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EVALUATION OF *IN VIVO* ANTI-INFLAMMATORY ACTIVITY OF WHOLE PLANT OF *Trianthema triquetra* ROTTLER EX WILD

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ABSTRACT

Trianthema triquetra Rottler Ex wild (Aizoaceae) is described in Ayurveda for the treatment of Rheumatism, gout, palsy and amenorrhea. Present study was aimed to investigate the *in vivo* anti-inflammatory activity of (sirusharanai) whole plant and to support its traditional use. The ethanolic extract of whole plant of *Trianthema triquetra* (TT) was investigated for its anti-inflammatory activity in carrageenan induced paw edema in Wister albino rats. Two doses of the ethanolic extract (TT 200 and 400mg/kg p.o) were used in the study and Diclofenac sodium (4.5mg/kg i.p) was used as standard. The ethanolic extract of *Trianthema triquetra* at the concentration of (400mg/kg p.o) significantly ($P<0.05$) reduced increased in paw volume induced by carrageenan. *Trianthema triquetra* at the dose of 400mg/kg showed potent anti-inflammatory action on comparison with the standard drug Diclofenac sodium.

KEYWORDS: *Trianthema triquetra*, Carrageenan, anti-inflammatory, Diclofenac, paw volume.

INTRODUCTION

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions as the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens.[1] Inflammation is usually associated with pain as a secondary process resulting from the release of analgesic mediators, non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immunosuppressant drugs which have been used usually in the relief of

inflammatory diseases by people around the world for a long time. However, these drugs were often associated with severe side effects such as upper gastrointestinal symptoms and lesions such as esophagitis, gastritis, peptic ulcers and their severe complication including bleeding and perforation.

Recently many natural medicines derived from medicinal plants were considered as effective and safer for the treatment of various diseases including inflammation and pain. The development of plant based anti-inflammatory drugs have been given importance in the global market. There are many plants which have not been so far subjected to scientific evaluation. *Trianthema triquetra* is a small prostate, branched herb widely distributed in India which belongs to the family Aizoaceae. It has been traditionally used for different medicinal purposes such as anti-oxidant, hepatoprotective activity. It contains phytoconstituents like flavonoids, steroids, terpinoids, fatty acid, phenolic, gums, resins, quinine's and saponins. The presence of flavonoids has been reported as the reason for their anti-oxidant and hepatoprotective activity.[2] The present study was to evaluate the anti- inflammatory activity of whole plant of *Trianthema triquetra*. The ethanolic extract was evaluated for their potency against inflammation in rats.

MATERIALS AND METHODS

Collection and identification of plant: Fresh whole plant of *Trianthema triquetra* was collected from the Koyambedu market, Chennai, Tamil Nadu (India), in the month of October 2017. It was identified and authenticated by Prof. P. Jayaraman., Ph.D., Plant Anatomy Research Centre, West Tambaram, Chennai-45.

Preparation of extracts: The whole plant was freshly collected and then cut into small pieces. After that, shade drying was carried out for a week. The dried plant was powdered. 60g powered plant material was weighed and extracted with petroleum ether (500 ml), ethyl acetate (500 ml) and ethanol (500 ml) separately for 24 hours in a soxhlet apparatus. All the extracts were evaporated and dried. Extracts were used for further *in-vitro* and *in- vivo* anti-inflammatory study. In *in-vitro* anti-inflammatory method the ethanolic extract of whole plant of *Trianthema triquetra* showed maximum significant activity in RBC membrane stabilization method and Protein denaturation (Heat hemolysis) method when compared with other two extracts. Hence, the ethanolic extract was further used for in- *vivo*-anti-inflammatory study.

Experimental Animals: The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee, Madras Medical College, Chennai-03 and this protocol met the requirements of national of CPCSEA (Approval No: 1917/ReBi/S/16/ CPCSEA/25.10.2016 and Protocol No: 6/AEL/IAEC/MMC, Date: 12/09/2017).

The Wistar rats (100-150g) used for this study were procured from Animal Experimental Laboratory, Madras Medical College, Chennai-03, India. The food withdrawn on the day before the experiment, however they were allowed free access to water and standard diet throughout the experiments. The animals were handled according to the prescribed CPCSEA ethical guidelines for laboratory animals.[3]

***In Vivo* Anti-Inflammatory Activity[4-9]**

Carrageenan induced paw edema in Wister albino rat was used as a model for screening of anti-inflammatory activity.

Drugs and chemicals

- 1% 0.2 ml of carrageenan induced at sub-plantar region.
- Diclofenac sodium 4.5mg/kg intra peritoneal (i.p) (standard drug).
- Ethanolic extract was dissolved in normal saline.

Carrageenan induced paw edema

Make a mark on left hind paw at the level of the lateral malleolus, so that every time the paw is dipped into the mercury column up to the fixed mark to ensure the constant paw volume measurement. Note the initial paw volume of each rat by mercury displacement method using plethysmograph. Treatment schedule and grouping of animals were tabulated below in **table.1.** Edema was produced by an injection of 0.2ml of 1% carrageenan into the left hind of each rat in the sub-plantar region after 30mins of drug administration. Calculate the percentage inhibition of edema of Diclofenac and test drug.

Percentage inhibition of edema was calculated as

$$\text{Inhibition of edema (\%)} = \frac{V_c - V_t}{Vc} \times 100$$

Where,

Vt is the paw volume of drug treated group.

Vc is the paw volume of control group.

Table .1: Treatment schedule and grouping of animals.

S. No	Group	Treatment	Number of Animals
1	**Disease control**	0.2ml of 1% carrageenan inject at sub plantar region.	6
2	**Standard drug**	0.2ml of 1% carrageenan inject at sub plantar region & Diclofenac sodium (4.5mg/kg i.p.)	6
3	**Test I**	0.2ml of 1% carrageenan inject at sub plantar region & low dose (200mg/kg) of ethanolic extract p.o.	6
4	**Test II**	0.2ml of 1% carrageenan inject at sub plantar region & high dose (400mg/kg) of ethanolic extract p.o.	6

Biochemical Parameters: The blood sample were collected and allowed to clot and centrifuged at 2000 rpm for 15-20 minutes using REMI (412 LAG) cooling centrifuge. The serum was kept at -80ºC until analyzed. Levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), serum Alkaline Phosphate (ALP) were determined with an Automated Analyzer (Hitachi 911, Japan).

Estimation of Aspartate Aminotransferase (Ast/Sgot)[10,11]

Aspartate aminotransferase (AST) also referred to as serum glutamate oxaloacetate transferase (SGOT), is an enzyme involved in amino acids metabolism, AST is widely distributed in liver, RBC, heart, pancreas and kidney. A high level of AST in blood is observed in severe liver disease, kidney disease and lung disease.

Principle: The reagent of NADH consumption is measured photo metrically and is directly proportional to the AST concentration in the sample.

Procedure: 800 µl of L- Aspartate and Malate dehydrogenase and 200 µl of α- Ketoglutarate are mixed together and incubated at 37ºC for 2 minutes and 100 µl of sample was added. The change in absorbance was measured at 340 nm.

Calculation

AST = Abs/min × 1764 (factor)

Estimation of Alanine Aminotransferase (Alt/Sgpt)[11]: Alanine aminotransferase / Serum Glutamate Pyruvate Transferase is an enzyme which is involved in amino acids metabolism.

Principle: The reagent of NADH consumption is measured photometrically and is directly proportional to the AST concentration in the sample.

Procedure: 800 µl of L- Alanine and Lactate dehydrogenase and 200 µl of α- Ketoglutarate are mixed together and incubated at 37ºC for 2 minutes and 100 µl of sample was added. The change in absorbance was measured at 340 nm.

Calculation

ALT = Abs/min × 1764 (factor)

Estimation of Alkaline Phosphatase (Alp)[12,13]

Principle: When the enzyme incubated with p-nitro phenyl phosphate and Tris buffer (pH 9.6) in alkaline condition inorganic phosphate and p-nitro phenol are formed by the catalytic action of alkaline phosphatase. Amount of p-nitro phenol liberated by the enzyme is measured at 420 nm.

Procedure: 1ml of p-nitro phenyl phosphate and 1.5 ml of buffer were added with 100 µl of homogenate. The mixture was incubated at 37ºC for 30minutes. Then the reaction was stopped by addition of 0.1 N NaOH. The absorbance of liberated p-nitro phenol was measured at 420 nm.

Calculation

ALP U/I = ΔA/ 2764

Statistical Analysis: All the values were expressed as mean ± SEM. The data was statistically analyzed by one way ANOVA followed by Dunnet's test. One way analysis of variance (ANO A) was used to correlate the statistical difference between the variables. $P<0.05$ was considered to be significant. Statistical analysis was done by Graph Pad prism.

RESULTS AND DISCUSSION

***In Vivo* Anti-Inflammatory Activity:** Carrageenan induced paw edema in Wister albino rat was used as a model for screening of anti-inflammatory activity. Group 3 and 4 of experimental animals received low dose (200mg), high dose (400mg) of ethanolic extract of T*rianthema triquetra*. Group 1 served as disease control and Group 2 received Diclofenac (4.5mg/kg/i.p). The paw volume was measured for Control, standard and low and high dose of test drug at one hour interval for six hours which tabulated in **Table.2.**

Table. 2: *In vivo* anti-inflammatory activity of ethanolic extract of *Trianthema triquetra.*

Treatment groups	Paw volume (ml)							
	0mins	30mins	60mins	120mins	180mins	240mins	300mins	360mins
Group 1 Disease Control	0	0.066± 0.0001	0.083± 0.002	0.120± 0.0033	0.160± 0.0033	0.220± 0.006	0.250± 0.004	0.30± 0.006
Group 2 Diclofenac (4.5mg/kg)	0	0.051± 0.002****	0.042± 0.0023****	0.033± 0.002****	0.030± 0.0022****	0.026± 0.002***	0.022± 0.002****	0.020± 0.003****
Group 3 Low dose (200mg/kg)	0	0.056± 0.002****	0.052± 0.002****	0.048± 0.0012****	0.046± 0.002****	0.045± 0.002**	0.042± 0.0024****	0.040± 0.002****
Group 4 High dose (400mg/kg)	0	0.053± 0.002****	0.048± 0.002****	0.042± 0.0023****	0.042± 0.002****	0.040± 0.0023**	0.038± 0.0012****	0.036± 0.0012****

Values are expressed by Mean ± SEM (n=6).

One way ANO A followed by Dunnet's test *p<0.05, **p<0.01, ***p<0.001,****p<0.0001 when compared to disease control.

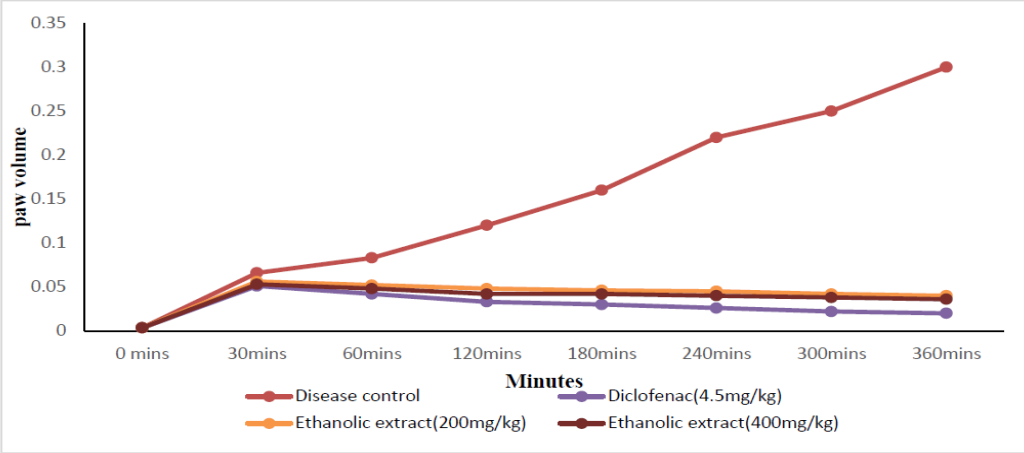

Fig. 1: Paw volume for experimental animals.

From the **Table.2 and Fig.1**, it is clear that high dose of ethanolic extract of *Trianthema triquetra* significantly decreases paw volume when compared with control. Though it showed significant decrease in paw volume, it was less significant than standard -Diclofenac.

Table. 3: Percentage inhibition of paw volume.

Treatment groups	Percentage inhibition of paw volume (%)							
	0mins	30mins	60mins	120mins	180mins	240mins	300mins	360mins
Group 1 - Diclofenac (4.5mg/kg)	0	22.70	49.39	72.50	81.20	88.18	91.20	93.30
Group3-Low dose of ethanolic extract of T.triquetra (200mg/kg)	0	15.15	37.57	60.83	71.25	79.54	83.20	86.60
Group4- High dose of ethanolic extract of T.triquetra (400mg/kg)	0	19.69	42.53	65.00	73.75	81.18	84.80	88.01

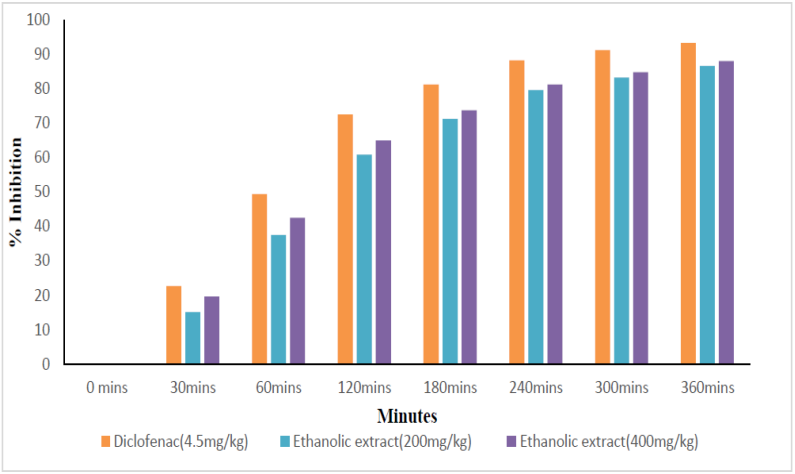

Fig. 2: Percentage inhibition of paw volume.

From the **Table.3 and Fig.2** it was seen that the high dose of ethanolic extract has shown increased percentage inhibition of paw volume than low dose when compared with standard.

Biochemical Estimation

After the completion of *in vivo* anti-inflammatory study the blood samples were collected by the retro-orbital puncture method and the serum was separated by using centrifuge at 2000rpm speed for 10mins. Separated serum was subjected to the estimation of Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Alanine Transferase (ALT).

Aspartate Aminotransferase (AST) evaluations

Table. 4: Aspartate Aminotransferase levels.

Group	Treatment	AST (IU/ml)
I	Normal	207±3.57
II	Disease control	242.0±2.08
III	Standard	230.66±3.38
IV	Low dose	232.66±2.90
V	High dose	228.0±4.58

Values are expressed by Mean ± SEM (n=6).

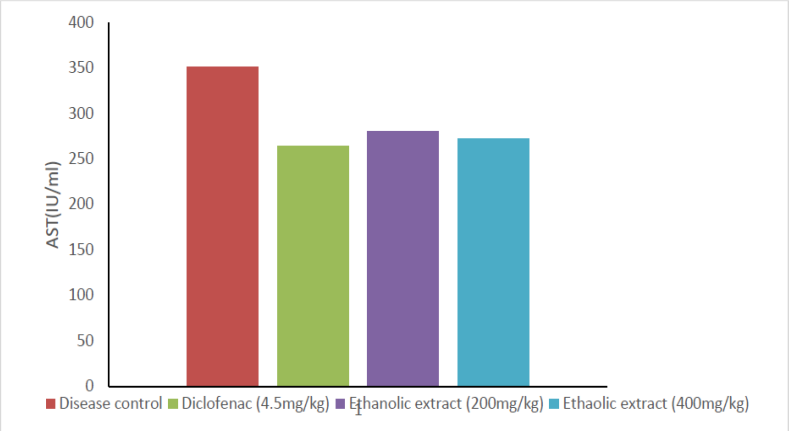

Fig. 3: Aspartate Aminotransferase levels.

In the **Table.4 and Fig. 3;** It was seen that AST level 242.0±2.08 was increased significantly in disease control when compared to normal group 207±3.57.Treatment with standard showed a significant decrease in the level of AST 230.66±3.38 as compared to the disease control. The ethanolic extract of *Trianthema triquetra* treated groups of animal's also showed significant decrease in the level of **AST232.66±2.90and 228.0±4.58** respectively. The reduction was more in the group treated with the high doses ethanolic extract of *Trianthema triquetra* 228.0±4.58, when compared to low dose treated groups.

Alanine Aminotransferase (ALT) evaluations

Table. 5: Alanine Aminotransferase levels.

Group	Treatment	ALT (IU/ml)
I	Normal	63.83±1.38
II	Disease control	89.66±0.88
III	Standard	70.66±1.20
IV	Low dose	84.66±2.02
V	High dose	80.66±1.45

Values are expressed by Mean ± SEM (n=6).

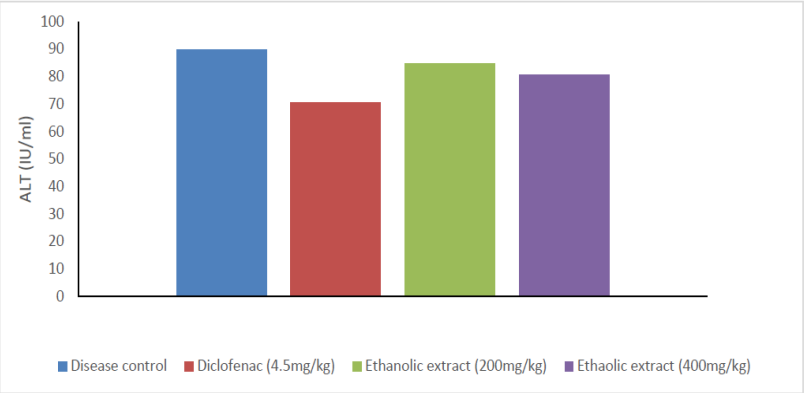

Fig. 4: Alanine Aminotransferase levels.

In the **Table.5 and Fig. 4** It was seen that ALT level89.66±0.88 has increased significantly in disease control animals when compared to normal group 63.83±1.38.Treatment with standard showed a significant decrease in the level of ALT 70.66±1.20 as compared to the disease control. The ethanolic extract of *Trianthema triquetra* treated groups of animal's also showed significant decrease in the level of **ALT84.66±2.02** and **80.66±1.45** respectively. The reduction was more in the group treated with the higher doses ethanolic extract of *Trianthema triquetra*80.66±1.45, when compared to low dose treated groups.

Alkaline phosphatase (ALP) evaluations

Table. 6: Alkaline phosphatase levels.

Group	Treatment	ALP (IU/ml)
I	Normal	228.2±1.38
II	Disease control	351.0±1.73
III	Standard	264.0±3.21
IV	Low dose	280.66±1.45
V	High dose	272.0±2.08

Values are expressed by Mean ± SEM (n=6).

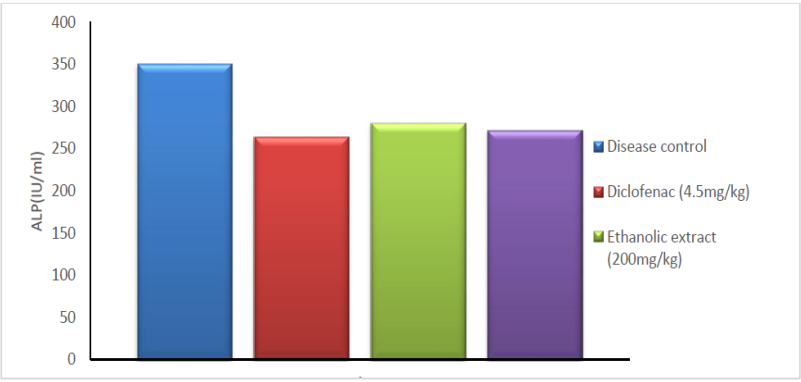

Fig. 5: Alkaline phosphatase levels.

In the **Table.6 and Fig.5** It was seen that ALP level 351.0±1.73 has increased significantly in disease control animals when compared to normal group 228.2±1.38.Treatment with standard showed a significant decrease in the level of ALP264.0±3.21 as compared to the disease control. The ethanolic *extract* of *Trianthema triquetra* treated groups of animal's also showed significant decrease in the level of ALP 280.66±1.45 and 272.0±2.08 respectively. The reduction was more in the group treated with the higher doses of ethanolic extract of *Trianthema triquetra* 272.0±2.08, when compared to low dose treated groups.

DISCUSSION

In vivo anti-inflammatory activity was carried out by carrageenan induced paw edema. The development of acute edema in the paw of the rat has been described as a three phase event. It is mainly attributed to the release of histamine, serotonin in the initial phase (0-2hr), cytokines in the second phase (3hr) and prostaglandin in the third phase (>4hr). Inflammatory effect induced by carrageenan is associated with free radicals and this induced inflammatory response is linked to neutrophil infiltration and the production of neutrophil derived free radical such as hydrogen peroxide, super oxide, hydroxyl radicals and other neutrophil derived mediators. Carrageenan (lambda), are a family of linear sulfated polysaccharides that are extracted from red edible seaweeds. Carrageenan cause inflammation and edema (14, 15). In the present study, the dose of 400mg/kg of ethanolic extract of *Trianthema triquetra* and 4.5mg/kg Diclofenac sodium treated experimental animals showed significant inhibition of carrageenan induced edema after 1,2,3, and 4 hours of edema induction. One hour after the edema induction, 400mg/kg of the ethanolic extract of plant showed no significant activity against edema. However, after the fourth, fifth and sixth hours the low and high dose ethanolic extract of *Trianthema triquetra* showed significant edema inhibition when compared to the control group **(Table.3 and Fig.2)**. This implies that ethanolic extract of whole plant of *Trianthema triquetra* possess anti-inflammatory activity in a dose dependent manner. This anti-inflammatory effect due to the presence of tannins, flavonoids, alkaloids and saponins.

Liver is an important organ involved in metabolism of many Xenobiotic. In this study analysis of possible hepatic injury, permeability cell membrane causing alteration in cell membrane was evaluated by estimation of AST, ALT and ALP. It was seen that AST, ALT and ALP levels has increased significantly in disease control animals which were compared to normal group. Treatment with standard showed a significant decrease in the level of AST,

ALT and ALP. The ethanolic extracts of *Trianthema triquetra* treated groups of animals also showed a significant decrease in the level of AST, ALT and ALP. The reduction was more in the group treated with the high dose of ethanolic extract of *Trianthema triquetra*, when compared to low dose treated groups. This indicates that there was no liver damage due to the administration of the extract.

CONCLUSION

In vivo anti-inflammatory activity was carried out by Carrageenan induced paw edema using ethanolic extract of whole plant of *Trianthema triquetra*. Two doses of the ethanolic extract i.e. 200mg/kg and 400mg/kg were used for anti-inflammatory activity evaluation. The studies indicated that ethanolic extract at the dose of 400mg/kg has significant anti-inflammatory activity which was comparable with that of the standard. Hence from these studies it was concluded that the ethanolic extract of whole plant of *Trianthema triquetra* possess significant anti-inflammatory activity. Further studies can be carried out in the future to elucidate the mechanism of action of ethanolic extract of whole plant of *Trianthema triquetra* Rottler ex wild which may be followed and clinical studies to establish its efficacy in humans.

REFERENCES

1. Thomas S kupper, Robert C Fuhlbrigge. Immune surveillance in the skin: mechanisms and clinical consequences. Nature reviews immunology, 2004; 4: 211-222.
2. Vinay Kumar, Abbas AK, Nelson fausto. Robbins and Cortan Pathologic Basis of disease.7th edition; Elsevier publication, 2008; 47-48.
3. http://caf.iisc.ernet.in/image/cpcsea-guidelines-latest.pdf.
4. Tamilselvan A, Rama Devi M, Siva Subramanian N, Srinivas L, Prathyusha K, Sri silam K, Lalappa T, Usha Yadav. Anti-inflammatory activity of *Pupalia lappacea L.Juss*. International journal of Allied medicinal sciences and clinical research Volume 2/ Issue 2/April-June-2014.
5. Gerhard Vogel H. Drug discovery and evaluation.2nd edition;Springer-Verlag Berlin Heidelbeg, 2002; 759-762.
6. Eze Charity Chinasa et al., Evaluation of anti-inflammatory property of the leaves of *Sansevieria liberica* ger. and labr. (fam: dracaenaceae). Asian Pacific Journal of Tropical Medicine, 2011; 791-795.

7. Winter CA, Risley EA, Nuess GW Carrageenan induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, 1962; 111: 544-547.
8. Gilligan J.P., Modulation of carrageenan induced paw edema by substance P; inflammation, 1994; 18: 285-292.
9. Gerhard H.Vogel; Drug discovery and evaluation (Pharmacological assays); Second completely Revised, Updated & Enlarged. Edition, 692-717&760.
10. Elamathi S, Jayashree N, Priyadharshini R. Evaluation of hepatoprotective activity of *pongamia pinnata* linn., seed extract against carbon tetrachloride induced hepatotoxicity in Wister rats, 2016; 5(8): 1542-1554.
11. Schmidit E and Schmidt FW. Enzyme Biology Clinical, 1963; 3:1.
12. Reitman S. and Frankel S. American Journal Clinical Pathalogy, 1957; 28-56.
13. Ezeko Vitalis C., Nwokocha Chukwuemeka R. Mounmbegna Philippe E, Nriagu Chinonso C. Effects of *Zingiber Officinale* on liver function of mercuric chloride-induced Hepatotoxicity in adult Wister Rats. Revised Electron Biomed / Electron Journal Biomedicine, 2007; 3: 40-45.
14. Mohini AP et al. *In-vivo* and *in-vitro* screening of medicinal plants for their anti-inflammatory activity: an overview. Journal of Applied Pharmaceutical Science, 2012; 2(6): 19-33.
15. Sosa Sequera MC, Suarez O, Dalo NL. Kaurenic acid: An in vivo experimental study of its anti-inflammatory and antipyretic effects, 2010; 42(5): 293-296.