4. RESULTS

The pharmacognostic, in vitro antioxidant, in vitro and in vivo pharmacological activities of leaf, fruit and seed of the medicinal plant Elaeocarpus serratus L. selected on the basis of ethnobotanical information compiled from folk medicine, are laid down for the first time in the present study.

Pharmacognostic study is the major and reliable criteria for identification of any plant drug. The pharmacognostic characters of the leaf, fruit and seed have been studied through various parameters and their results were depicted. The macroscopic, microscopic, physiochemical and phytochemical analysis of the plant samples were carried out in the present research. The heavy metals, vitamins and fatty acids were quantified in the plant parts. The chemical profiling of the plant samples was carried out using GC-MS analysis. The in vitro and in vivo anti-arhritic and anti-diabetic activities of the plant extracts were assessed. Further, the cardio protective activity of the plant was evaluated in vivo.

4.1. Pharmacognostical investigation of Elaeocarpus serratus

4.1.1. Macroscopic evaluation

The macroscopic characters are useful in the quick identification of plant material and also serve as an important criterion for standardization. Elaeocarpus serratus L. is a tree that grows up to 18m tall in evergreen to semi-evergreen forests up to 1600m. Bark brownish, smooth; blaze orange red. Branchlets terete, glabrous, with scars of fallen leaves. Leaves simple, alternate, spiral, clustered at twig ends; petiole 1.2-4cm long, swollen at both ends, glabrous; lamina elliptic, apex acuminate or obtuse, base acute, margin serrate, glabrous, red when senescent; midrib slightly raised above; secondary nerves 5-9 pairs. Inflorescence racemes; flower petals white, laciniate; peduncle about 8cm long; pedicels 5-8mm long. Calyx of 5 lobes, free, 5-7mm long, ovate-lanceolate, reddish, hairy. Petals 5 free, stamens many, free, filamentous; anthers 2-celled, with tufts of hairs at tips. Fruit drupe, oblong, ellipsoid or ovoid about 2.5cm long; containing a much tubercled, 1-seeded stone. Flowering: September-October, Fruiting: January (Gamble, 2005), Table 1 and Plate 2, 3, 4.
4.1.2. Microscopic evaluation of fresh plant parts

Microscopic evaluation allows more detailed examination of a drug and it could be used to identify the drug by its known histological characters of plant origin. The histological studies were made from very thin sections of leaf, fruit and seed of *Elaeocarpus serratus* L. The leaf showed the following anatomical features:

The leaf is dorsiventral. It has a thick midrib. The midrib of the lamina is plano convex in sectional view. It is flat adaxially and broadly conical abaxially (Plate 5 - 5.1). The midrib is 700µm and 900µm wide. The epidermal layer of the midrib consists of cells which are small, elliptical and thick walled. The ground tissue is parenchymatous; the cells are circular, compact and thin walled. Tannin is distributed in most of the cells. The vascular system includes abaxial and adaxial strands which are just opposite with their xylem (Plate 6). Phloem occurs on the outer boundary of the xylem. The entire vascular strands are surrounded by a thick sclerenchyma sheath.

Lamina (Plate 5 - 5.2): The lamina is even and smooth. It is 300µm thick. The adaxial epidermis is fairly thick, the cells being squarish and the cell lumen is often filled with mucilage. The abaxial epidermis is thin and the cells are small and circular. Palisade cells are in three rows; they are cylindrical and possess tannin contents. The spongy parenchyma cells are in 6 to 7 layers; they are small and lobed, loosely arranged with wide air chambers.

Crystals (Plate 7): Calcium oxalate crystals are abundant in the leaf mesophyll and veins. In the mesophyll the crystals are druses (Plate 7.1, 7.2). They are up to 30µm in diameter. Along the veins, the crystals are prismatic type (Plate 7.3). They occur in parenchyma cells that cover the veins. The crystals are up to 20µm long.

Venation (Plate 8): The major lateral veins and vein islets are thick and straight. The vein-islets are wide, polygonal in outline and have distinct vein boundaries (Plate 8.1, 8.2). The vein-terminations are mostly repeatedly branched forming dendroid outline with in the islets (Plate 8.3).
Petiole (Plate 9): The basal (proximal) part of the petiole is boat shaped in sectional view, with two adaxial-lateral short thick wings and flat adaxial side (Plate 9.1). It is 130µm thick and 140µm wide. It consists of thick darkly stained epidermal cells. The ground tissue is homogenous and parenchymatous. The cells are angular, thick walled and compact. Some of the cells have dense amorphous inclusions. Such cells are surrounded by a rosette of parenchyma cells (shown by arrow in Plate 9.2).

The vascular strands are thick and wide, triangular in outline and conjoint, collateral and closed. There are several, closely arranged parallel lines of xylem elements, each line possessing 6 or more cells. Phloem occurs in thick sheath enclosing the xylem. This is a circular, small single bundle placed within the wing. The wing bundle is also collateral, comprising a cluster of xylem and a cap of phloem (Plate 9.2). The proximal (upper) end of the petiole is basically similar to the lower end in structure and outline Plate (10-10.1). The triangular vascular cylinder is cleaved narrowly at the adaxial end of the strand (Plate 10.2).

Stem (Plate 11- 11.1, 11.2, 11.3): The stem measured 2.6mm in thickness. It consists of thick and fissured periderm. The periderm consists of narrow and thin walled tubular cells. The periderm is 70µm thick. There is a broad parenchymatous cortex comprising of elliptic cells with dense accumulation of tannin. The cortex is 250µm wide. The cortex is followed by a zone of dense sclereids which is gradually transformed into phloem zone. The phloem zone consists of mixed masses of sclerenchyma and radial fibre of sieve elements (Plate 12-12.1). Secondary xylem exhibits a distinct growth ring, which is demarcated by a line of thick walled cells in the beginning of the growth (Plate 11.1). The vessels are angular and thick walled and occur mostly in radial multiples of 2 - 6 cells. Xylem rays are fairly prominent; the ray cells are wide, thick walled and straight (Plate 12.2). Xylem fibres are thick walled, lignified and occur in radial rows.

Fruit (Plate 13): The fruit is a drupe with epicarp, mesocarp and endocarp. Epicarp is thin and membranous. The epidermal cells are small and less prominent. The cuticle is thin (Plate 13.1, 13.2). The mesocarp is thick, fleshy and parenchymatous. The cells are thin walled angular and compact. The cells possess dense deposition of tannin. In the midst of tannin bearing cells, there are groups of
tannin free cells (Plate 14 -14.1, 14.2). The tanniniferous and tannin free cells are random in distribution. The mesocarp tissue is small with vascular strands. The vascular strands possess four or five xylem elements and about six phloem elements. Associated with the vascular strands are partially encircling sclereids (Plate 15 -15.1). They are also sclereid clusters which are not associated with any vascular elements (Plate 15.2).

Seed (Plate 16): The endosperm is cellular. It is a thick segment comprising of vertical rows of parenchymatous cells. The surface layer of the endosperm appears to have epidermal layer; inner to the epidermal layer are small squares of parenchymatous cells arranged in compact parallel lines (Plate 16.1, 16.2). Towards the inner position, the cells become slightly longer and are in most conspicuously featured parallel lines (Plate 17-17.1,17.2). The conspicuous feature of the endosperm cells is the presence of spherical bodies of smaller and larger sizes (Plate 18-18.1, 18.2). The chemical nature of the spherical bodies is not known. Calcium oxalate crystals are frequent in the outer zone of the endosperm (Plate 19-19.1). The crystals are similar to druses. They are circular bodies comprising of thin needles radiating the central dark circular body which is ergastic substance. This type of crystal is known as rosettes (Plate 19.2). The rosettes are only one in a cell. They are 15µm in diameter. Starch grains are also sparsely seen in the endosperm. The grains are circular and the hilum is in the center (Plate 19.3). The grains are 25µm in diameter.

4.1.2.2. Microscopic evaluation of dry powder plant parts

Leaf powder

The leaf powder (Plate 20) consists of thin and small fragments of epidermal peelings. The abaxial peeling of the epidermis consists of polygonal cells with thick anticlinal walls. The stomata are cyclocytic type with a circle of subsidiary cells (Plate 20.1, 20.2). Adaxial epidermal peeling is apostomatic. The epidermal cells are polygonal with thick straight anticlinal walls. Minute prismatic crystals are sparsely seen on the epidermal cells (Plate 21-21.1, 21.2). Epidermal trichomes are unicellular, unbranched, wide and straight with pointed tip. The trichome has granular inclusions (Plate 21.3).
**Stem powder**

The stem powder includes the following elements: Plate 22, 23, 24.

i. Wide fibres (Plate 22.1): Extremely wide and thick walled fibres with wide lumen are seen. The wide fibres are 600µm long and 40µm wide.

ii. Narrow fibres (Plate 22.2): The narrow fibres have thick walls and reduced lumen. They are uniform in thickness and tapering at the ends. They are up to 780µm long and 10µm thick.

iii. Thick pieces of periderm tissue are seen in surface view. They consist of rectangular or squarish cells arranged in compact parallel rows. Their walls are thick (Plate 23.1).

iv. Sclereids (Plate 23.2): Long, fibriform (fibre-like) sclereids are frequently seen. They are long, narrow and pointed at the tip. The cell walls are thick and possess wide canal-like simple pits.

v. Vessel elements (Plate 24 - 24.1, 24.2): The vessel elements are long, wide and cylindrical. They have wide, circular multiseriate, alternate bordered pits. The perforation plate is simple, circular and oblique. Some of the vessel elements have long pointed tails. The vessel measures are up to 600µm long.

**Fruit Powder**  (Plate 25)

Fruit powder consists of parenchymatous cells of different shapes and sizes. The cells are thin walled. The cells have no inclusions (Plate 25.1, 25.2).

**Seed Powder**  (Plate 26)

The seed coat powder includes only brachy sclereids of varying shapes and sizes (Plate 26.1, 26.2, 26.3). They have thick lignified walls, canal-like pits and wide lumen.
4.1.3. Physio-chemical Parameters

The determination of physio-chemical parameters is important in determination of adulterants and improper handling of drugs.

4.1.3.1. Organoleptic evaluation

Organoleptic investigation of the colour of powdered leaf, fruit and seed was green, light brown and reddish brown were represented in the Table 2 and Plate 27. The fruit had an aromatic and pleasant odour whereas the leaf and seeds had a characteristic smell. All the three plant parts had a characteristic taste.

4.1.3.2. Behaviour of the powdered leaves, fruit and seed of *E. serratus* with different chemical reagents

In the present study the leaf, fruit and seed powders treated with chemicals like hydrochloric acid, nitric acid, sulphuric acid, ferric chloride, acetic acid, ammonia, potassium hydroxide solution, iodine solution, ethyl acetate, silver nitrate, α-naphthol solution, sodium hydroxide, potassium iodide and water showed various shades of green, yellow, orange, brown and black when seen with the naked eye Table 3.

4.1.3.3. Determination of ash values

Table 4 displayed the physio-chemical properties like ash values and solubility of leaf, fruit and seed powder of *E. serratus*. An ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The total ash content was highest in the seed (2.75 ± 0.23%) followed by leaf (2.63 ± 0.33%) and fruit (2.33 ± 0.2%). The water soluble ash value was more or less the same in the leaf, fruit and seed (45.23 ± 0.27, 45.66 ± 0.34 and 44.63 ± 0.29%, respectively). The acid soluble ash values of the plant parts [leaf (32.56 ± 1.25%), fruit (32.33 ± 2.66%) and seed (32.66 ± 2.09%)] were lower than the acid insoluble ash values [leaf (47.52 ± 2.14%), fruit (47.65 ± 1.72%) and seed (47.55 ± 3.24%)]. The percentage solubility of the plant parts in alcohol [leaf (68.12 ± 3.02%), fruit (67.88 ± 2.03%) and seed (67.53 ± 3.01%)] was higher
than the value of solubility percentage in water [leaf (50.23 ± 2.02%), fruit (50.66 ± 3.03%) and seed (50.21 ± 4.03%).

4.1.3.4. Extractive value

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.

Leaf, fruit and seed of *E. serratus* were subjected to successive solvent extraction using the solvents hexane, petroleum ether, benzene, chloroform, acetone, ethanol and water based on the increasing order of polarity using soxhlet apparatus. Their extractive values were determined and the results are given in Table 5. The average values are expressed as percentage of air-dried materials. The maximum extractive value was obtained using the solvent ethanol (leaf, fruit and seed being 70.21 ± 4.02, 70.33 ± 2.01, 70.23 ± 5.04%, respectively), followed by water [leaf (56.42 ± 3.03%), fruit (56.33 ± 4.01%) and seed (56.41 ± 2.02%)] and acetone [leaf (45.23 ± 2.04%), fruit (45.64 ± 4.01%) and seed (44.53 ± 4.05%)]. The minimum extractive value was observed in benzene and petroleum ether extracts of leaf (8.9 ± 0.16% and 8.02 ± 9.11%, respectively). In general, the polar solvents showed higher extractive values than non-polar solvents.

4.1.3.5. Estimation of minerals in the leaf, fruit and seed of *E. Serratus*

A perusal of data in Table 6 indicates that the mineral contents in the analysed plant parts are in wide range. A total of 10 (inclusive of macro and micro elements) were determined in the powdered samples of various parts of *E. serratus*. The concentration of mineral elements analyzed in the study decreased in the order Ca > Na > Mg > Fe > Zn > Mn > Se. The concentration of macro elements was as follows: calcium [leaf(125.6±2.4mg/g), fruit(125.6±6.1mg/g) and seed (125.6±5mg/g)], magnesium [leaf(42.6±3.04mg/g), fruit(43.5±4.02mg/g) and seed (42.6±2.01mg/g)], manganese [leaf (0.113±0.003mg/g), fruit (0.113±0.014mg/g) and seed (0.123 ± 0.003mg/g)] and sodium [leaf(45.3±3.2mg/g), fruit(45.6 ± 5.1mg/g) and seed (46.3 ± 4.4mg/g)]. The concentration levels of the microelements iron and zinc was maximum in the leaf (4.82± 1.01, 0.14 ± 0.02mg/g, respectively). Selenium was
present only in trace amounts in the plant parts studied. Boron, cobalt and organic 
carbon are absent in the analyzed samples. Among the various macro elements 
studied calcium was present in high amount. Iron was present in higher concentration 
among the micro elements studied.

4.1.3.6. Estimation of heavy metals in the leaf, fruit and seed of *E. serratus*

Table 7 expresses the presence or absence of heavy metals in the various parts 
of *E. serratus*. The concentration of arsenic was less than 1ppm in all the plant parts 
studied. Mercury was 0.35 ppm (leaf), 0.34 ppm (fruit) and 0.36 ppm (seed). The 
heavy metals like nickel and cadmium were completely absent in the study material.

4.1.3.7. Estimation of vitamins in the leaf, fruit and seed of *E. Serratus*

The water soluble vitamins B₁, B₂, B₆ and B₁₂ were detected in the leaf, fruit 
and seed of *E. serratus* Table 8. Among the plant parts studied, the leaf contained the 
maximum amount of Vitamins B₁ (thiamin) and Vitamin B₁₂ (cyanocobalamin) 
(0.315 ± 0.21; 1.324 ± 0.21mg/g, respectively). Similarly, the vitamins B₂ (riboflavin) 
and vitamin B₆ (pyridoxamine) was found to be higher in the fruit sample 
(0.113 ± 0.11; 0.045 ± 0.04mg/g). Generally the seeds possessed moderate amounts of 
B-complex vitamins than the leaf and fruit.

4.1.3.8. Estimation of fatty acids in the leaf, fruit and seed of *E. Serratus*

Table 9 summarizes the results obtained from the estimation of fatty acids in 
the leaf, fruit and seed powder of *E. serratus*. The table shows the concentration of 
fatty acids such as palmitic acid, oleic acid, linolenic acid and stearic acid. All the 
four fatty acids were present in maximum level in fruit sample (3.11±1.01; 2.20±1.01; 
3.11±1.01 and 1.34±0.1%, respectively). The leaves also showed appreciable amounts 
of fatty acids.
4.1.4. Phytochemical analysis of *E. serratus*

4.1.4.1. Qualitative phytochemical study

The pharmacological properties of medicinal plants are due to the presence of secondary metabolites. Tables 10, 11 and 12 depicts the phytochemical screening of powder of leaf, fruit and seed of *E. serratus* in various extracts i.e. hexane, petroleum ether, benzene, chloroform, acetone, ethanol and water. The result showed the presence or absence of carbohydrate, protein, alkaloids, terpenoids, triterpenes, phenols, flavonoids, tannins, saponins, steroids, glycosides, coumarin, quinine, anthraquinone, starch, gum and fixed oils indicated by (+) or (-) symbol. Generally the solvents ethanol, water and acetone were more efficient in extracting the phytochemicals than the other solvents.

4.1.4.2. Quantitative phytochemical study

The quantitative estimation of phytoconstituents in the various plant parts of *E. serratus* is depicted in Table 13. Noticeable quantity of carbohydrate was seen in the leaf (9.52 ± 0.39%), fruit (9.46 ± 0.26%) and seed (9.45 ± 0.15%). Appreciably high amount of protein was registered in the leaf sample (13.56 ± 0.33%) followed by the seed (13.45 ± 0.25%) and fruit (13.11 ± 0.18%). In general, the leaf sample recorded high amounts of protein and carbohydrates. The maximum content of phytochemicals was as follows: tannin (0.123 ± 0.014%) in leaf; flavonoid (0.204 ± 0.001%), phenols (0.113 ± 0.002%), saponin (0.304 ± 0.002%), starch (5.66 ± 2.01%) in fruit and alkaloid (0.946 ± 0.02%), steroids (0.855 ± 0.005%) and anthraquinone (0.006 ± 0.002%) in seed. Presence of the phytoconstituents contributes significantly to the antioxidant capacity of this plant. These phytochemical compounds are known to support biological activities in medicinal plants and this might be responsible for the antioxidant activities of these plant extracts used in this study.

4.1.5. GC-MS analysis of leaf, fruit and seed of *Elaeocarpus serratus*

The bioactive phytocomponents present in the ethanolic extract of leaf, fruit and seed of *Elaeocarpus serratus* were identified by GC-MS analysis and the GC and
MS running time was 46.16, 44.85 and 46.20 minutes for leaf, fruit and seed, respectively. The GC-MS chromatogram of ethanolic extracts of *E. serratus* (leaf, fruit and seed) are presented in Figures 1, 2 and 3. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of leaf, fruit and seed of *E. serratus* are presented in Tables 14, 15 and 16. The spectra of the compounds are matched with Wiley 9.0 and NIST libraries.

Thirty compounds were detected in ethanolic extract of *E. serratus* leaf Table 14 Fig. 1. The most prevailing major compounds were methanol (20.57%), n-dotriacontanol (10.70%), n-octadecanol (10.08%), docosanoic acid, 1,2,3-propanetriyl ester (9.07%), n-hexadecene (8.52%), bis-(3,5,5-trimethylhexyl) ether (6.30%), ethanone, 1-cyclopentyl-(4.81%), cyclohexane, ethyl-(4.05%) etc. and pharmacologically important components like hexadecanoic acid methyl ester (0.80%), ricinoleic acid (0.77%), citronellyl isobutyrate (0.69%) and farnesol (0.51%) were also detected in minor amounts in the leaf sample.

The crude ethanolic extract of *E. serratus* fruit was analysed using GC-MS Table 15 and Fig. 2. The extract had the following major composition: n-octanol (25.91%), n-hexadecene (9.73%), n-dodecanol (9.24%), n-pentadecanol (8.04%), 3-pentyl-1-cyclohexanone (7.78%), n-hexadecanol (6.11%), n-nonadecanol (4.06%), benzene, chloro- (4.04%). While n-octanol was first which came out from the column (RT= 3.04 min.), n-pentadecanol was retained in the column for the longest of all (RT= 40.22 min.). Minor components like 1,3-dicyano-2-selenabicyclo[3.1.0] hex-3-ene (0.48), N-(2-deuterioallyl)-2-fluoro-N-methylaniline (0.52%), 2-butoxy-7,8-dimethoxy-chroman-6-ol(0.53), (+-) -trans-2-(2,5-Octdiynyl)-3-undecyloxirane (0.57) were also detected. Tricosane a pharmacologically active compound was noticed in the fruit extract.

Table 16 and Fig. 3 depicted the GC-MS analysis of ethanolic extract of seed of *E. serratus*. Some of the identified components were n-propanol (19.12%), n-hexadecene (12.08%), n-octadecanol (10.70%), n-decyl prop-2-ynoate (9.61%), n-undecanol (6.41%), methyl (R)-3-methyl-2-oxo[4,4,4-D3] butanoate (4.91%), N-allyloxymethylacrylamide(4.21%),di(1-adamantyl)aceticacid(4.03%), pentadecanal
(1.27%), docosane (2.19%), 4-methyl-4-nitro-5-oxoheptanal (0.86%), n-tricosane (0.82%). The highest peak area percentage of 19.12% was obtained by n-propanol (RT= 3.04min.) and lowest peak area percentage of 0.61% was obtained by 1-propylthio-3,3,3-trifluoropropyl acetate (RT= 11.57min.). Most of these compounds have not been reported from this plant. So far however there may be variation in the chemical composition based on topography.

4.2. Evaluation of In vitro Antioxidant Activity of E. serratus

4.2.1. Hydroxyl Radical (‘OH) Scavenging Activity

Hydroxyl radicals are the major oxygen species causing lipid peroxidation and enormous biological damage. The IC\textsubscript{50} values of various solvent extracts of leaf, fruit and seeds of E. serratus are depicted in Fig. 4. The ethanol extracts of the leaf, fruit and seed inhibited degradation by hydroxyl radicals with IC\textsubscript{50} values of 24.30 ± 0.19µg/ml, 24.08 ± 0.27µg/ml and 33.75 ± 0.24µg/ml, respectively. The ethanol extracts were more efficient in scavenging the hydroxyl radicals than the acetone and water extracts. The antioxidant activity confirmed the medicinal importance of the plant. The observed scavenging effect could be explained by understanding the nature and generation of radicals as well as study of different chemical properties of naturally occurring antioxidants.

4.2.2. Superoxide Radical (O\textsubscript{2}−) Scavenging Activity

Superoxide radical is a highly toxic species and is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzyme that catalyse the breakdown of superoxide radical. Superoxide scavenging activity of ethanol, acetone and water extracts of different parts of E. serratus is presented in Fig. 5. The various extracts of E. serratus were capable of scavenging the superoxide radicals in a dose-dependent manner. The ethanol extract of seed showed the maximum scavenging of superoxide radicals with an IC\textsubscript{50} value of 41.56 ± 0.18µg/ml, followed by acetone extract of leaf (IC\textsubscript{50}=59.52 ±0.79µg/ml). The scavenging ability of the ethanol extract of seed (IC\textsubscript{50}=41.56 ± 0.18µg/ml) was more or less equal to the standard antioxidant gallic acid (IC\textsubscript{50}=38.27 ± 0.28µg/ml). Higher inhibiting effects shown by the extracts on
superoxide anion formation have possibly rendered them promising antioxidant characteristics.

4.2.3. Nitric oxide (NO˙) Radical Scavenging Activity

The antioxidant capability of various parts of *E. serratus* was measured by nitric oxide radical scavenging assay. In the present study, *E. serratus* extracts competed with oxygen to react with nitric oxide and thus inhibited generation of the anion. The scavenging activity of the extract against nitric oxide was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in the reacting mixture. Fig. 6 shows NO˙ scavenging activity of *E. serratus* extracts. The highest NO˙ scavenging activity was noticed in the acetone extract of seed (IC₅₀=36.23±0.26µg/ml). The activity was even better than the standard BHA (IC₅₀=43.37±1.26µg/ml) and closer to that of gallic acid standard (IC₅₀=29.76±0.81µg/ml). The ethanol extract of leaf (IC₅₀=45.94±0.16µg/ml) and acetone extract of fruit (IC₅₀=64.12±0.44µg/ml) also exhibited good scavenging activity which was comparable to that of the standards. The results indicated that the extracts might contain compounds capable of inhibiting nitric oxide and offered scientific evidence for the use of the leaves in curing diseases.

4.2.4. ABTS˙⁺ Radical Scavenging Activity

ABTS˙⁺ is a blue chromophore and has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals. The ability of the different solvent extracts of leaf, fruit and seed of *E. serratus* to effectively scavenge the ABTS radical cation is displayed in Table 17. Addition of the plant extract to the pre-formed ABTS˙⁺ reduced it to ABTS in a concentration-dependent manner. *E. serratus* exhibited potent ABTS˙⁺ scavenging activity with maximum activity seen in the ethanol extract of leaf (18428.1±23.08µmol/g) and water extract of fruit (18270.8±32.2µmol/g). The scavenging of ABTS˙⁺ by other extracts namely, acetone extract of leaf (16144.2±42.2µmol/g), ethanol extract of fruit (12321.4±111.3µmol/g) and ethanol extract of seed (10867.6±112.9µmol/g) was found to be higher indicating high antioxidant activity. The lowest ABTS˙⁺ scavenging activity was noted in the acetone extract of seed (7578.8 ± 99.5µmol /g).
4.2.5. β-carotene / Linoleic Acid Peroxidation Inhibition Activity

In β-carotene/linoleic acid bleaching assay, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The inhibition of the lipid peroxidation by different solvent extracts of leaf, fruit and seed of *E. serratus* is presented in Table 18. The ethanol and water extracts of leaf showed the maximum inhibition of lipid peroxidation (79.43 ± 5.27 and 78.33 ± 9.67 %, respectively). In general, all the extracts showed above 60% inhibition of β-carotene bleaching activity. The inhibition percentage of the standards BHA and gallic acid were 92.39 ± 2.1 and 95.16 ± 1.8 %, respectively and greater than that of the plant extracts.

4.3. Evaluation of Pharmacological Activity of *E. serratus*

4.3.1. Toxicological study of *E. serratus*

A single dose (1000, 2000, 3000, 4000, and 5000 mg/kg b.w. p.o./day) of ethanolic extracts of leaf, seed and fruit of *E. serratus* administered to Albino rats showed no death up to 72 hours. At these doses, there were no abnormal clinical signs which include changes in skin colour and behavioral changes like alertness, grooming, restlessness, tremors, convulsions and writhing at any time during the observation period. The effect of plant extracts on touch response, torch response, pain response, and righting reflex, gripping strength, pinna reflex, corneal reflex, pupils, urination, salivation and lacrimation were also found to be normal. These results indicated that the plant extracts were quite safe even at a high dose of 5000 mg/kg b.w.p.o. and had no acute toxicity (Table 19).

4.3.2. Evaluation of *In vitro* Anti-arthritic Activity

4.3.2.1. Inhibition of Protein Denaturation and Proteinase Inhibitory Activity

The data on the effect of ethanolic extracts of *Elaeocarpus serratus* leaf and seed on inhibition of protein denaturation and anti-proteinase activity are shown in Fig. 7. The maximum percentage of protein denaturation was observed in the leaf extract (68.32%) at 400μg/ml followed by the seed extract 62.13% at 400μg/ml. But the low doses (200μg/ml) of both the extracts gave moderate value 56.74% and
52.17% in leaf and seed, respectively). When compared to the standard indomethacin which showed an inhibition percentage of 75.45% at 100µg/ml. Denaturation of protein is one of the causes of inflammation. From the results of the present study, it is known that the extracts are capable of controlling the denaturation of protein in inflammatory disease effectively.

The ethanolic extracts of the leaf and seed of *E. serratus* inhibited moderate anti-proteinase activity with an inhibition percentage of 51.16, 50.12 and 64.71, 59.34% at 200 and 400mg/ml b.w., respectively. On comparison with the crude plant extracts, the standard indomethacin showed maximum inhibition of 72.41% at 100µg/ml concentration.

**4.3.3. Evaluation of In vivo Anti-arthritic Activity of E. serratus**

**4.3.3.1. Freund’s complete adjuvant (FCA)-induced Paw Edema Assay**

Paw swelling is one of the arthritic symptoms. The determination of paw swelling is apparently simple sensitive and quick procedure in evaluating the degree of the inflammation and assessing the therapeutic effect of drugs. Measurement of paw only gives induction of edematous changes in this region. In this present study, Freund’s Complete Adjuvant (FCA) model was used. Standard drug is indomethacin and the anti-arthritic potential of ethanolic extracts of *E. serratus* leaf and seed (200, 400 mg/kg b.w. p.o.) were assessed Table 20. Following FCA-induction, the animals showed arthritis development as seen by the increase of paw volume from the 1st day onwards. Observations of paw volume (Plates 28, 29, 30, 31 and 32) were recorded in the regular interval from the day of adjuvant injection. The paw diameter reached maximum up to 11th day of adjuvant injection and after that it was slightly decreased. The chronic inflammation developed on the 10th day in group II (11.84 ± 0.07mm). The *E. serratus* leaf and seed ethanolic extracts inhibited chronic inflammation response. The leaf extract at 400 mg/kg b.w. reduced the paw volume on 7th day (8.08 ± 0.05mm) group V but the seed extract at 400 mg/kg b.w. showed reduction in paw volume after the 7th day (8.31 ± 0.07mm) group VII. On comparison with the indomethacin standard which showed an inhibition of 5.12 ± 0.06mm at 10 mg/kg p.o., the leaf and seed extracts caused a significant decrease in the paw
volume (5.55 ± 0.05 and 5.86 ± 0.04 mm, respectively) at 400 mg/kg b.w. on the 15th day.

4.3.3.2. Percentage Protection of Paw Volume

The investigation is based on the need for newer anti-arthritic agents from natural source with potent activity and lesser side effect. The percentage protection of paw volume by the ethanolic extracts of leaf and seed was 50.86, 60.84, 47.07 and 58.75%, respectively at 200 and 400 mg/kg p.o. In the leaf and seed extracts the maximum protection percentage of paw edema due to FCA administration, was more pronounced at 400 mg/kg b.w. which was comparable with the standard drug indomethacin as depicted in Fig. 8.

4.3.3.3. Evaluation of Biochemical Parameters in Blood Serum of E. serratus

In order to explore the effect of antioxidant defenses in the body of experimental animals during inflammation process, the antioxidant levels (SOD, CAT, GPx, GST and LPO) in the blood serum of normal group (group I) and experimental groups (group II to group VII) of rats were evaluated and depicted in Table 21. This table shows a highly significant reduction (p< 0.05) in the antioxidant status (SOD, CAT, GPx, and GST) in the FCA-induced arthritic rats (group II) as compared with the control animals (group I). However, the oral administration of the both the doses (200 and 400 mg/kg p.o.) of ethanolic extract of leaf and seed of E. serratus (group IV to group VII) significantly increased (p<0.05) the levels of SOD, CAT, GPx, and GST enzymes to near normalcy as in group III treated with the reference drug indomethacin (10 mg/kg b.w. p.o.). High dose (400 mg/kg p.o.) of leaf extracts exhibited remarkable antioxidant enzyme augmenting ability which was comparable to the standard drug.

On the contrary, the level of LPO was found to be significantly increased (p<0.05) in the induced group (group II) as compared to the control rats (group I). The administration of high and low doses (200 and 400 mg/kg p.o.) of the ethanolic extract of leaf and seed (group IV to group VII) was significantly reduced (p<0.05) the levels of lipid peroxidation comparable to the standard. High dose of the leaf
extract reduced the LPO level to 12.24 ± 0.09µmoles/mg protein which was closer to that of the standard drug treated group (12.09 ± 0.11µmoles/mg protein).

4.3.4. Evaluation of Cardio protective activity of E. serratus

4.3.4.1. Investigation of Lipid Profile in Isoprenaline-induced Wistar Albino Rats

Table 22 depicts the diagnostics of lipid profile in plasma of normal and experimental group of rats. Rats treated with isoprenaline showed a significant increase (p<0.05) in the levels of total cholesterol (TC), triglycerides (TGL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) and significant decrease (p<0.05) in high density lipoprotein (HDL) level than control group rats.

The animals pretreated with ethanolic leaf and seed extracts of *Elaeocarpus serratus* produced a significant decrease (p<0.05) in the level of total cholesterol, TGL, LDL cholesterol and VLDL cholesterol than the group II after 30 days of administration of extracts in a dose-dependent fashion. The decrease in serum lipids in extracts treated groups showed the cardio protective nature of the extracts. However, serum HDL level was decreased in isoprenaline-treated group II rats and administration of ethanolic samples of extracts (leaf and seed) at high dose and low dose resulted in a significant elevation (p<0.05) of HDL level. Administration of the pure ethanoilc leaf and seed extracts without isoprenaline at 400 mg/kg p.o. (group V and group VIII) did not cause significant damage as the induced group and is comparable with that of the control group, which further prints to the cardio protective activity of *E. serratus* leaf and seed extracts.

4.3.4.2. Evaluation of Biochemical Parameters in Blood Serum

In the isoprenaline-treated animals (group II) there was a significant elevation (p<0.05) in the level of serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels when compared to control rats. A significant decrease (p<0.05) in the levels of serum biochemical marker enzymes was noted in the animals pretreated with ethanolic leaf and seed extracts of *E. serratus* (200 and 400 mg/kg b.w.) when compared to group II. However, the groups-V and VIII administrated with the plant extracts alone without isoprenaline showed normal
levels of AST, ALT and ALP comparable with the control group. Therefore it confirms that the plant extracts do not alter the physiological conditions of the heart when the extracts alone were given at the dose of 400 mg/kg b.w. (Table 23).

Isoprenaline is a well-known cardio toxic agent due to its ability to destruct the myocardial cells. As a result of this, cytosolic enzymes such as lactate dehydrogenase (LDH) and creatine phosphokinase (CK-MB) were released in to blood serum which serves as diagnostic marker of myocardial tissue damage. Significant (p<0.05) increase was observed in the level of marker enzymes LDH and CK-MB in group II as compared with group I. Pretreatment of animals with E. serratus leaf and seed extracts at doses of 200 and 400 mg/kg b.w. p.o. significantly decreased (p<0.05) the LDH and CK-MB levels as compared with group II. Thus the leaf and seed extracts showed a significant (p<0.05) protective effect against myocardial infarction. The pure samples of leaf and seed extracts (without isoprenaline) retained the activity of these enzymes to near normal level comparable with the control group. The results indicate that E. serratus leaf and seed extracts have the tendency to reduce the elevated levels of cardio marker enzymes proving its cardio protective effect.

4.3.5. Evaluation of In vitro Anti-diabetic Activity of E. serratus

The results showed that ethanolic extract of fruit of Elaeocarpus serratus exhibited different degree of α-amylase and α-glucosidase inhibitory activities in in vitro assays using starch and p-nitrophenyl glucopyranoside as a substrate, respectively.

4.3.5. 1. Assessment of α-Amylase Inhibitory Activity

The in vitro studies demonstrated that ethanol fruit extract of E. serratus had α-amylase inhibitory activity. The percentage inhibition at 20, 40, 60, 80 and 100µg/ml concentrations of plant extract showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration of 100µg/ml tested showed a maximum inhibitory activity of 89.54 ± 2.99%. Acarbose is a known standard drug for α-amylase inhibitor which showed 94.65 ± 0.82% at 100µg /ml (Fig.9).
4.3.5.2. Assessment of α-Glucosidase Inhibitory Activity

Fig. 10 shows the inhibitory activity of ethanolic extract of fruit of *E. serratus* on α-glucosidase. From the figure it is clear that the ethanolic fruit extract (at concentrations 20-100µg/ml) showed moderate α-glucosidase inhibitory activity from 6.68 ± 0.59 to 25.93 ± 0.34%. The standard acarbose (at concentrations 20-100 µg/ml) showed α-glucosidase inhibitory activity from 43.12 ± 0.59 to 75.80 ± 1.01%.

The plant extract exhibited weak α-glucosidase enzyme inhibition when compared to α-amylase enzyme inhibition. According to the experimental results, it was certainly confirmed that the ethanolic fruit extract of the plant had both α-amylase and α-glucosidase inhibitory activities.

4.3.6. Evaluation of In vivo Anti-diabetic Activity of *E. serratus*

4.3.6.1. Assessment of Body Weight and Blood Glucose Level

Changes in the body weight and blood glucose levels of rats fed with ethanolic extract of fruit of *E. serratus* and Streptozotozin (STZ) are presented in Table 24. There was a significant increase (p<0.05) in the body weight of control rats and rats fed with ethanolic extract of fruit of *E. serratus* while there was a significant decrease (p<0.05) in the body weight of rats treated with STZ. Among the treated groups, highest body weight was recorded in groups V (170.09 ± 0.01g) that were administered with 400 mg/kg b.w. of the extract followed by group IV (163.78 ± 1.02g) administered with 200 mg/kg b.w. of the fruit ethanolic on the 30th day. Thus, the administration of the ethanolic fruit extract of *E. serratus* significantly (p<0.05) increased the body weight of the STZ-treated animals in a dose-dependent pattern.

Table 25 shows that the blood glucose level in the normal control group (groups I) did not show any significant variation in the blood glucose throughout the experimental period. Administration of streptozotozin (STZ) (50 mg /kg b.w.) led to a significant elevated (p<0.05) glucose level almost four times on 30th day (470.83±6.31mg/dl) than group I. Administration of the glibenclamide (600µg/kg b.w.) significantly reduced (p<0.05) the blood glucose levels. A decrease
in blood glucose level in the group IV and group V could be observed from the 10th day onwards registering a significant decrease (p<0.05) on 30th day of treatment. It is evident that the administration of high dose of the plant extract showed maximum reduction (202.50 ± 5.79mg/dl) in the blood glucose level.

**4.3.6.2. Evaluation of Liver Marker Enzymes in Blood Serum**

Table 26 shows the effect of ethanolic extract of *E. serratus* fruit on total protein, total albumin and liver function enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). It could be observed that diabetes caused a significant decrease (p<0.05) in total protein and total albumin levels (mg/dl) in serum of diabetic rats. Treating diabetic rats with high and low dose (200 and 400 mg/kg p.o.) of ethanolic extract of *E. serratus* fruits caused significant increase (p<0.05) in total protein (6.63 ± 0.23 and 8.07 ± 0.10g/dl, respectively) total albumin (2.19 ± 0.06 and 3.62 ± 0.03g/dl, respectively) levels compared with diabetic control rats. Diabetic rats showed a significant (p<0.05) elevation in the activities of serum liver marker enzymes AST, ALT and ALP as compared to normal rats. However, treatment with the ethanolic extract of fruit of *E. serratus* (200 and 400mg/kg b.w.) significantly reduced (p<0.05) the level of these enzymes. The curative effect of ethanolic extract of *Elaeocarpus serratus* fruit could easily be noticed through the normalization of all enzymes tested compared to the control and standard drug treated group.

**4.3.6.3. Assessment of Carbohydrate Metabolizing Enzymes in Blood Serum**

The activity of carbohydrate metabolizing enzymes such as hexokinase (HK), phospho glucoisomerse (PGI), glucose-6-phosphatase (G-6-P) and fructose-1,6-diphosphatase (F-1,6-DP) in the blood serum is depicted in Table 27.

There was a significant (p<0.05) decrease in the activities of HK and PGI and significant (p<0.05) increase in the activities of G-6-P and F-1, 6-DP in the liver of blood serum in diabetic rats as compared with normal control rats. However, the high dose (400 mg/kg b.w. p.o. of the fruit extract showed significant enzyme augmenting ability which was comparable with the standard drug glibencalmide.