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Pharmacological and Biochemical evaluation of anti-arthritic activity of Justicia gendarussa extract in FCA induced arthritis in Wistar rats

Ashish[1], Anjali[1], Praveen K Dixit*[1], Nagarajan K[2]

[1]Department of Pharmacology, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, Affiliated to Dr APJ Abdul Kalam Technical University, Lucknow, India
[2]Department of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, Affiliated to Dr APJ Abdul Kalam Technical University, Lucknow, India

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ABSTRACT

The present study is carried out to evaluate anti-inflammatory and anti-arthritic potential of *Justicia gendarussa*. Leaves and stem extracted with ethanol and chloroform. These extract is tested against in-vitro (HRBC membrane stabilization method and Inhibition of protein denaturation method) and in-vivo (Carrageenan induced paw edema model) anti-inflammatory models. To assess the anti-arthritic activity, FCA induced arthritis model is used. Assessment of arthritis is done by paw volume, joint diameter, body weight, biochemical parameters, hematological parameters (Hb, RBC, WBC, ESR). The effect of in-vitro anti-inflammatory model depends on concentration. Both test extract and standard Diclofenac sodium has been shown concentration dependent effect. The maximum anti-inflammatory effect of the test extract achieved at 2000μg/ml. The test (JGLE, JGLC, JGSE) has been shown inhibition of paw edema induced by carrageenan at 50mg/kg body weight. The extract JGLE, JGLC, JGSE at 50mg/kg body weight and 100mg/kg body weight orally showed the significant ($P< 0.05$) and dose dependent inhibitory effect against FCA induced arthritis model. Diclofenac sodium 20mg/kg body weight orally is used as a standard. JGLE exhibit more significant and most promising anti-arthritic and anti-inflammatory effect than other extracts these effects support the traditional role of *J. gendarussa* in arthritis and other inflammatory condition.

*Corresponding Author

Name: Praveen K Dixit
Phone: +91 8192026467
Email: praveendixit87@gmail.com

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INTRODUCTION

Inflammation is a response of immune system that protects the host against the foreign substance. The major four vital signs of acute inflammation are as follows, Calor (heat), Rubor (redness), tumor (swelling) and dolor (pain), leading to Functiolaesa loss (or impairment) of function (Mittal *et al.*, 2014). Inflammatory process which is successful or controlled in manner is needed that leads to the removal of dangerous stimuli and also help in the restoration of normal physiology (cellular or tissue physiology) (Tasneem *et al.*, 2019).

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune

disorder which shows the chronic inflammatory responses and also causes the symmetrical polyarthritis primarily in the synovial joints. Some triggering events initiate the inflammatory pathway where the immune cells play the greater role and the some inflammatory mediators like cytokines, some enzymes proteinase play a crucial role in the joint destruction (Majithia and Geraci, 2007). The major targets of rheumatoid arthritis is synovial joints which leads to the destruction of the synovial joints and their membrane causes joint disability, arthralgia, swelling, redness and also limit the joint movement (Guo *et al.*, 2018). Arthritis is a common term with a joint paint as a primary feature which includes osteoarthritis (OA), rheumatoid arthritis (RA) and psoriatic arthritis (Li *et al.*, 2016). In traditional medicine system the plant aerial part of *Justicia gendarussa* (f. Acanthaceae) is very well known for its various therapeutic activities (Ramees *et al.*, 2019). Leaves contain, alkaloids, flavonoids, saponin, steroids, O-distributed aromatic amine i.e., 2-(2'-amino-benzylamino) benzyl alcohol and their respective O-methyl ethers, stigmasterol, lupeol, 16-hydroxylupeol, 28β-sitosterol (Ashish *et al.*, 2020). The plant shows the various therapeutic activity like, anti-arthritic (Paval *et al.*, 2009a,b), anti-inflammatory (Kavitha *et al.*, 2011), anti-bacterial (Kumar *et al.*, 2018), dyspepsia (Kumar *et al.*, 2012), cytotoxic (Ayob *et al.*, 2014), anti-angiogenic (Mangai, 2018), anti-fungal (Ayob *et al.*, 2013), Cough (Patel and Zaveri, 2012), hepatoprotective (Krishna *et al.*, 2010; Phukan *et al.*, 2014), anti-anxiety activity (Jothimanivannan *et al.*, 2013), anti-depressant effect (Mythili and Jothimanivannan, 2017), sedative and hypnotic activity (Jothimanivannan *et al.*, 2013), anti-diabetic (Islam *et al.*, 2015).

MATERIALS AND METHODS

The leaf and stem part of the plant *Justicia gendarussa* purchased from the market of Delhi, India. The part of the plant is authenticated from the NISCAIR (National Institute of Science Communication & Information Resources) Delhi, India with authentication No. (Ref. No.-NISCAIR/Consult/2019/3539-40).

Preparation of Extract

Fresh and dried leaf and stem part of *Justicia gendarussa* were coarsely power by mechanically. Extraction of the leaves and stem part is done by using the solvent ethanol (95%) and chloroform (95%) by soxhlet apparatus. Distillation process is used to concentrate the extract. The extract evaporates to dryness. Extract (JGLC, JGLE and JGSE) was kept in vacuum desiccators until use (Paval *et al.*, 2009b).

HRBC (Human Red Blood Cell) membrane stabilization method

Reagents Preparation

For the preparation of the Alsevers solution dissolve 2gm dextrose, 0.8gm sodium citrate, 0.05gm citric acid and 0.42gm sodium chloride dissolve in distilled water and make-up the volume upto 100ml. Preparation of the Hypotonic saline is done by dissolving the 0.36gm of sodium chloride in 100ml distilled water. To prepare the Isotonic solution dissolve the 0.85gm of Sodium chloride in 100ml distilled water. For the preparation of the phosphate buffer (pH 7.4) take 2.38gm disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8gm of sodium chloride and dissolve in 100ml distilled water (Gautam *et al.*, 2013). Human red blood cell (HRBC) is used to determine the in-vitro anti-inflammatory activity of the test compound (plant extract) (Mittal *et al.*, 2013). Blood collected from the healthy human volunteer who has been not taken any sort of NSAIDs (Non steroidal anti-inflammatory drugs) 2 week prior from the experiment. The freshly collected blood is added in the equal volume of Alsever solution. The whole mixture (Blood and Alsever solution) is centrifuged at the 3000rpm (10min). The separated packed cells obtained after the centrifuge were washed with three time freshly prepared isosaline solution (.85%, pH 7.2). The total volume of the blood is measured and make the 10% v/v suspension with isosaline solution (Kota *et al.*, 2018). The total reaction mixture contain the 1mL of phosphate buffer, 2mL of hyposaline [0.36%], 0.5mL of HRBC suspension and 0.5mL of different concentration of plant extract (100, 200, 400, 800 and 1600μg/ml) and reference compound Diclofenac sodium (50, 100, 200, 400, 800 and 1600 μg/ml) (Mittal *et al.*, 2013). The whole reaction mixture was incubated at the 37^0C for the period of 30min and after the completion of the incubation the mixture is centrifuged at the 3000rpm for the time period of 20min. The supernatant is decanted and the total Hb content of the middle layer of the suspension is estimated spectophotometrically at 560nm (Nainwani *et al.*, 2014).

The total % of hemolysis of HRBC membrane stabilization can be calculated by using the formula ,

% Hemolysis =

$$\frac{(Optical\ density\ of\ Test\ sample)}{Optical\ density\ of\ control} \times 100$$

The total % of HRBC membrane stabilization calculated by,

% Protection =

$$100 - \left[\left(\frac{Optical\ density\ of\ test\ sample}{Optical\ density\ of\ control}\right) \times 100\right]$$

Evaluation of anti-inflammatory activity by protein denaturation Inhibition method

The total reaction mixture (50mL) contains the 2mL of fresh egg albumin (from hen's fresh egg), 28mL of phosphate buffer saline (PBS, pH 6.4) and add 20mL of varying concentration of JGLC, JGLE and JGSE to be the final concentration to become 10, 50, 100, 200, 400, 800, 1000, and 2000 μg/mL. Same volume of double distilled water has been taken as control. The whole reaction mixture was incubated by using the BOD incubator (37$\pm$2) °C for 15 min and heated at 70 °C for 5 min on water bath, kept aside for cooling and measure the absorbance at 660nm under UV 1800 spectrophotometer. Reference diclofenac sodium at the concentration of 10, 50, 100, 200, 400, 800, 1000, 2000 μg/mL was used and take a similar treatment to determine the absorbance and the viscosity were also determined for both the sample (test and standard) through the Ostwald viscometer (Mittal *et al.*, 2013).

The total % inhibition of the protein denaturation was determined by,

% Inhibition =

$$100 \times \left(\frac{Vt}{Vc} - 1\right)$$

Vt = Total absorbance of test sample

Vc = Total absorbance of control sample

Experimental Animals

Wistar rat body weight 200-250gm taken from the animal house facility, KIET School of Pharmacy, Ghaziabad. The animals are stored in the cage which is made up of polypropylene under standard laboratory condition (12hr. light and 12hr. dark) and had a free access for food and water. The temperature of animal house maintained at 25 $\pm$ 2^{o}C. The approval of the protocol to be approved by the Institutional Animal Ethical Committee (IAEC) of KIET School of Pharmacy (IAEC/KSOP/E/10/06), Ghaziabad.

Carrageenan Induced Paw Edema Model

Male Wistar rats having body weight 200-300gm is used for the assessment of hind paw edema. The procedure of Kumar *et al.* (2012) is employed in this model. For the induction of edema in the right hind paw 0.1ml of 1% carrageenan in 0.9% saline aponeurosis injection is injected (Kumar *et al.*, 2012). Injection is inserted in right hind paw after 1hr. of oral administration of plant extract which is extracted in various solvent. Extract of plant *Justicia gendarussa* evaluated for its anti-inflammatory in the solvent ethanol and chloroform (JGLE, JGLC, and JGSE) at the concentration of 50mg/kg body weight orally (Sarkar, 2012). Each groups having 6 animals in the groups (n=6). Reference compound diclofenac sodium at the concentration of 20mg/kg/b.w is used by oral route before 1 h to carrageenan induction. The volume of paw edema is measured at time interval of 1, 3, and 5 h by using the dial Vernier calliper and mercury plethymometer (Kavitha *et al.*, 2011).

Group I (Normal control) received 0.9% Normal control, Group II (Inflammation control) received Carrageenan + 0.9% Normal saline, Group III (Standard control) received Carrageenan + Diclofenac sodium (20mg/kg), Group IV (JGLE) received JGSE extract (50mg/kg body weight orally), Group V (JGLC) received JGSE extract (50mg/kg body weight orally), Group VI (JGSE) received JGSE extract (50mg/kg body weight orally).

The calculation of the total swelling inhibition rate and the total edema inhibition rate to be done by,

% edema inhibition =

$$\frac{(Vc\ -\ Vt)}{Vc} \times 100$$

Where, Vc = Volume of control

Vt = Volume of test.

FCA (Freund's Complete Adjuvants) Induced Arthritis Model

FCA (Freund's Complete Adjuvants) Induced Arthritis Model is used to performed anti-arthritic effect of test drug by using the wistar rats. Animals divided into 9 groups and every group having the 6 animals in each group. Arthritis induction is achieved by the use of Full Adjuvants (FCA) from the Freund. In the FCA induced arthritis model that has 10 mg of dry heat-killed sterile paraffin oil Mycobacterium butyricum / ml is injected into the plantar surface of the animal's left hindfoot. Treatment of the animals starts from 20th day of arthritis and the treatment is continued for 20th days. During this 20th day of treatment body weight of animal and the paw volume to be takes at the regular interval. The blood is obtained by retro-orbital puncture and various hematological parameters such as, Hb, RBC, WBC, ESR to be performed at the end of the test [9]. Anti-arthritic activity assessment of extract (JGLE, JGLC, JGSE) is done at 50mg/kg body weight orally and 1000mg/kg body weight orally and standard Diclofenac sodium (20mg/kg/b.w) is used orally (Paval *et al.*, 2009b; Kavitha *et al.*, 2011).

Group I (Normal control) receive Normal saline, Group II (Arthritic control) FCA 0.1ml + Normal saline, Group III (Standard control) FCA 0,1ml + Diclofenac sodium 20mg/kg/b.w., Group IV (JGLEH) JGLE extract 100mg/kg body weight orally, Group V (JGLEL) JGLE extract 50mg/kg body weight orally, Group VI (JGLCH) JGLC extract 100mg/kg body weight orally, Group VII (JGLCL) JGLC extract 50mg/kg body weight orally, Group VIII (JGSEH) JGSE extract 100mg/kg body weight orally, Group IX (JGSEL) JGSE extract 50mg/kg body weight orally.

Behavioral Assessment

Paw Volume

The paw (hind paw) volume is measured before and the after FCA injection. Paw volume is measured by using the plethysmometer (Choudhary *et al.*, 2014).

Joint Diameter

The measurement of the joint diameter is done by using the screw gauge micrometer 0, 7^{th}, 14^{th}, and 20^{th} days (Gautam *et al.*, 2018).

Body Weight

The overall change in the body weight is measured at every week (0, 7^{th}, 14^{th}, 20^{th} day) of the study (Ekambaram *et al.*, 2010).

Biochemical Analysis

On the 20^{th} day of study by using the anaesthesia the rats is anaesthetized and blood is collect by retro-orbital puncture and the sample is used for the evaluation of the parameters likes, Serum glutamate pyruvate transaminase (SGOT) and serum glutamate oxaloacetate transaminase (SGPT) (Battiwala *et al.*, 2013).

Hematological Parameters

Various parameters (Hemtological parameters) like Hb, RBC, WBC, and ESR are evaluated at the end of the study (Jalalpure *et al.*, 2011).

Statistical Analysis

The experimental results were shown as a Mean ± Sem. Where applicable, data were shown to one way analysis of variance (ANOVA) followed by dunnett's test. Data were considered statistically significant P < 0.05.

Standardization Parameters

Foreign matter Determination

Take total 500 g of drug (crude) material and spread out into thin layer. Inspection of the crude drug to be done by using the 6X lens and remove the foreign material as much as possible. Weight the crude drug and determine the total % of foreign organic material.

Determination of Moisture content

Take 2gm of crude drug sample into the petric disc and then heat this into hot air oven (105^{o}C) for 1 hour. Take out petric disc from the oven and allowed to cool down. Calculate the total moisture by using this formula,

% Moisture =

$$\frac{(Wt\ of\ petri\ dish\ +\ Wt\ of\ Sample\ -\ Dried\ wt)}{Wt\ of\ Sample} \times 100$$

Extractive value Determination

5gm of fresh dried powdered drug macerate in 100ml of (Ethanol, water, chloroform, pet. ether) in a close flask and leave for 24hr. Shake the flask starting 6hr and then leave for 18hr. Filter the whole mixture. 25ml of filtrate evaporate up to dryness at 105^{o}C. Calculate the total % of obtained extract by using the formula,

$$\frac{(wt\ after\ drying\ -wt\ of\ petridish)}{wt\ of\ sample\ \times\ vol.\ of\ filtrate} \times 100$$

Ash value Determination

Take 2gm of crude drug in pre-weigh silica disc and burn this at temperature (not exceeding, more than) 450^{o}C for 5hr. Calculate the total ash by using the formula,

$$\frac{(wt\ after\ drying\ -wt\ of\ crucible)}{wt\ of\ sample} \times 100$$

Acid Insoluble Ash value

Soluble total ash in 25ml of 2M HCL and then filter this by using the ash less filter paper. Wash filter paper till filtrate become neutral. Burn this for 15min at 450^o C and allow to-cool in a desiccator. Calculate the acid insoluble ash by using the formula,

$$\frac{(wt\ after\ drying\ -wt\ of\ crucible)}{wt\ of\ sample} \times 100$$

Carbohydrate Detection

Fehling' Test

Boil 1ml of each Fehling's solution (A &B) or agent, add 2 ml of extracts than heat for 10 min over boiling water bath and the yellow and then brick red precipitate shows availability of reducing sugars.

Test for Amino acids and Proteins

Ninhydrin Test

Take 3 ml test extracts individually, and add 3-4 drops of (5% Ninhydrin solution) in the test extract. Whole mixtures are heated on water bath for around 8-10 mins, then the purple or bluish color obtained.

Test for Phytosterols

Salkowshi's Test

Take 1 ml of plant test extract in chloroform add 2-3 drops of sulfuric acid (concentrated). Formation of brown ring shows availability of phytosterols.

Test for Glycosides

Baljet's Test

Take few drops of plant test extract and add sodium picrate, so that, the yellow to orange color observed.

Test for Saponins

Foam Test

Few drops of plant extract are shaken vigorously in water and measure foam intensity.

Test for Flavonoids

Ferric Chloride Test

Plant test solution shows intense green color with ferric chloride solution.

Tannins & Phenolic Compounds Test

Nitric acid test

Take an extract of 2–3 ml and add a few drops Dilute HNO3 shows reddish to yellow color.

Alkaloids Test

Mayer's Test

Take 1 ml Mayer reagent (potassium mercuric iodide solution) in an extract (test) of 1 ml, and Whitish-yellow or precipitate cream shows presence of alkaloids.

Dragendorff's Test

Take 1 ml of plant sample extract with 1 ml of the reagent Dragendorff (potassium bismuth iodide solution). Orange or red precipitate is a symbol of the presence of alkaloids.

Wagner's Test

1 ml Wagner's reagent with 1 ml extract (iodine in potassium iodide solution) mixtures gives reddish brown precipitate which shows alkaloids presence (Shalavadi and Muchchandi, 2018; Kaushik *et al.*, 2016; Kumar, 2013).

RESULTS AND DISCUSSION

The effect of extract JGLE, JGLC, JGSE on HRBC membrane stabilization has been shown in Figures 1, 2, 3, 4, 5 and 6 and the extract JGLE, JGLC, JGSE shows the maximum effect at 2000μg/ml. Protein denaturation Inhibition effect is shown in the Figures 7, 8 and 9 and it shows the maximum % inhibition effect of the extract JGLE, JGLC, JGSE at 2000μg/ml. Effect

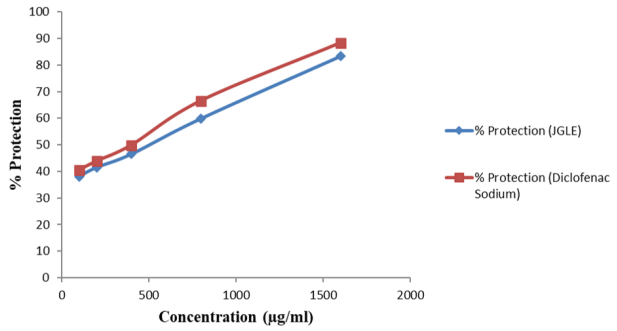

Figure 1: The % protection effect of standard, (Diclofenac sodium), JGLE by HRBC membrane stabilization method

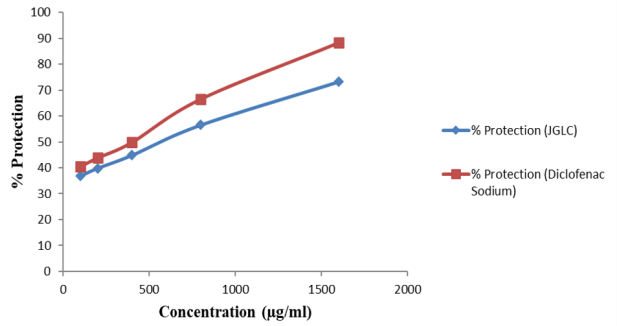

Figure 2: The % protection effect of standard, (Diclofenac sodium), JGLC by HRBC membrane stabilization method

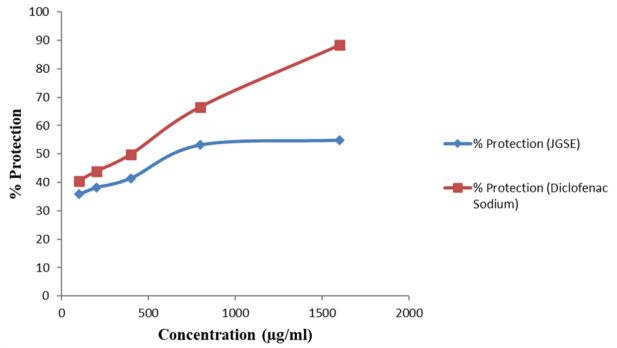

Figure 3: The % protection effect of standard, (Diclofenacsodium), JGSE by HRBC membrane stabilization method

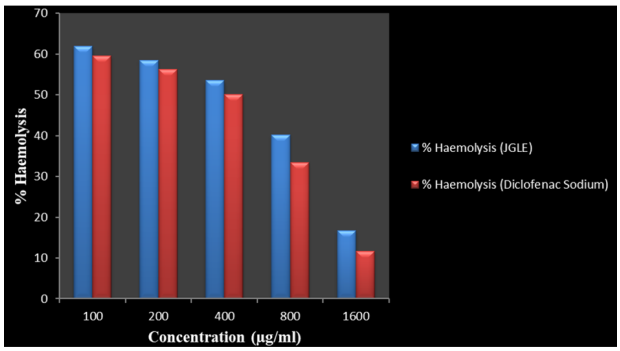

Figure 4: The % hemolysis effect of standard, (Diclofenac sodium), JGLE by HRBC membrane stabilization method

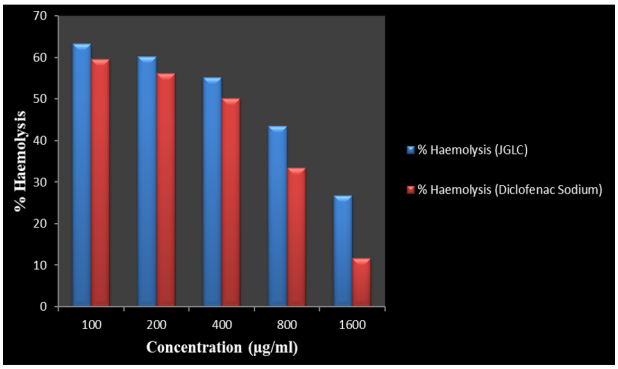

Figure 5: Bar diagram representing the % hemolysis effect of standard, (Diclofenacsodium), JGLC by HRBC membrane stabilization method

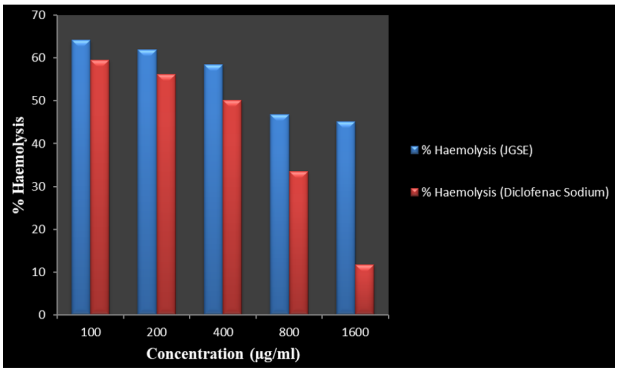

Figure 6: The % hemolysis effect of standard, (Diclofenacsodium), JGSE by HRBC membrane stabilization method

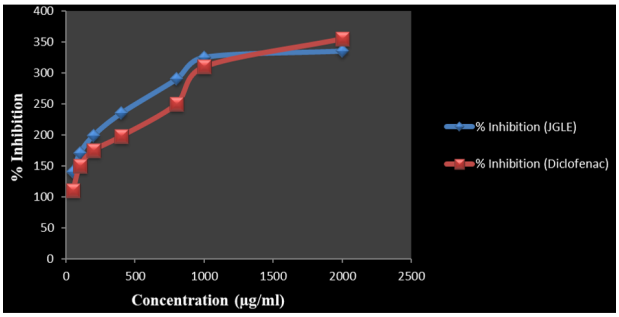

Figure 7: The % Inhibition effect of standard (Diclofenac sodium), JGLE in Protein Denaturation Inhibition method

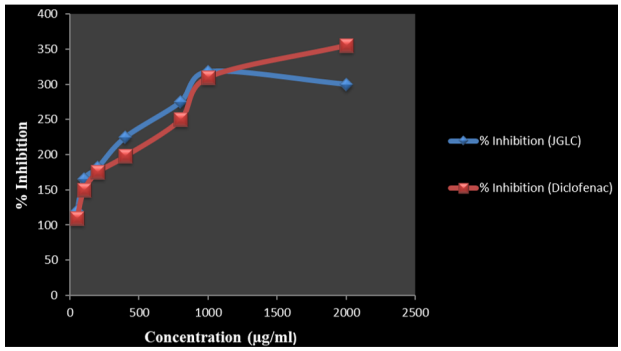

Figure 8: The% Inhibition effect of standard (Diclofenacsodium), JGLC in Protein Denaturation Inhibition method

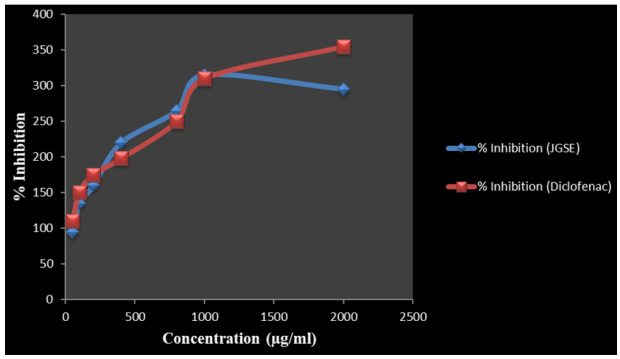

Figure 9: The% Inhibition effect of standard (Diclofenacsodium), JGSE in Protein Denaturation Inhibition method

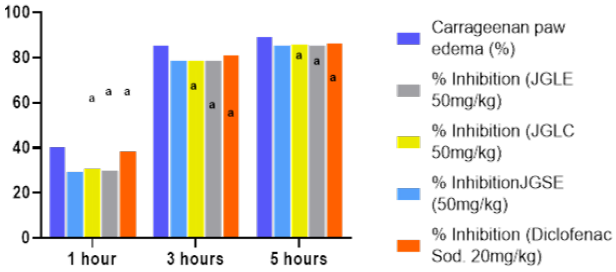

Figure 10: The total % Inhibition of standard, (Diclofenacsodium), JGLE, JGLG, JGSE on Carrageenan induced paw edema model at 1hr, 3hr,and 5hr time interval

of extract JGLE, JGLC, JGSE on carrageenan induced paw edema model at 1hr, 3hr, and 5hr time interval shown in the Figure 10. The effect of the extract is time dependent and the effect of the extract JGLE, JGLC, LGSE on body weight change. Values were expressed Mean± SEM $^{a}P < 0.05$ significant as compared to normal control group, $^{b}P < 0.05$ significant as compared to positive control, $^{c}P < 0.05$ significant as compared to positive control at 50mg/kg is much higher than the standard, diclofenac sodium at 20mg/kg. Figure 11, body weight change, Fig-

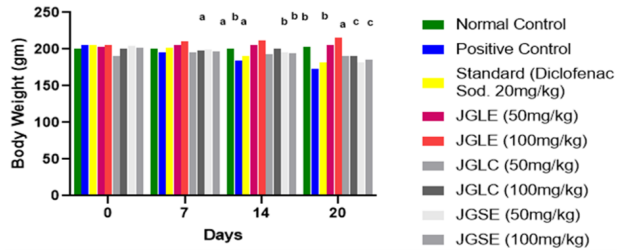

Figure 11: The effect of standard (Diclofenac sodium),JGLE, JGLC, LGSE on body weight change

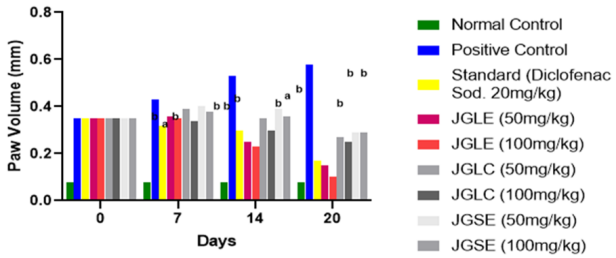

Figure 12: The effect of standard (Diclofenac sodium),JGLE, JGLC, LGSE on change in paw volume

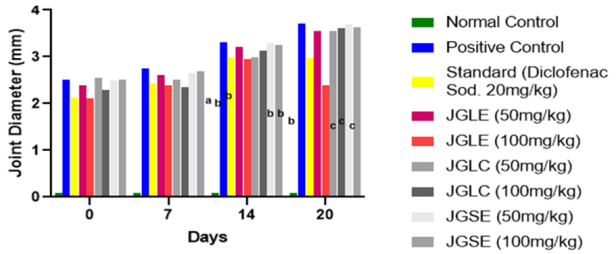

Figure 13: The effect of standard (Diclofenac sodium),JGLE, JGLC, LGSE on change in joint diameter

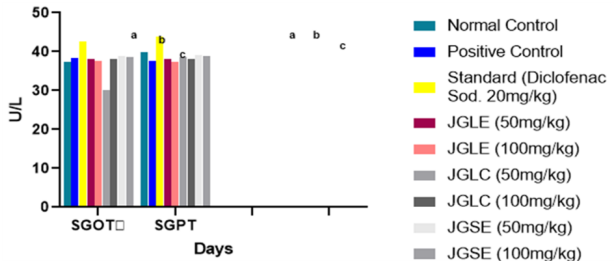

Figure 14: The effect of standard (Diclofenac sodium),JGLE, JGLC, LGSE on change in biochemical parameters

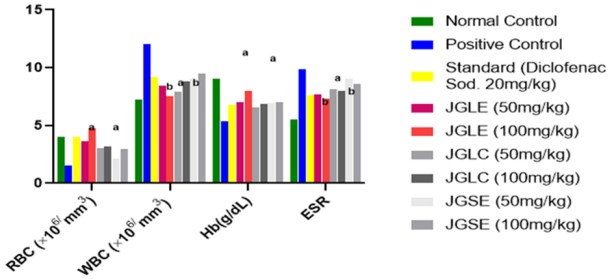

Figure 15: The effect of standard (Diclofenac sodium),JGLE, JGLC, LGSE on change in Hematological parameters

Table 1: Standardization Parameters

S. No.	Parameters	Value Obtained (Justicia gendarussa)	
		Leaf	Stem
1.	Foreign Matter	0.06%	0.09%
2.	Moisture Content	5.80%	6.88%
3.	Extractive Value (Methanol)	72%	50%
4.	Extractive Value (Ethanol)	24%	23.06%
5.	Extractive Value (Chloroform)	32	28.04%
6.	Extractive Value (Petroleum Ether)	24%	20.22%
7.	Extractive Value (Water)	70%	53%
8.	Total Ash Value	1.69%	4.60%
9.	Acid Insoluble Ash Value	0.65%	1.50%

Table 2: Phytochemical Screening

S. No.	Phytochemicals	Present (+)/ Absent (-)	
		Leaf	Stem
1.	Carbohydrates	+	+
2.	Proteins & amino acids	+	+
3.	Fixed oil & Fats	-	-
4.	Phytosterols	+	-
5.	Glycosides	+	+
6.	Saponins	++	+
7.	Flavonoids	+	-
8.	Tannins & Phenolic compounds	+	+
9.	Alkaloids	+	+

ure 12 shows that, JGLE, JGLC, LGSE on change in paw volume. Values were expressed Mean± SEM $^{a}P < 0.05$ significant as compared to normal control group, $^{b}P < 0.05$ significant as compared to positive control, $^{c}P < 0.05$ significant as compared to positive control paw volume, Figure 13 Shows that, JGLE, JGLC, LGSE on change in joint diameter. Values were expressed Mean± SEM $^{a}P < 0.05$ significant as compared to normal control group, $^{b}P < 0.05$ significant as compared to positive control, $^{c}P < 0.05$ significant as compared to positive control joint diameter, Figure 14 Shows that, JGLE, JGLC, LGSE on change in biochemical parameters. Values were

expressed Mean ± SEM biochemical parameters, Figure 15 shows that, JGLE, JGLC, LGSE on change in Hematological parameters. Values were expressed Mean± SEM $^{a}P < 0.05$ significant as compared to normal control group, $^{b}P < 0.05$ significant as compared to positive control, $^{c}P < 0.05$ significant as compared to positive control hematological parameters also summarized the effect of extract JGLE, JGLC, JGSE on FCA Induced arthritis model at 50mg/kg and 100mg/kg. Tables 1 and 2 shows the standardization and phytochemical screening parameters respectively.

CONCLUSIONS

The current work investigated the anti-inflammatory work of various extract *Justicia gendarussa* by using the in-vitro and in-vivo models and the extract JGLE, JGLC, and JGSE is used to evaluate for their anti-inflammatory activity. In the anti-inflammatory (in-vitro) study there are 2 models (Inhibition of protein denaturation and Human red blood cell membrane stabilization method) are used and the effect of these extracts for these in vitro-study were measured or evaluated at concentration (50, 100, 200, 400, 800, 1000, 2000 μg/ml) and (50, 100, 200 400, 800, 1600 μg/ml) respectively. Effect of the test extract increases with the concentration and the extract JGLE to be found most effective at the concentration of 2000μg/ml in both the models and the results of the extracts was compare with the reference drug, Diclofenac sodium, at the same concentration. Carrageenan paw edema model is used as in-vivo method for the evaluation the anti-inflammatory potential of the test extract JGLE, JGLC, and JGSE which is obtained from the aerial part of *Justicia gendarussa* plant. The plant extract (JGLE, JGLC and JGSE) concentration 50mg/kg/b.w.p.o is used and the result is compare with the reference compound Diclofenac sodium at 20mg/kg/b.w.p.o. The extract JGLE has been shown more paw edema inhibition than the other extracts. The anti-arthritis ability of the plant *Justicia gendarussa* extract as JGLE, JGLC and JGSE was evaluated using the arthritis model induced by FCA in Wistar rats. To evaluate the anti-arthritic effect of test extract various parameters like, Hematological parameters (Hb, WBC, RBC, ESR), biochemical parameters, body weight, paw volume, and paw thickness (joint diameter) are used. The concentration at which the test extract exert its anti-arthritic potential is 50mg/kg/b.w.p.o and 100mg/kg/b.w.p.o is used and the whole test extract data is compared with the reference drug Diclofenac sodium at 20mg/kg/b.w.p.o is used. The ethanolic extract of the *Justicia gendarussa* shows the much better improvement in the parameters than other extract. Saponins (Apigenin) are well known for their anti-inflammatory activity, which effectively reduce oxidative stress and express multiple factors that potentiate inflammation and also play a crucial role in reducing these factors, they are responsible for the inflammation and other disease related complications. A flavonoid (vitexin) is also present which is potent anti-inflammatory agents vitexin successfully encounters the inflammatory activity. They inhibit 5-lipoxygenase pathway, which increase the synthesis of inflammatory mediators along with the COX-2 pathway. This mechanism strongly supports the carrageenan induced inflammation. By successfully inhibiting the pathway of 5-lipoxygenase (5-LOX) and cyclooxygenase-2 (COX-2), extracts is successfully inhibited the synthesis of various inflammatory mediators like, prostaglandins (PGs), Interleukins (ILs), tumor necrosis factors (TNF-α), leukotrines (LTA_4, LTB_4, LTC_4, LTD_4, LTE_4), TXA_2 etc. Increase synthesis of these inflammatory mediators contributes process of inflammation and also makes the disease more complicated like arthritis.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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