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EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF *BOSWELLIA SERRATA* METHANOL EXTRACT IN EXPERIMENTALLY INDUCED ARTHRITIS ANIMALS

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ABSTRACT : Arthritis is a term often used to mean any disorder that affects the joints. *Boswellia*, also known as Indian frankincense is an herbal extract taken from the *Boswellia serrata* tree, appears to be a novel inhibitor of a pro-inflammatory enzyme called 5-Lipoxygenase and may possess other anti-inflammatory effects. The present study was conducted to evaluate the anti-inflammatory activity for the gum of the plant, *Boswellia serrata*, extracted by employing the following: water, methanol, n-hexane and petroleum ether. Rats of both sexes, in separate cages were given a single oral limit dose of 5,000 mg/kg body weight of the different *Boswellia serrata* gum resins extracts while a control animal received distilled water. Collagen-induced arthritis was used as the method to induce rheumatoid arthritis in the rats by a single injection with an emulsion that contained type II collagen and complete Freund's adjuvant. Plethysmometer was used to detect the paw volume of the animals as a sign of rheumatoid arthritis. This appears after 10–14 days depended the age of animal, the signs included. Statistical analysis was employed by using One-Way ANOVA. In order to determine the *in-vivo* changes, the Assessment of the biochemical markers such as Lipid peroxidase (LPO), Glutathione (GSH), Catalase (Cat), Superoxide dismutase (SOD) and Nitric oxide (NO). Immunological changes that occurred due to the inflammation was estimated by the Assessment of inflammatory markers such as Interleukin 1 Beta (IL-1 β), Interleukin 6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Interleukin 10 (IL-10) and Interfer on gamma (IF-γ) using Enzyme-Linked Immunosorbent Assay.

Key words : *Boswellia serrata*, arthritis and anti-inflammatory.

INTRODUCTION

Arthritis is a painful case characterized by inflammation, which leads to pain and toughness in most of the moveable joints of the human body. i.e arthritis is a widespread joint disorder that occurs due to an inflammation (Blackham *et al*, 2017). There are more than 100 diagnosed kinds of arthritis in the world, the most common forms are osteoarthritis and rheumatoid arthritis (Hayer *et al*, 2016). The illness mostly occurs during age and affects moving parts such as fingers, knees and hips. Rheumatoid arthritis is a widespread autoimmune disorder that predominately that affects the moving parts of the human body, for instance hands and feet (Ashbrook, 2016). In order to investigate the importance of such non-steroidal future drugs, it had been decided to study the *Boswellia serrata* various crude extracts on animals that induced by arthritis. To achieve such a purpose, *Boswellia serrata* needs to undergo quantitative and qualitative tests such as TLC, HPLC and GC-MS investigations to evaluate all of the extracts, then evaluation to be done on animals already induced by arthritis using collagen induced arthritis model. Both of *in-vitro* (human red blood cell membrane stabilization method) and in-vivo (Biochemical and immunological tests) examinations have been employed to compare between various animals that were divided into different groups using several scientific criteria.

The treatment of arthritic rats with *Boswellia serrata* and diclofenac sodium has shown noticeable improvement, which is obvious from the reduced pro-inflammatory cytokine and increased enzyme levels. This may be attributed to the potent anti-inflammatory fractions of the pharmacologically active principles present in the plant extract.

In the histopathological study, it had been observed sub-plantar injection of collagen induced arthritis in the form of accumulation of inflammatory cells like neutrophils whereas *Boswellia serrata* treated rats decreased the cellular infiltrates.

MATERIALS AND METHODS

1. Extraction of natural plant products

All the extracts were screened using phytochemical tests for the existence of different secondary metabolites

using regular phytochemical methods (Woolley *et al*, 2012). Qualitative compound examinations are carried out in favor of every extracts to recognize a variety of chief plant constituents approximating alkaloid, glycoside, carbohydrate, phenolic, tannin, phytosterol, fixed oil, fat, protein, amino acid, flavonoid, saponin, etc. using different identification or confirmative test (Borrelli *et al*, 2016).

2. Assessment of the Biochemical Markers such as LPO, GSH, Cat, SOD and NO

The biochemical testing for the enzymes involved in oxidative stress all of Methanol, Petroleum ether, Aqueous and n-Hexane extracts were measured by biochemical assay using specific markers such as LPO, GSH, Cat and SOD (Lund *et al*, 2011).

3. Assessment of inflammatory markers such as IL-1â, IL-6, TNF-á, IL10, IF-ã

Immunological testing for the cytokines involved in the inflammation in rats, all of groups were undergoes immunological assay using specific markers such as IL-1â, IL-6, TNF-á, IL10 and IF-ã (Kundu *et al*, 2012).

4. X-Ray analysis

Paw of the rat was scanned under the dental x-ray to compare the swelling size and bone shape between the different animal groups (Blackham *et al*, 2017). That have been categorized, before and after the treatment using methanol extract.

5. Histopathology study

At the end of the study, all animals from each group were sacrificed for gross and histopathological examination. A detailed gross pathological examination was conducted for external surfaces and orifices. All synovial tissues were weighed individually immediately after necropsy and they were preserved in 10% Formalin for histopathological examinations. Histopathological inspection was performed on paraffin-embedded blocked by Haematoxylin and eosin staining technique (Bekana *et al*, 2014).

The following steps were employed in order to investigate the histopathological chnges:

- All the experimental rats were employed to collect the Paw region of the animals after being sacrificed through cervical dislocation.
- Neutralized formalin was employed to fix the specimens, dehydrated along with ethanol and embedded in paraffin wax (56°C).
- Sectioning:
- Haematoxylin and eosin used to stain the Serial sections.
- The stained sections were observed under microscope and the histological changes were recorded with the help of a pathologist.

7. Statistical analysis

Data of Antioxidant Activity, Paw volume and HRBC membrane protection testing was analyzed by using One-Way ANOVA calculator. The results are revealed as Mean ±S.E.M (mean standard error). $p < 0.05$ was shown to be significant ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

RESULTS AND DISCUSSION

1. Anti-Inflammatory activity of collagen induced arthritis

Few investigations have been initiated around the globe into researching, screening and analyzing the local plants with anti-inflammatory values. The anti-inflammatory effects of some of the medicinal plants have been validated and others disproved (Etzel, 2016). However, in spite of therapeutic potential of *Boswellia serrata*, its effect on inflammation has not been studied in detail. Therefore, the present study screened and identified the anti-inflammatory efficacy of *Boswellia serrata* preparations against collagen induced arthritis in experimental rat models. The methanol extract at the dose levels of 45, 90 and 180 mg/kg showed reduction in paw volume at all time intervals as compared to untreated group (negative control), but significant reduction in pawvolume was noticed in 45 mg/kg at 1st and 3rd hour observation 24.13% and 23.71% respectively shown in Fig. 1.

The group treated with Hexane extract of *Boswellia serrata* at the dose levels of 45, 90 and 180 mg/kg showed reduction in paw volume at all time intervals as compared to untreated group (negative control), but significant reduction in paw edema volume was noticed in 180mg/kg body weight at 1st and 3rd hour observation 31.03% and 19.59%, respectively.

This study shown that, the methanol fraction prepared from *Boswellia serrata* resins contains high amounts of total phenolics and total flavonoids and it exhibited strong reducing power and antioxidant activity and anti-inflamatory activity. Thus, methanol seems to be most promising solvent for extraction and isolation of naturalanti-oxidative compounds from *Boswellia serrata* gum.

2. Assessment of the biochemical markers such as LPO, GSH, Cat and SOD

In order to achieve, *in vivo* biochemical testing for the enzymes involved in oxidative stress all of Methanol, Petroleum ether, Aqueous and n-Hexane extracts were

undergoes biochemical assay using specific markers such as LPO, GSH, Cat and SOD.

Table 1 shows the *in vivo* Biochemical assay for enzymes involved in oxidative stress of Methanol extract at 400 mg/kg dose as compared with normal at 10 ml/kg Saline dose, positive control at 2.5 mg/kg dose and negative control at 10 ml/kg.

Saline dose, results of the sample was 41.50±4.346, 0.64±0.067, 24.06±1.619 and 110.22±10.923 for LPO, GSH, Catalase and SOD, respectively. When collagen induced arthritis rats treated with *Boswellia serrata* methanol extract the researcher observe significant, changing in the biochemical markers value for instance LPO in the normal group was 19.83±3.800 this value increased after the induction of rheumatoid arthritis to 54.83±5.962 and deceased to 41.50±4.346 after treatment but the positive control shows a significant reduced 27.61±3.277, which was more than methanol extract. In case of GSHin the normal group was 1.13±0.123. This value decreased after the induction of rheumatoid arthritis to 0.26±0.056 and increased to 0.64±0.067 after treatment using methanol extract but the positive control shows a significant increase 0.85±0.062, which was more than methanol extract. In case of catalasein the normal group was 37.97±5.176 this value decreased after the induction of rheumatoid arthritis to 15.05±1.296 and increased to 24.06±1.619 after treatment using methanol extract but the positive control shows a significant increase 30.14±3.333, which was more than methanol extract. In case of SOD in the normal group was 159.09±17.068 this value decreased after the induction of rheumatoid arthritis to 78.40±14.138 and increased to 110.22±10.923 after treatment using methanol extract but the positive control shows a significant increase 137.50±14.570, which was more than methanol extract.

Table 3 shows the *in vivo* Biochemical assay for enzymes involved in oxidative stress of Hexane Extractat 400 mg/kg dose as compared with normal at 10 ml/kgSaline dose, positive control at 2.5 mg/kg dose and negative control at 10 ml/kg Saline dose, results of the sample was 42.50±4.223, 0.61±0.023, 22.03±1.821 and 101.34±11.268 for LPO, GSH, Catalase and SOD, respectively. After the collagen induced arthritis rats been treated with *Boswellia serrata* hexane extract the researcher observe significant, changing in the biochemical markers value for instance LPO in the normal group was 19.83±3.800this value increased after the induction of rheumatoid arthritis to 54.83±5.962 and deceased to 42.50±4.223 after treatment but the positive control shows a significant reduced 27.61±3.277, which was more than hexane extract.

In case of GSH in the normal group was 1.13±0.123 this value decreased after the induction of rheumatoid arthritis to 0.26±0.056 and increased to 0.61±0.023after treatment using methanol extract but the positive control shows a significant increase 0.85±0.062, which was more than hexane extract.In case of catalasein the normal group was 37.97±5.176 this value decreased after the induction of rheumatoid arthritis to 15.05±1.296 and increased to 22.03±1.821after treatment using hexane extract but the positive control shows a significant increase 30.14±3.333, which was more than hexane extract.In case of SODin the normal group was 159.09±17.068 this value decreased after the induction of rheumatoid arthritis to 78.40±14.138 and increased to 101.34±11.268 after treatment using methanol extract but the positive control shows a significant increase 137.50±14.570, which was more than hexane extract.

Table 3 shows the *in vivo* Biochemical assay for enzymes involved in oxidative stress of petroleum Ether extractat 400 mg/kg dose as compared with normal at 10 ml/kg Saline dose, positive control at 2.5 mg/kg dose and negative control at 10 ml/kgSaline dose, results of the sample was 39.35±3.893, 0.62±0.089, 23.45±1.856 and 109.54±09.187 forLPO, GSH, Catalase and SOD, respectively. After the collagen induced arthritis rats been treated with *Boswellia serrata* hexane extract the researcher observe significant, changing in the biochemical markers value for instance LPO in the normal group was 19.83±3.800 this value increased after the induction of rheumatoid arthritis to 54.83±5.962 and deceased to 39.35±3.893 after treatment but the positive control shows a significant reduced 27.61±3.277, which was more than petroleum ether extract.In case of GSH in the normal group was 1.13±0.123 this value decreased after the induction of rheumatoid arthritis to 0.26±0.056 and increased to 0.62±0.089 after treatment using methanol extract, but the positive control shows a significant increase 0.85±0.062, which was more than petroleum ether extract. In case of catalase in the normal group was 37.97±5.176 this value decreased after the induction of rheumatoid arthritis to 15.05±1.296 and increased to 23.45±1.856 after treatment using hexane extract but the positive control shows a significant increase 30.14±3.333, which was more than petroleum ether extract. In case of SOD in the normal group was 159.09±17.068 this value decreased after the induction of rheumatoid arthritis to 78.40±14.138 and increased to 109.54±09.187 after treatment using methanol extract but the positive control shows a significant increase 137.50±14.570, which was more than petroleum ether extract.

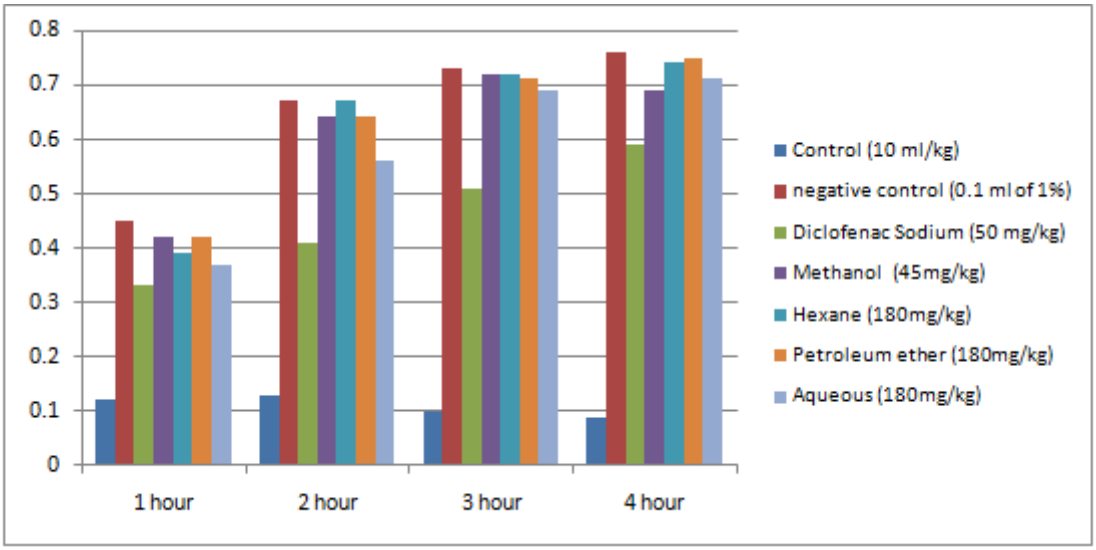

Fig. 1 : Effect of different extracts of *Boswellia serrata*on paw volume in wistar albino.

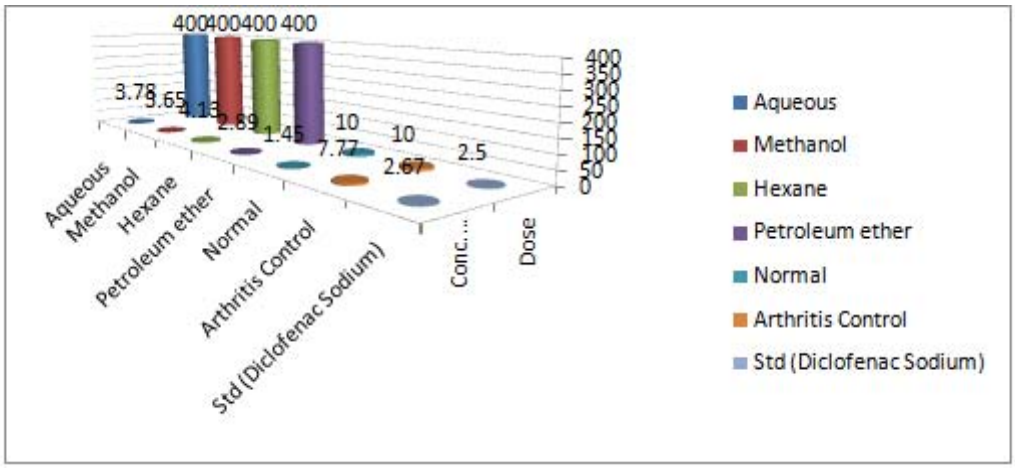

Fig. 2 : IL – 6 estimation.

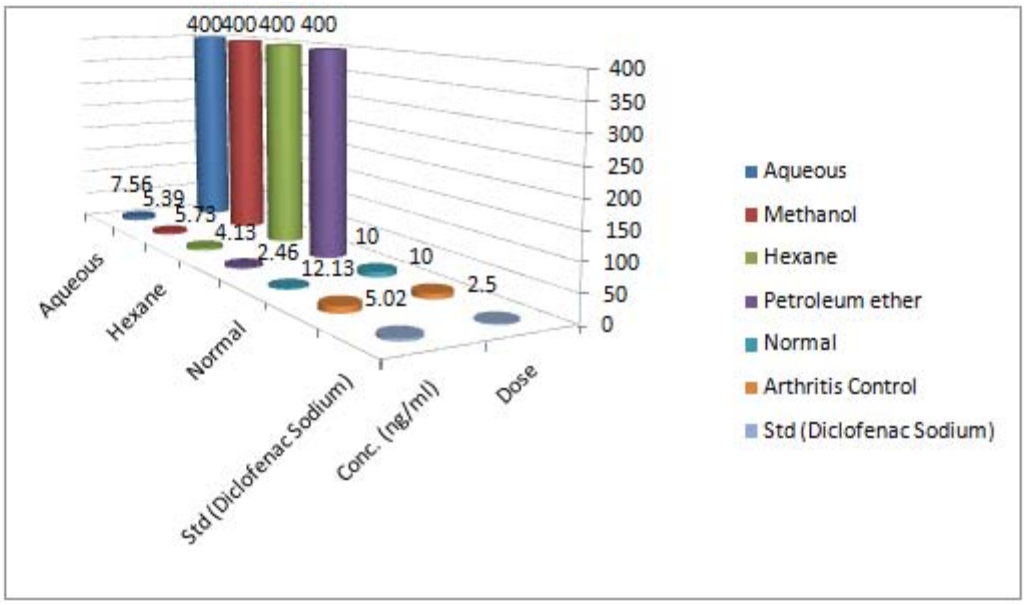

Fig. 3 : TNF – á estimation.

Table 1 : *In vivo* Biochemical Assay for enzymes involved in oxidative stress of methanol extract.

S.No.	Treatment	Dose	LPO (nMMDA/g wet tissue)	GSH (nmol/g wet tissue)	Catalase(unit/g wet tissue)	SOD (unit/g wet tissue)
1.	Normal	10 ml/kg Saline	19.83±3.800	1.13±0.123	37.97±5.176	159.09±17.068
2.	Arthritis Control	10 ml/kg Saline	54.83±5.962	0.26±0.056	15.05±1.296	78.40±14.138
3.	Std (Diclofenac Sodium)	2.5 mg/kg	27.61±3.277	0.85±0.062	30.14±3.333	137.50±14.570
4.	Sample	400 mg/kg	41.50±4.346	0.64±0.067	24.06±1.619	110.22±10.923

Table 2 : *In vivo* Biochemical Assay for enzymes involved in oxidative stress of Hexane Extract.

S.No.	Treatment	Dose	LPO (nMMD A/g wet tissue)	GSH (nmol/g wet tissue)	Catalase(unit/g wet tissue)	SOD (unit/g wet tissue)
1.	Normal	10 ml/kg Saline	19.83±3.800	1.13±0.123	37.97±5.176	159.09±17.068
2.	Arthritis control	10 ml/kg Saline	54.83±5.962	0.26±0.056	15.05±1.296	78.40±14.138
3.	Std (Diclofenac sodium)	2.5 mg/kg	27.61±3.277	0.85±0.062	30.14±3.333	137.50±14.570
4.	Sample	400 mg/kg	42.50±4.223	0.61±0.023	22.03±1.821	101.34±11.268

Table 3 : *In vivo* Biochemical assay for enzymes involved in oxidative stress of petroleum ether extract.

S.No.	Treatment	Dose	LPO (nMMD A/g wet tissue)	GSH (nmol/g wet tissue)	Catalase(unit/g wet tissue)	SOD (unit/g wet tissue)
1.	Normal	10 ml/kg Saline	19.83±3.800	1.13±0.123	37.97±5.176	159.09±17.068
2.	Arthritis control	10 ml/kg Saline	54.83±5.962	0.26±0.056	15.05±1.296	78.40±14.138
3.	Std (Diclofenac sodium)	2.5 mg/kg	27.61±3.277	0.85±0.062	30.14±3.333	137.50±14.570
4.	Sample	400 mg/kg	39.35±3.893	0.62±0.089	23.45±1.856	109.54±09.187

Table 4 : *In vivo* Biochemical Assay for enzymes involved in oxidative stress of aqueous extract.

S.No.	Treatment	Dose	LPO (nMMD A/g wet tissue)	GSH (nmol/g wet tissue)	Catalase(unit/g wet tissue)	SOD (unit/g wet tissue)
1.	Normal	10 ml/kg Saline	19.83±3.800	1.13±0.123	37.97±5.176	159.09±17.068
2.	Arthritis control	10 ml/kg Saline	54.83±5.962	0.26±0.056	15.05±1.296	78.40±14.138
3.	Std (Diclofenac sodium)	2.5 mg/kg	27.61±3.277	0.85±0.062	30.14±3.333	137.50±14.570
4.	Sample	400 mg/kg	38.77±3.766	0.58±0.089	21.42±1.765	107.33±08.163

Table 4 shows the *in vivo* Biochemical assay for enzymes involved in oxidative stress of Aqueous Extractat 400 mg/kg dose as compared with normal at 10 ml/kg Saline dose, positive control at 2.5 mg/kg dose and negative control at 10 ml/kg Saline dose, results of the sample was 38.77±3.766, 0.58±0.089, 21.42±1.765 and 107.33±08.163 forLPO, GSH, Catalase and SOD, respectively. After the collagen induced arthritis rats been treated with *Boswellia serrata* hexane extract the researcher observe significant, changing in the biochemical markers value for instance LPO in the normal group was 19.83±3.800 this value increased after the induction of rheumatoid arthritis to 54.83±5.962 and deceased to 38.77±3.766 after treatment, but the positive control shows a significant reduced 27.61±3.277, which was more than Aqueous extract. In case of GSHin the normal group was 1.13±0.123 this value decreased after the induction of rheumatoid arthritis to 0.26±0.056 and increased to 0.58±0.089 after treatment using methanol extract, but the positive control shows a significant increase 0.85±0.062, which was more than Aqueous extract. In case of catalasein the normal group was 37.97±5.176 this value decreased after the induction of rheumatoid arthritis to 15.05±1.296 and increased to 21.42±1.765 after treatment using hexane extract but the positive control shows a significant increase 30.14±3.333, which was more than Aqueous extract. In case of SOD in the normal group was 159.09±17.068 this value decreased after the induction of rheumatoid arthritis to 78.40±14.138 and increased to 107.33±08.163 after treatment using methanol extract but the positive control shows a significant increase 137.50±14.570, which was more than Aqueousextract.

3. Assessment of inflammatory markers such as IL-1β, IL-6, TNF-α, IL10 and IF-γ

In order to achieve *in vivo* immunological testing for the cytokines involved in inflammation all of Methanol, Petroleum ether, Aqueous and n-Hexane extracts were

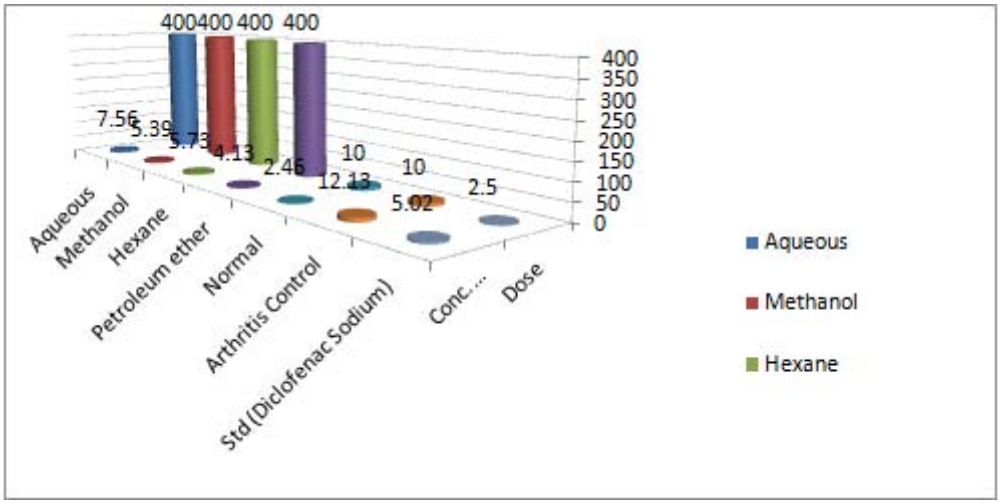

Fig. 9 : IL-10 estimation.

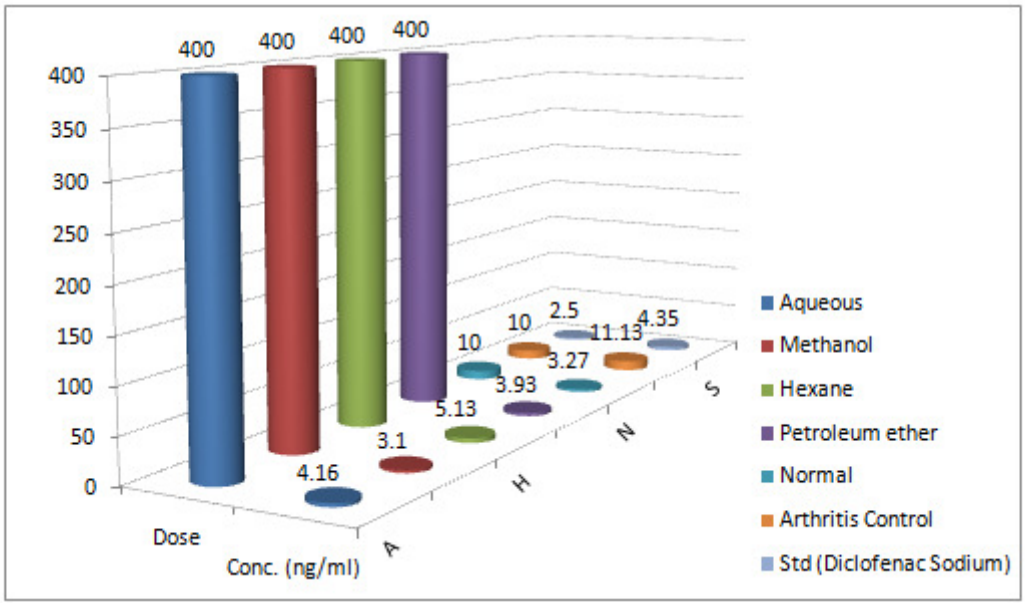

Fig. 5 : IF-γ estimation.

undergoes immunological assay using specific markers such as IL-1β, IL-6, TNF-α, IL10 and IF-γ.

The methanol extract of oleo-gum-resin showed maximum inhibitory response as compared to other fractions. The result strongly suggests that the oleo-gum-resin can be used efficiently as anti-inflammatory agent.4.35±0.152, 4.77±0.205, 3.78±0.203, 1.97±0.167, 11.43±0.078 and 5.17±0.456, respectively. Among these *Boswellia serrata*gum extracts petroleum ether shows the best result in a value of 3.78±0.203 followed by methanol extract 4.35±0.152. Among these *Boswellia serrata* gum extracts methanol shows the best result in a value of 2.65±0.202 followed by petroleum ether extract 2.89±0.198.

Figure 8 shows the comparing between the extracts (methanol, hexane, petroleum ether and aqueous), normal and the standard based on their activity against IL – 6 in experimentally induced arthritis rats employing IL – 6 rat kit.

Fig. 4 shows the comparing between the extracts (methanol, hexane, petroleum ether and aqueous), normal and the standard based on their activity against TNF – á in experimentally induced arthritis rats employing TNF – á rat kit that mentioned in chapter two. Among these *Boswellia serrata* gum extracts methanol shows the best result in a value of 4.39±0.189 followed by petroleum ether extract 5.13±0.198.

Fig. 5 shows the comparing between the extracts (methanol, hexane, petroleum ether and aqueous), normal and the standard based on their activity against IL-10 in experimentally induced arthritis rats employing IL-10 rat kit that mentioned in chapter two.Among these *Boswellia serrata* gum extracts methanol shows the best result in a value of 3.10±0.125 followed by petroleum ether extract

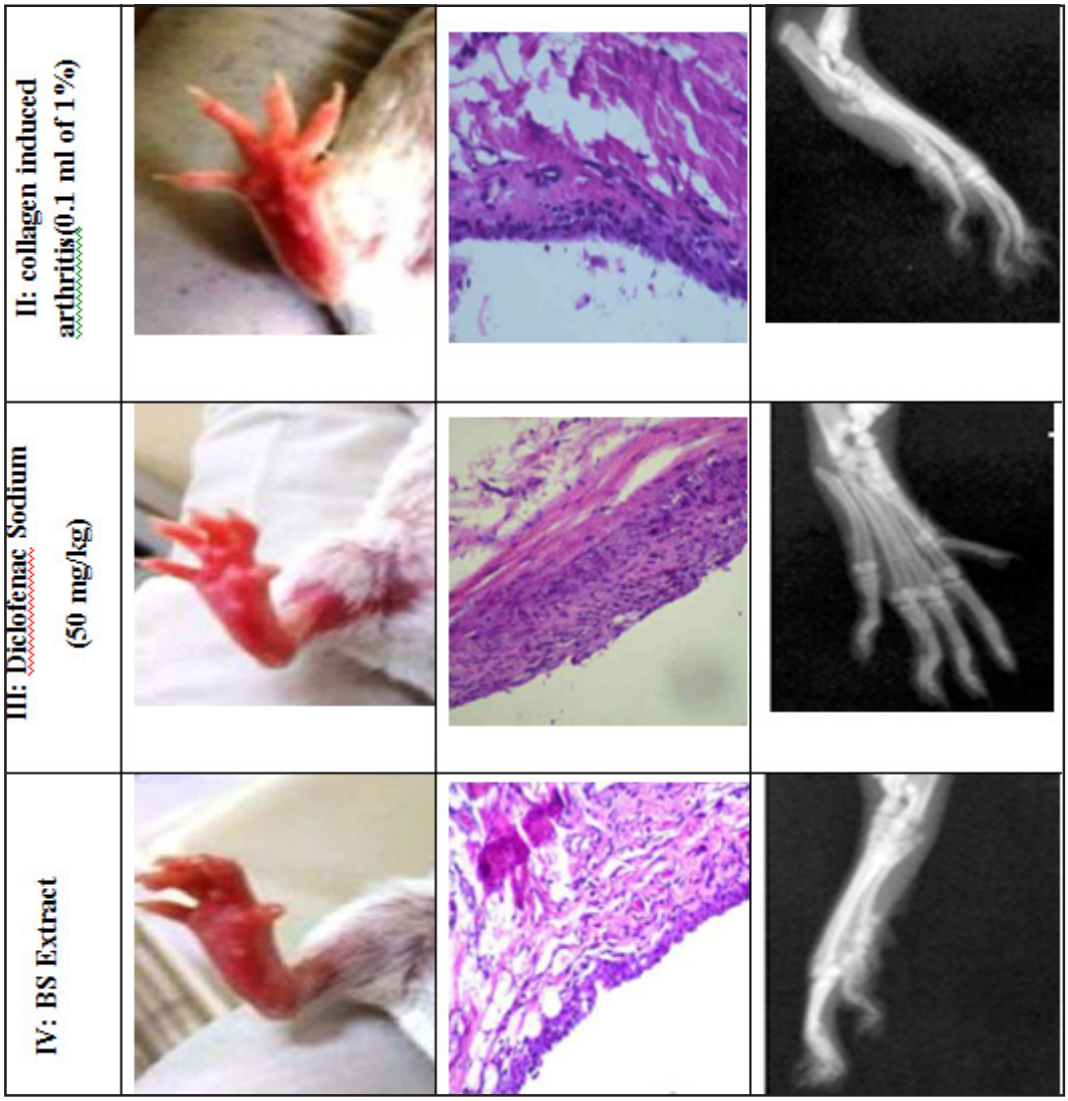

Fig. 6 : The anti-inflammatory activity of *Boswellia serrata* was evaluated in collagen induced arthritis.

3.93±0.134.

Fig. 5 shows the comparing between the extracts (methanol, hexane, petroleum ether and aqueous), normal and the standard based on their activity against IF-γ in experimentally induced arthritis rats employing IF-γ rat kit that mentioned in chapter two. The above acute model result was indicate that methanol extract of *Boswellia serrata* at the dose levels of 45, 90 and 180 mg/kg showed reduction in arthritis at all time intervals as compared to collagen control but significant reduction in paw volume was noticed in 45 mg/kg at 1st and 3rd hour observation 24.13% and 23.71% respectively shown in Fig. 6. The groups treated with Hexane extract of *Boswellia serrata* at the dose levels of 45, 90 and 180 mg/kg showed reduction in arthritis volume at all time intervals as compared to collagen control, but significant reduction in arthritis volume was noticed in 180mg/kg body weight at 1st and 3rd hour observation 31.03% and 19.59%, respectively.

4. X-ray study

Paw of the rat was scanned under the dental x-ray to compare the swelling size and bone shape between the different animal groups, that have been categorized as mentioned earlier in, before and after the treatment using methanol extract as shown in the Fig. 6.

Differentiation was noticed due to the anti-inflammatory activity of the methanol extract as well as diclofenac sodium which used as positive standard, that reduce the swelling volume and suppress the arthritic activity and it was so close to the shape and volume of the positive standard.

6. Histopathological study

The histopathological changes were revealed under the microscope and the anti-inflammatory effect of *Boswellia serrata* was evaluated in collagen induced

arthritis, as present in the Fig. 6, which indicates increase in the paw volume of collagen induced arthritis group and decease in the swelling volume of the groups treated by the standard and *Boswellia serrata* extracts. In the histopathalogical study, it had been observed sub-plantar injection of collagen induced arthritis in the form of accumulation of inflammatory cells like neutrophils whereas *Boswellia serrata* treated rats decreased the cellular infiltrates at different concentrations and found to be much greater in higher concentrations. The development of collagen-induced arthritis is a two-phase event. The first phase (0-1 h) is related to the release of serotonin,bradykinin, histamine and substance P. The second phase (after 1h) is mainly due to the neutrophil infiltration into the inflammatory site and the production of large pro-inflammatoryamounts mediators such as PGE2 and various cytokines for instance IL-1â, IL-6, IL-10 and TNF-á.

Fig. 6 shows a brief for the main idea of entire thesis, it compares between *Boswellia serrata* methanol extract with normal, positive and negative control using several parameters such as morphology, histopathology and X-ray.

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