvents extracts of *Ocimum tenuiflorum*. The phyto chemical constituents show positive results in preliminary screening were quantitatively estimated using spectrophotometric analysis.

Steroids

The amount of the total steroids present in the extracts are expressed in terms of the standard steroid i.e., Cycloartenol equivalent units. Standard calibration curve for Cycloartenol was obtained in the concentration range of 0.2-1.2µg/mL with regression equation of y = 1.590x - 0.054. The preliminary screening suggests that the chloroform and methanolic extracts shows positive results for steroids and hence were used for quantification study. The amount of steroids present in the extracts was determined using the standard regression equation. The amount of steroids was estimated to be 11.85 mg/g and 57.69 mg/g respectively for chloroform and methanol extracts respectively. The quantitative estimation of steroids present in leaf extracts of *Ocimum tenuiflorum* confirms that steroids were observed in less quantity in chloroform extract whereas high quantity was noticed in methanolic extract.The standard calibration curve results were presented in table 2 and calibration curve was presented in figure 5.The result noticed in the quantification of steroids was presented in table 3.

Table 2: Standard calibration curve for Steroids

Concentration in µg/ml	Absorbance
0.2	0.258
0.4	0.605
0.6	0.889
0.8	1.215
1.0	1.507
1.2	1.878

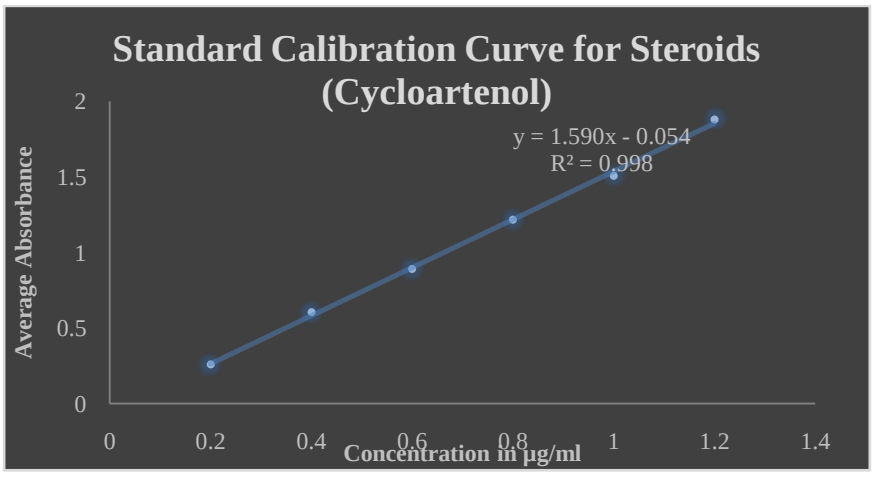

Figure 2: Standard Calibration Curve of Steroids

Table 3: Estimation of Steroids in leaf extracts of *Ocimum tenuiflorum*

S No	Extract	Absorbance observed	Amount estimated in mg/g
1	Chloroform	0.134	11.85
2	Methanol	0.863	57.69

Alkaloids

Total Alkaloids present in the extracts are expressed in terms of the standard alkaloid i.e., Atropine equivalent units. Standard calibration curve for Atropine was obtained in the concentration range of 4-9µg/mL with regression equation of y = 0.080x + 0.071 (Table 4.5). The amount of steroids present in the extracts was determined using the standard regression equation. The preliminary screening suggests that the methanol and water extracts shows positive results for alkaloids and hence were used for quantification study. The amount of alkaloids present in the extracts was determined using the standard regression equation. The amount of alkaloids was estimated to be 121.69 mg/g and 12.30 mg/g respectively for methanol and water extracts respectively. The quantitative estimation of alkaloids present in leaf extracts of *Ocimum tenuiflorum* confirms that alkaloids were observed in less quantity in water extract whereas high quantity was noticed in methanolic extract. The standard calibration curve results were presented in table 4 and calibration curve was presented in figure 6.The result noticed in the quantification of steroids was presented in table 5.

Concentration in µg/mL	Absorbance
4	0.391
5	0.481
6	0.559
7	0.631
8	0.721
9	0.799

Table 4: Standard calibration curve of Alkaloids

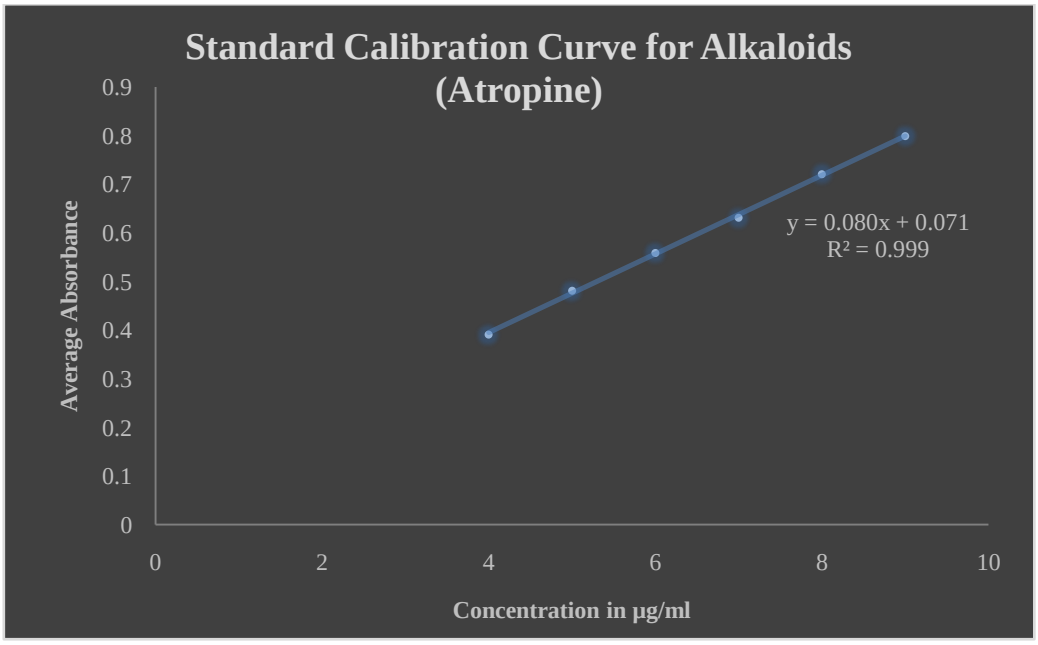

Figure 6: Standard Calibration Curve of alkaloids

S No	Extract	Absorbance observed	Amount estimated in mg/g
1	Methanol	0.268	121.69
2	Water	0.091	12.30

Table 5: Estimation of alkaloids in leaf extracts of *Ocimum tenuiflorum*

Phenolics:

Phenolic compounds present in the extracts are expressed in terms of the standard catechol equivalent units. Standard calibration curve for catechol was obtained in the concentration range of 2-12µg/mL with regression equation of y = 0.0351x + 0.0203. The amount of phenolic compounds present in the extracts was determined using the standard regression equation. The preliminary screening suggests that only methanol extract shows positive results for phenolic compounds and hence was used for quantification study. The amount of phenolic compounds present in methanol extract was determined using the standard regression equation. The amount of phenolic compounds was estimated to be 71.32 mg/g for methanol extract. The quantitative estimation of phenolic compounds present in leaf extracts of *Ocimum tenuiflorum* confirms that phenolic compounds were observed in high quantity in methanolic extract. The standard calibration curve results were presented in table 6 and calibration curve was presented in figure 7.The result noticed in the quantification of steroids was presented in table 7.

Concentration in µg/ml	Absorbance
2	0.089
4	0.166
6	0.228
8	0.298
10	0.371
12	0.443

Table 6: Standard Calibration curve of Phenolic compounds

Table7: Estimation of phenolic compounds in leaf extracts of *Ocimum tenuiflorum*

S No	Extract	Absorbance observed	Amount estimated in mg/g
1	Methanol	0.521	71.32

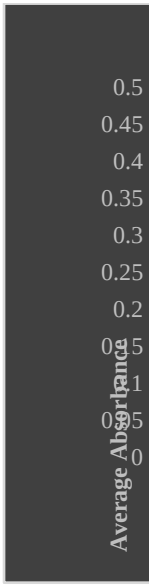

Figure 7: Standard Calibration Curve of Phenolic compounds

Flavonoids:

The amount of the total Flavonoids present in the extracts are expressed in terms of the standard flavonoid i.e., Quercetin equivalent units. Standard calibration curve for Quercetin was obtained in the concentration range of 0.1-0.6µg/mL with regression equation of y = 0.484x + 0.133.The preliminary screening suggests that the chloroform and methanolic extracts shows positive results for flavonoids and hence were used for quantification study. The amount of flavonoids present

in the extracts was determined using the standard regression equation. The amount of flavonoids was estimated to be 3.65 mg/g and 116.60 mg/g respectively for chloroform and methanol extracts respectively. The quantitative estimation of flavonoids present in leaf extracts of *Ocimum tenuiflorum* confirms that flavonoids were observed in less quantity in chloroform extract whereas high quantity was noticed in methanolic extract. The standard calibration curve results were presented in table 8 and calibration curve was presented in figure 8. The result noticed in the quantification of steroids was presented in table 9.

Table 8: Standard calibration curve of Flavonoids

Concentration in µg/ml	Absorbance
0.1	0.185
0.2	0.227
0.3	0.278
0.4	0.327
0.5	0.374

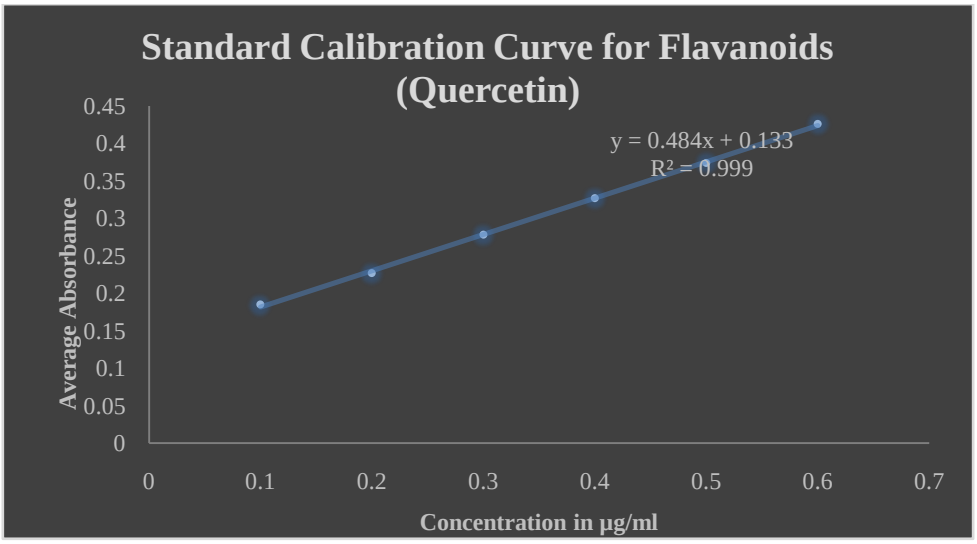

Figure 8: Standard Calibration Curve of Flavonoids

S No	Extract	Absorbance observed	Amount estimated in mg/g
1	Chloroform	0.151	3.65
2	Methanol	0.698	116.60

Table9: Estimation of Flavonoids in leaf extracts of *Ocimum tenuiflorum*

Antioxidant Activity:

It is commonly accepted that reactive oxygen species, such as superoxide ($O2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) radicals, are produced under oxidative stress. Reactive oxygen species play important roles in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, diabetes, and inflammation. Some natural antioxidants and compounds with radical scavenging activity have been identified over the last few years.

Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers.

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. Howeverover production of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA

Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities. Hence antioxidant activity of different solvent extracts of *Diospyros chloroxylon* Roxb was studied using DPPH free radical scavenging activity.

The DPPH method was evidently introduced nearly 50 years ago and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The parameter IC50 (efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color).The inhibition of DPPH radical by test samples was observed at arrange of 5-100µg/mL, and at the same dosage there was a similar free radical scavenging activity for Ascorbic acid which is well-known antioxidant compounds was also studied. The absorbance results noticed in DPPH activity study was presented in table 10. Table 116 represents the DPPH activity study results observed in the study and table 12 presents the IC50 values obtained in DPPH activity study. The DPPH activity graphs were presented in figure 9 to 11 respectively for chloroform, methanol and water extarcts respectively whereas figure 12 presents the comparative antioxidant results observed for various solvents leaf extracts of *Ocimum tenuiflorum*.

S No	Concentration in µg/mL	Absorbance observed			
		Ascorbic Acid	Chloroform extract	Methanol extract	Water extract
1	5	0.901	0.971	0.885	0.964
2	10	0.834	0.965	0.812	0.951
3	20	0.725	0.934	0.652	0.907
4	40	0.542	0.886	0.523	0.819
5	60	0.398	0.798	0.523	0.733
6	80	0.213	0.695	0.336	0.609
7	100	0.098	0.584	0.199	0.483

Table10: Absorbance observed in DPPH activity study

S No	Concentration in µg/mL	% DPPH inhibition			
		Ascorbic Acid	Chloroform extract	Methanol extract	Water extract
1	5	7.68	0.51	6.15	1.26
2	10	14.55	1.13	9.32	2.55
3	20	25.72	4.30	16.80	7.09
4	40	44.47	9.22	33.20	16.10
5	60	59.22	18.24	46.41	24.93
6	80	78.18	28.79	65.57	37.59
7	100	89.96	40.16	79.61	50.52

Table 11: DPPH Activity results for *Ocimum tenuiflorum*

S No	Sample	IC50 concentration in µg/mL
1	Standard ascorbic acid	49.98
2	Chloroform extract	130.84
3	Methanol extract	61.99
4	Water extract	103.58

Table 12: IC50 results observed in DPPH activity study

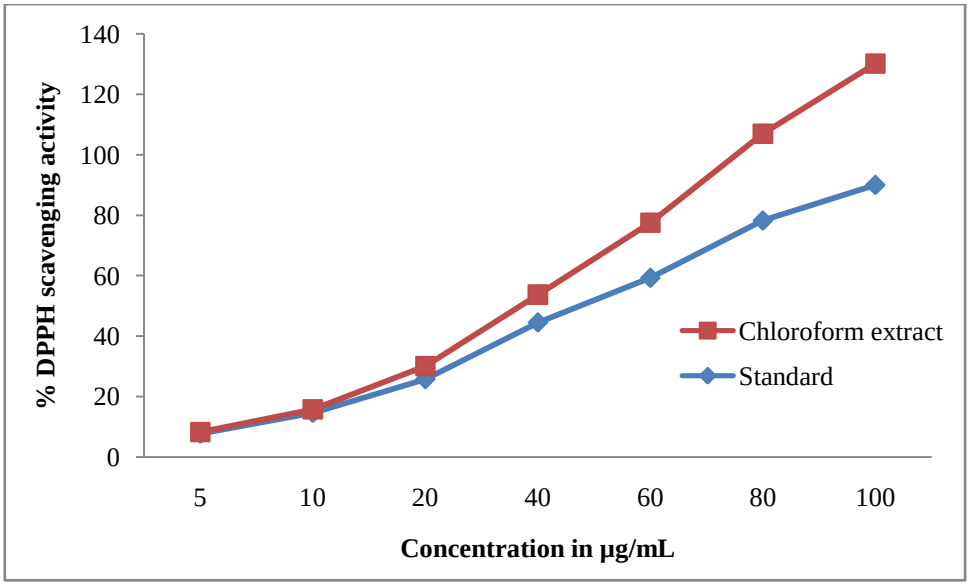

Figure 9: DPPH activity results observed for chloroform extract

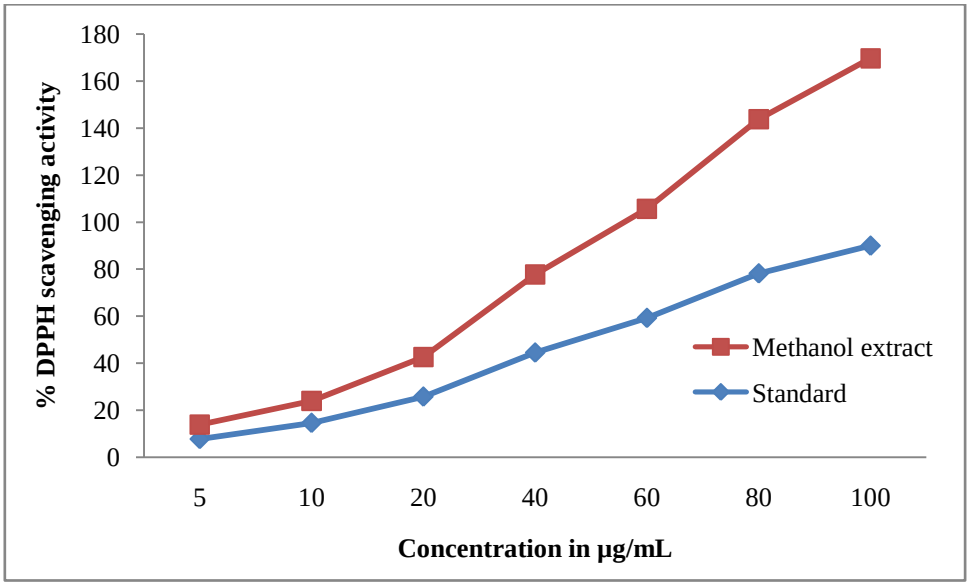

Figure 10: DPPH activity results observed for methanol extract

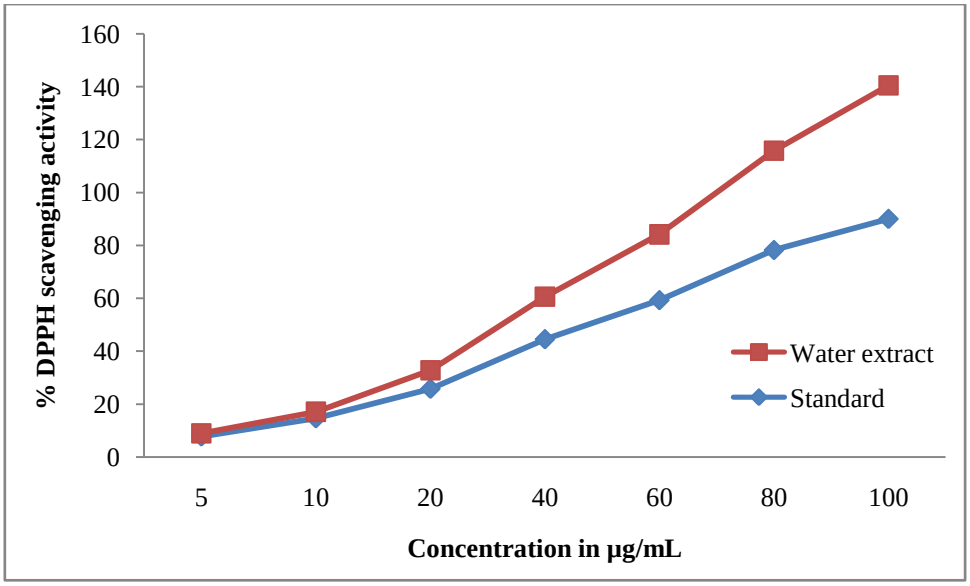

Figure 11: DPPH activity results observed for water extract

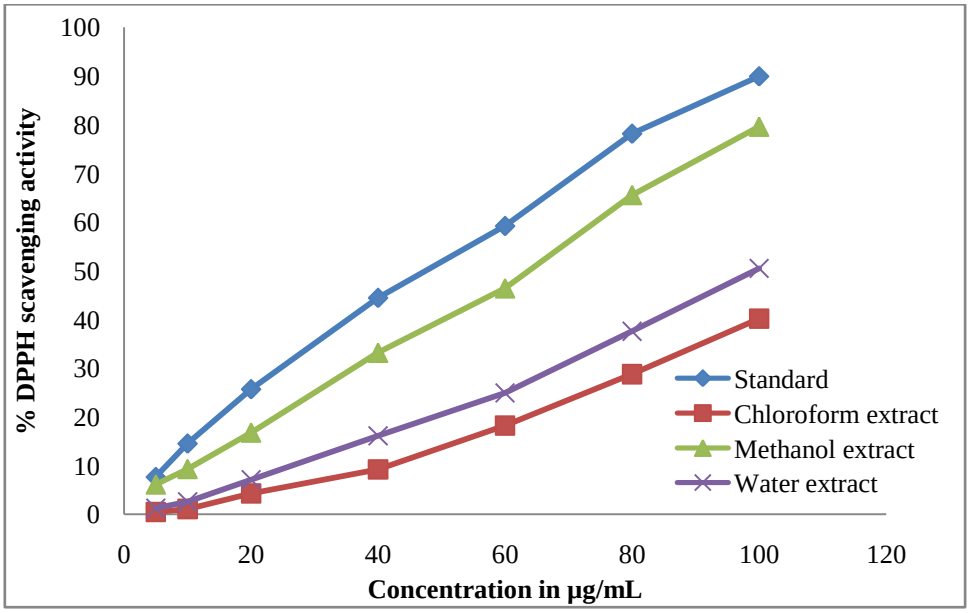

Figure 12: Comparative DPPH activity results observed for different solvent extracts of *Ocimum tenuiflorum*

5.5 Anti-inflammatory activity:

Inflammation is a defense mechanism that enables the body to protect itself against infection, burn, toxic chemical allergens, or any other harmful stimuli. Inflammation is a substantial reaction to damage, disease or destruction portrayed by heat, redness, pain, swelling and disturbed physiological functions. Denaturation of protein has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulfide bonding. Denaturation of protein causes the production of auto antigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited. In the present study, two types of drugs (NSAIDs and steroids) were used as reference drugs. NSAIDs prevent inflammation by blocking the cyclooxygenase enzyme activity. However, these drugs cause side effects of ulceration, hemorrhage, perforation and obstruction.

The anti-inflammatory activity of chloroform, methanol and water extracts of *Ocimum tenuiflorum* was performed using albumin denaturation method. The activity of methanolic extract was noticed to be significantly high then other extracts studied. The IC50 values were calculated for standard diclofenac as well as the extracts studied. The IC50 concentration was noticed to be 50.16µg/mL, 185.59µg/mL, 93.36µg/mL and 162.19µg/mL respectively for standard diclofenac, chloroform

extract, methanol extract and water extract respectively. The IC50 concentration of methanol extract was noticed to be very close to standard suggest that the activity of methanol extract was very high. The absorbance results noticed in anti-inflammatory activity study was presented in table 13. Table 14 represents the anti-inflammatory activity study results observed in the study and table 15 presents the IC50 values obtained in DPPH activity study. The anti-inflammatory activity graphs were presented in figure 13 to 15 respectively for chloroform, methanol and water extracts respectively whereas figure 16 presents the comparative antioxidant results observed for various solvents leaf extracts of *Ocimum tenuiflorum.*

S No	Concentration in µg/mL	Absorbance observed			
		Diclofenac	Chloroform extract	Methanol extract	Water extract
1	20	0.467	0.733	0.697	0.671
2	40	0.413	0.706	0.514	0.671
3	60	0.360	0.654	0.422	0.622
4	80	0.283	0.603	0.373	0.573
5	100	0.227	0.565	0.317	0.491
6	120	0.147	0.496	0.251	0.447
7	150	0.109	0.401	0.185	0.362
8	200	0.067	0.375	0.128	0.320

Table13: Absorbance observed in anti-inflammatory activity study

S No	Concentration in µg/mL	%DPPH			
		Diclofenac	Chloroform extract	Methanol extract	Water extract
1	20	36.85	0.81	5.67	5.67
2	40	44.07	4.47	30.44	9.15
3	60	51.29	11.50	42.90	15.84
4	80	61.69	18.40	49.47	22.40
5	100	69.28	23.49	57.12	33.55
6	120	80.05	32.90	66.03	39.58
7	150	85.19	45.72	74.91	51.01
8	200	90.94	45.72	82.68	56.70

Table 14: Anti-inflammatory Activity results for *Ocimum tenuiflorum*

S No	Sample	IC50 concentration in µg/mL
1	Standard Diclofenac sodium	50.16
2	Chloroform extract	185.59
3	Methanol extract	93.36
4	Water extract	162.19

Table 15: IC50 results observed in anti-inflammatory activity study

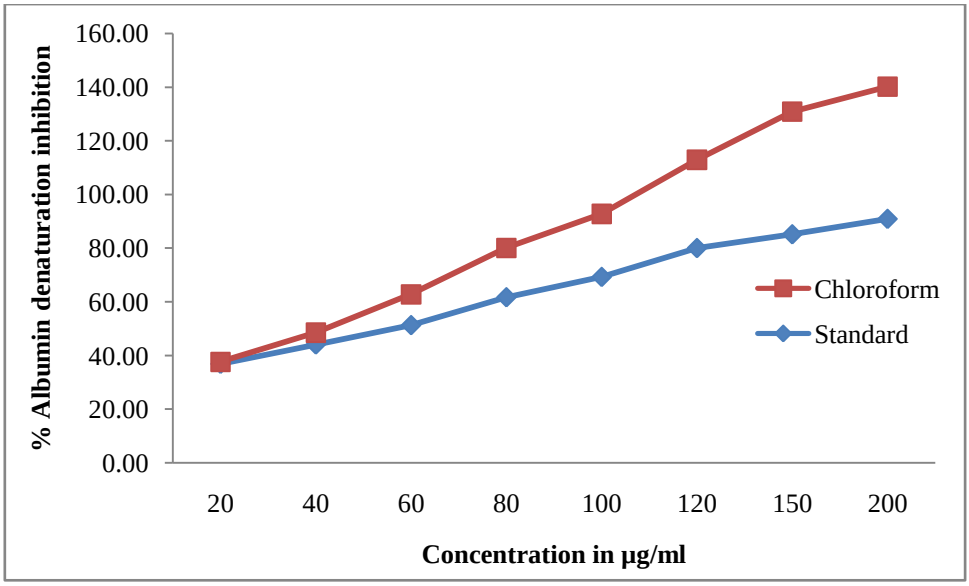

Figure 13:Anti-inflammatory activity results observed for chloroform extract

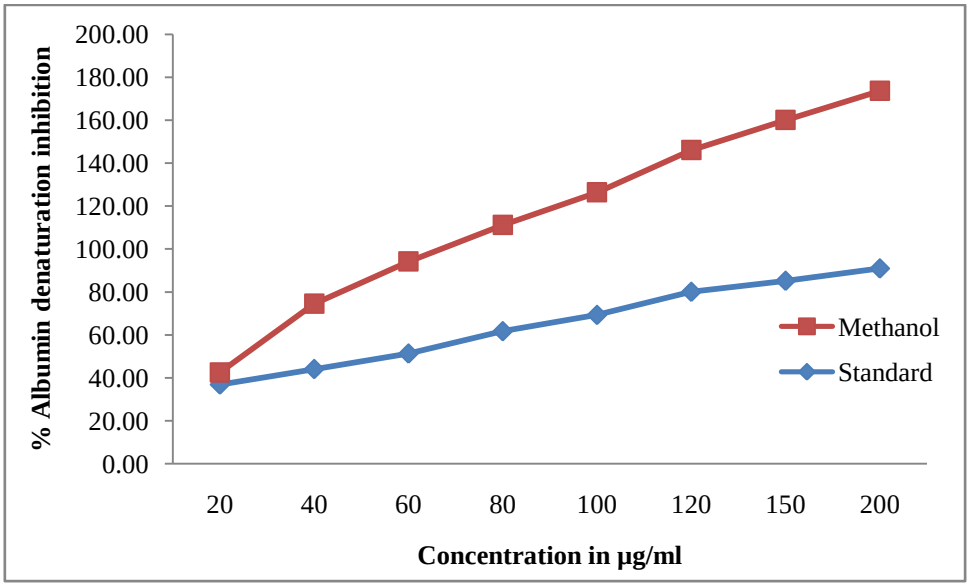

Figure 14: Anti-inflammatory activity results observed for methanol extract

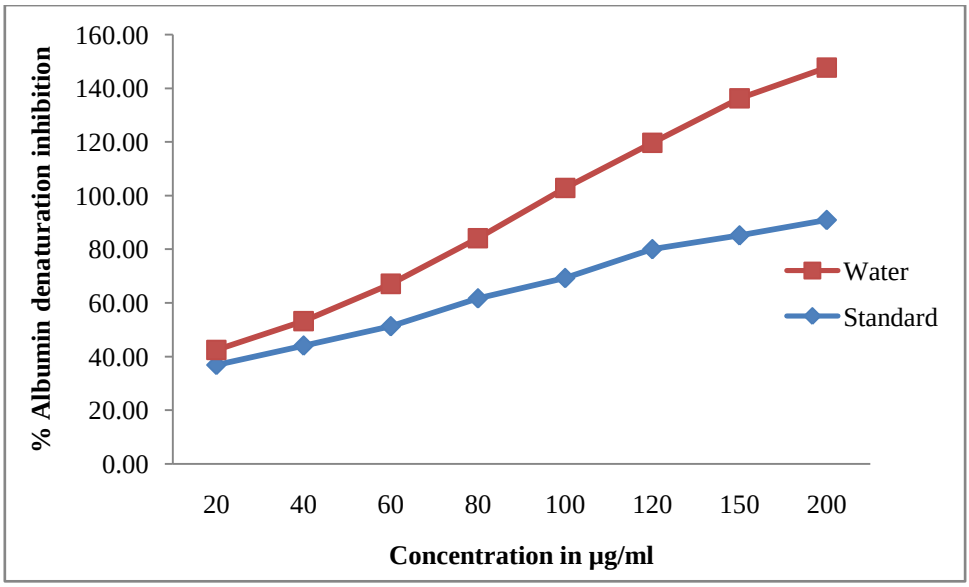

Figure 15: Anti-inflammatory activity results observed for water extract

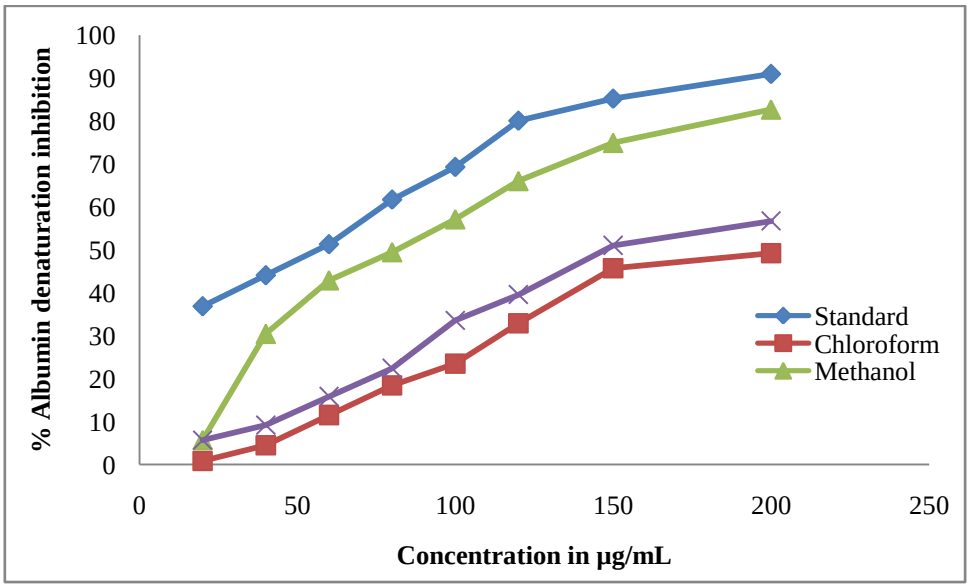

Figure 16: Comparative anti-inflammatory activity results observed for different solvent extracts of *Ocimum tenuiflorum*

5.6*In-vivo* hepatoprotective activity of *Ocimum tenuiflorum*:

The chloroform, methanol and aqueous extracts of *Ocimum tenuiflorum* were evaluated for its hepatoprotective activity against carbon tetrachloride induced hepatic damage. Healthy adult male albino rats of Wistar strain weighing 180-220 g were used for the study. Silymarin was used as positive control. The plant extracts at two different dose levels (200, 400 mg/kg, po), showed significant hepatoprotective activity as evidenced by an alteration in the serum enzyme levels.

The effect of *Ocimum tenuiflorum* both the dose levels on marker enzymes in serum against carbon tetrachloride induced hepatotoxicity was shown in Table16. Liver damage induced by carbon tetrachloride significantly increased the marker enzymes like AST, ALT and ALP in serum ($P<0.05$). Oral administration of the plant extracts of *Ocimum tenuiflorum* significantly decreased the level of marker enzymes AST, ALT and ALP ($P<0.01$) in serum. The total bilirubin level was significantly increased ($P<0.05$) in carbon tetrachloride treated animals. The *Ocimum tenuiflorum* extracts treated animals showed a significantly lower bilirubin level in serum. The total protein level in serum was considerably reduced in carbon tetrachloride toxicity. The *Ocimum tenuiflorum* extracts treated animals significantly increased ($P<0.05$) the total protein level in serum.

The present study revealed a significant increase in the marker enzymes like AST, ALT, ALP and serum bilirubin levels, on exposure to carbon tetrachloride, indicating considerable hepatocellular injury. Oral administration of *Ocimum tenuiflorum* at two different dose levels attenuated the increased levels of the marker enzymes produced by carbon tetrachloride and caused a subsequent recovery towards normalization almost like that of standard silymarin treatment. The decreased total protein level observed in the rats treated with carbon tetrachloride may be due to the decrease in the number of hepatocytes which in turn may result in decreased hepatic capacity to synthesis protein. On administration of extracts *Ocimum tenuiflorum* showed significant increase in total protein level, which indicates the increase in hepatocyte levels, accounting for its hepatoprotective effect. The subsequent recovery towards normalization of these enzymes strongly suggests the possibility of the extracts being capable of conditioning the hepatocytes so as to cause accelerated regeneration of parenchymal cells. The results showed that the extracts of the plant drugs *Ocimum tenuiflorum* different dose levels offer hepatoprotection. Figure 17, 18 and 19 presents the comparative AST, ALP and total protein content (TP) results observed in the study.

Group	Drug and Dose	AST (U/L)	ALP (U/L)	TP (U/L)
1	Control	47.76±0.222	48.78±0.135	6.81±0.031
2	Hepatotoxicant carbon tetrachloride (600 mg/kg)	100.34±0.343	76.31±0.297	0.76±0.003
3	Chloroform extract of *Ocimum tenuiflorum* (200 mg/kg)	77.3±0.371	76.31±0.297	6.80±0.025
4	Chloroform extract of *Ocimum tenuiflorum* (400 mg/kg)	51.12±0.200	50.11±0.156	1.31±0.004
5	Methanol extract of *Ocimum tenuiflorum* (200 mg/kg)	100.23±0.166	47.8±0.200	1.31±0.004
6	Methanol extract of *Ocimum tenuiflorum* (400 mg/kg)	100.23±0.166	99.1±0.104	1.31±0.004
7	Water extract of *Diospyros chloroxylon* (200 mg/kg)	77.30±0.314	75.55±0.259	6.00±0.021
8	Water extract of *Ocimum tenuiflorum* (400 mg/kg)	56.11±0.249	49.12±0.183	0.86±0.013
9	Positive control (silymarin) (25 mg/kg)	50.12±0.219	31.89±0.139	0.96±0.004

Table 16: Enzymatic study results

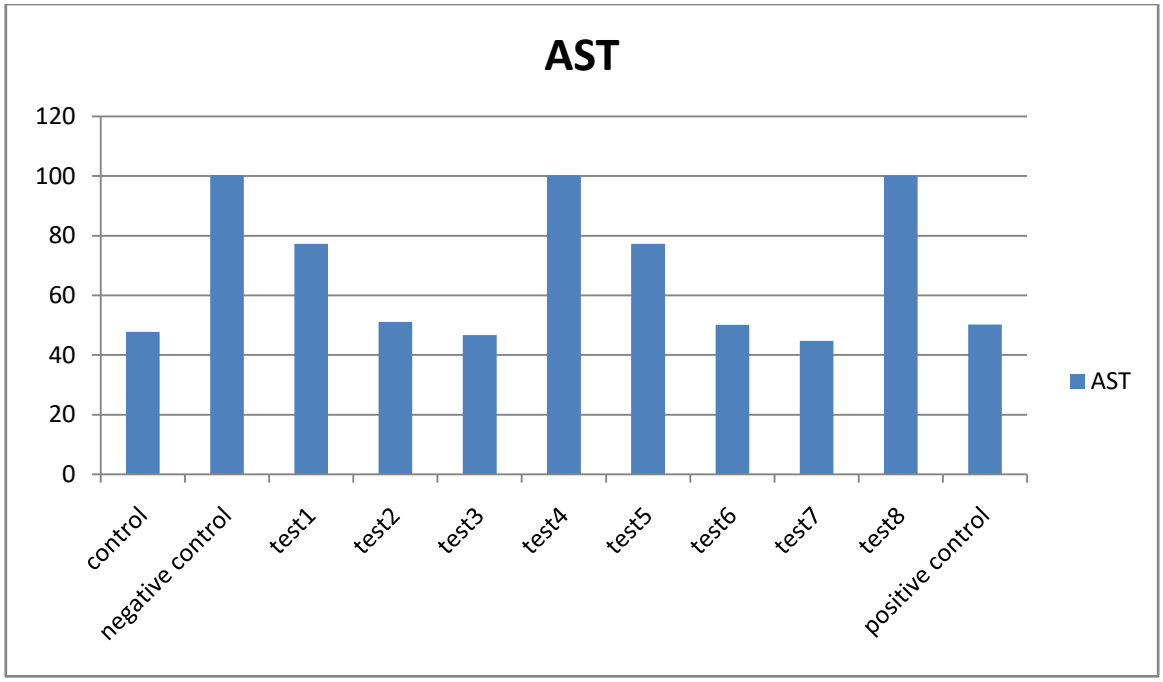

Figure 17: Comparative AST (U/L) results observed in the study

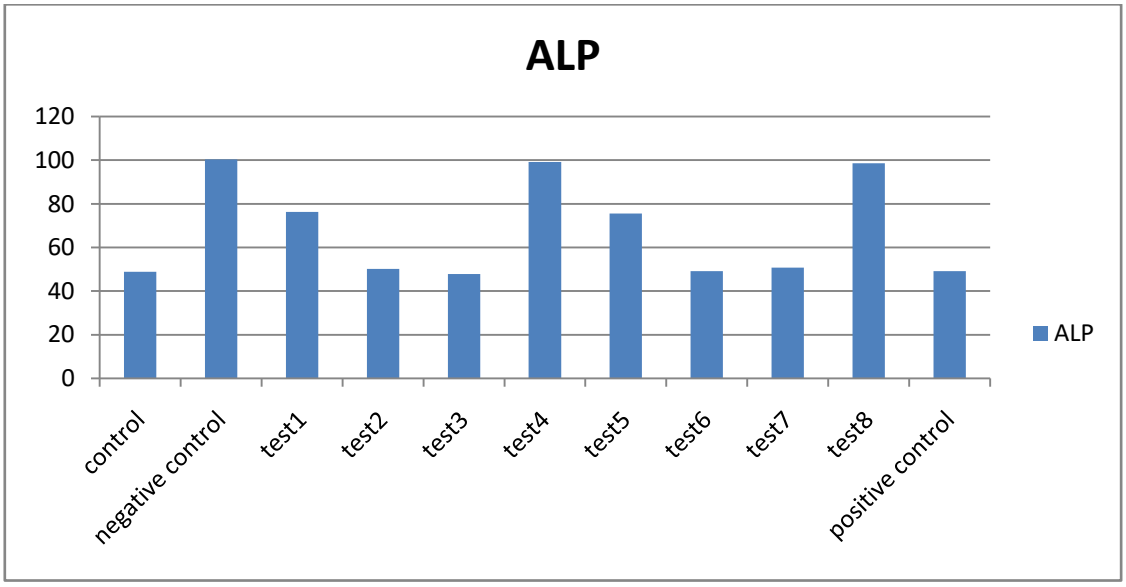

Figure 18: Comparative ALP (U/L) results observed in the study

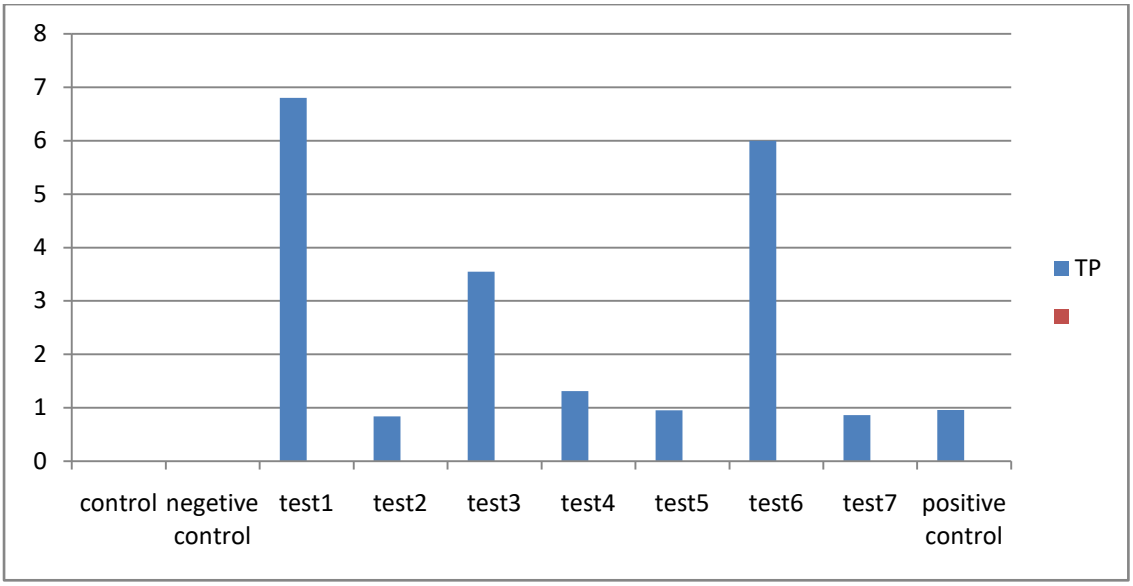

Figure 19: Comparative protein content observed in the study

Histopathological studies:

The histopathological observation of control group rat shows normal liver with central vein and cords of hepatocytes (Figure 20). The liver of hepatotoxicant carbon tetrachloride treated rats show severe hepatocellular degeneration and the fatty changes were identified for hepatocytes (Figure 21).

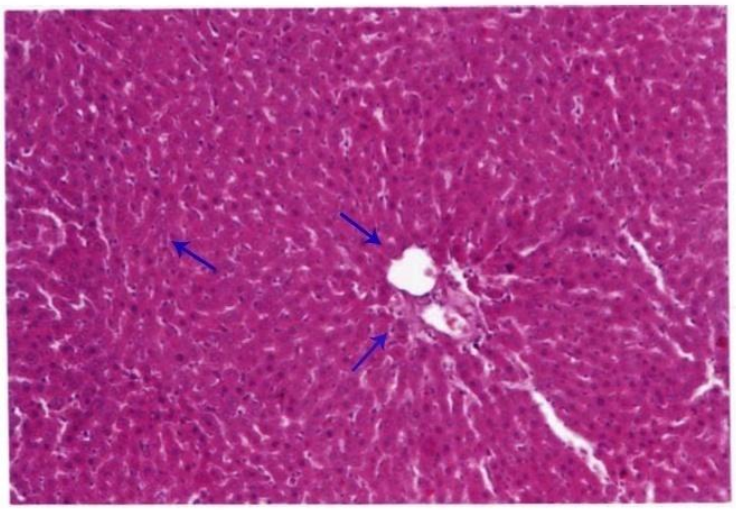

Figure 20: Liver section of control group rat shows normal liver with central vein and cords of hepatocytes

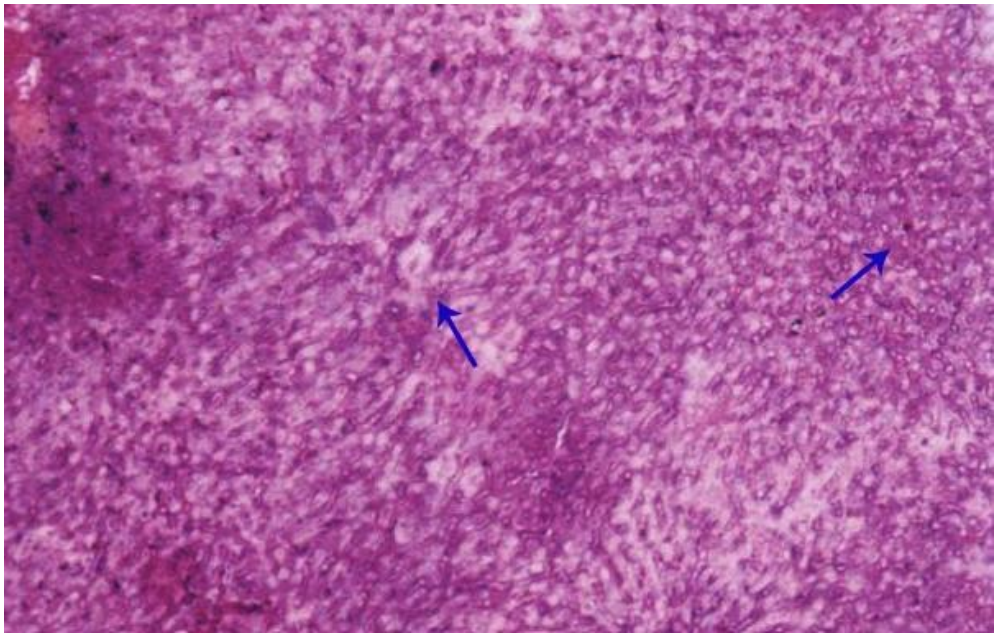

Figure 21: Liver section of hepatotoxicant carbone tetrachloride treated rats show severe hepatocellular degeneration and the fatty changes were identified for hepatocytes

The group of rats treated with hepatotoxicant and chloroform extract of *Ocimum tenuiflorum* at a dose level of 200 mg/kg body was noticed to be hepatocellular degeneration whereas at dose level 400 mg/kg body weight showed mild perilobular hepatocellular fatty changes.

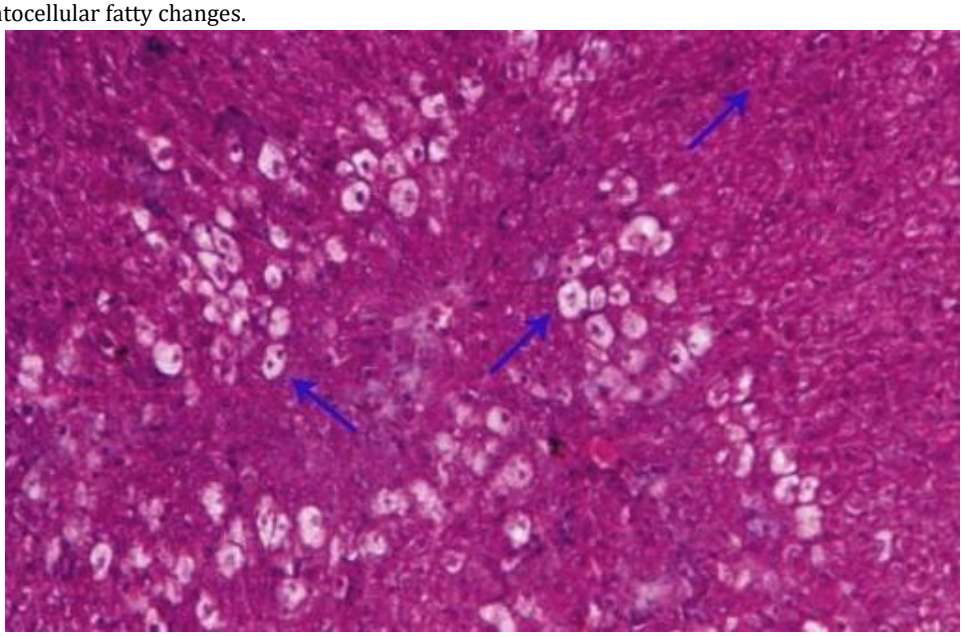

Figure 22: Liver section of hepatotoxicant and chloroform extract of *Ocimum tenuiflorum* shows hepatocellular degeneration

The group of rats treated with hepatotoxicant and methanol extract of *Ocimum tenuiflorum* at a dose level of 200 mg/kg body was noticed to bedilated central vein and mild perilobular hepatocellular fatty changes and at a higher dose of 400 mg/kg body weight showed regeneration of hepatocellular fatty changes. The regeneration of liver cells was noticed to be high in the group treated with methanol extract of *Ocimum tenuiflorum* at a dose level of 400 mg/kg.

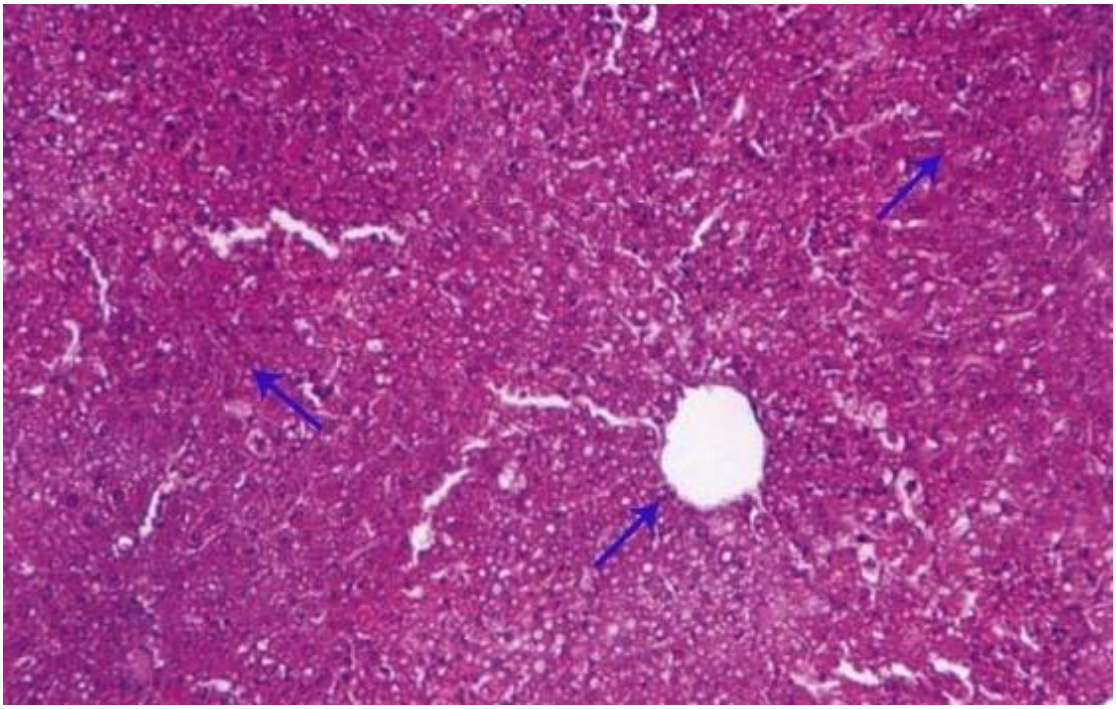

Figure 23: Liver section of hepatotoxicant and methanol extract of *Ocimum tenuiflorum* shows regeneration of fatty changes

The group of rats treated with hepatotoxicant and water extract of *Ocimum tenuiflorum* at a dose level of 200 mg/kg body was noticed to be hepatocellular degeneration whereas at dose level 400 mg/kg body weight showed mild perilobular hepatocellular fatty changes.

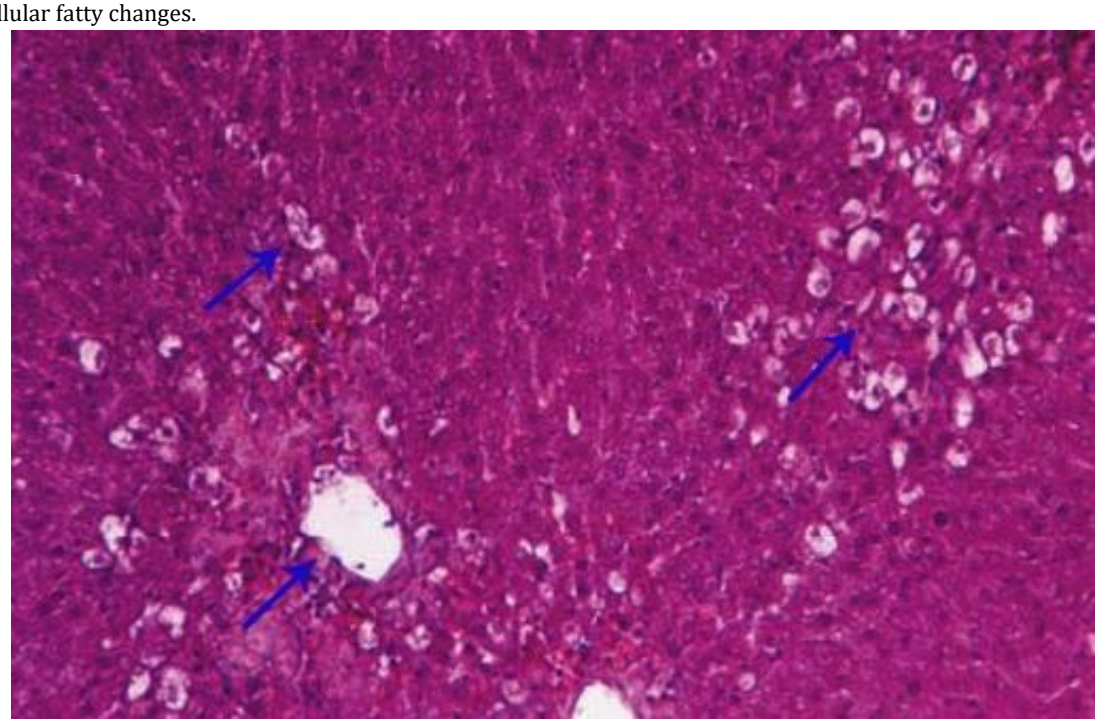

Figure 24: Liver section of hepatotoxicant and water extract of *Ocimum tenuiflorum* shows hepatocellular degeneration

The present investigation, it can be concluded that the histopathological study of the liver of the extract treated rats showed normal structure which also confirms the hepatoprotective nature of the extracts. Among the extracts, methanol extract was noticed to be significant high hepatoprotective than chloroform and water extracts in the study. The high hepatoprotective activity methanol extract may be attributed to the presence of flavonoids and phenolic compounds that are present in high quantity in methanol extract . A large number of these compounds are known to possess strong antioxidant and hepatoprotective properties. Hence, the observed antioxidant and hepatoprotective activity of the various extracts of *Ocimum tenuiflorum* may be due to the presence of any of these compounds.

CONCLUSION:

Ocimum tenuiflorum is a medicinal plant with therapeutic potential, belongs to the family Lamiaceae also known as, tulsi and is known for its anti-oxidant and hepatoprotective activity. I have been selected groups were divided into two different doses, test groups (200 and 400 mg/kg) of the crude extracts, the standard drug (silymarin 100 mg/kg), and the hepatotoxicant carbone tetrachloride was negative control. The result of anti-oxidant and hepatoprotective activity suggested that effects of *Ocimum tenuiflorum* leaves extract were tested on phytochemical, results showed the presence of alkaloid, saponins, steroid, phenolic compounds flavonoids, tannins as chemical constituents may have anti-oxidant and hepatoprotective activity, which is not evaluated till now.Amoung the results, shows The amount of steroids was estimated to be 11.85 mg/g and 57.69 mg/g respectively for methanol extracts respectively. The amount of alkaloids was estimated to be 121.69 mg/g and 12.30 mg/g respectively for methanol extracts respectively. The amount of phenolic compounds was estimated to be 71.32 mg/g for methanol extract. The amount of flavonoids was estimated to be 116.60 mg/g respectively for methanol extracts respectively. The IC50 concentration of methanol extract was noticed to be very close to standard suggest that the activity of methanol extract was very high. The 80% methanol extract decreased the absolute and relative weight of the liver of mice at the doses of 200 and 400 mg/kg ($p< 0.01$ and $p< 0.001$, respectively). It also suppressed the plasma levels of AST and ALT ($p< 0.001$) in the aforementioned doses. Among extracts, the methanol fraction showed maximum hepatoprotective activity in its dose of 400 mg/kg ($p< 0.001$, in all cases). *Ocimum tenuiflorum* is endowed with hepatoprotectiveactivity, probably mediated via its antioxidant and anti-inflammatory activity. Thus, *Ocimum tenuiflorum* can be taken as one candidate for the development of hepatoprotective agents because of its good safety profile.

Present study indicates that the comparisons of standard drug Silymarin positive control (25mg/kg) to test drug, Methanol extract of *Ocimum tenuiflorum* (400 mg/kg), AST (U/L), ALP (U/L), TP (U/L) respectively doses of 100.23±0.166, 99.1±0.104, 1.31±0.004.at Methanol extract of *Ocimum tenuiflorum* (200 mg/kg), AST (U/L), ALP (U/L), TP (U/L) respectively values of 51.12±0.200, 50.11±0.156, 1.31±0.004.such as that when compare standard drug to test drug apart from that 400 mg/kg, and the 80% methanol extract decreased the absolute and relative weight of the liver of rat at the doses of 100.23±0.166, 99.1±0.104, 1.31±0.004,AST (U/L), ALP (U/L), TP (U/L).

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Conflict consent
Not applicable

Animal Ethical statement no:
IAEC-HCOP/2023/06

Author contribution
All authors contributed equally

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