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Pharmacological evaluation for anti-bacterial and anti-inflammatory potential of polymeric microparticles

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The objective of the study was to evaluate anti-bacterial and anti-Inflammatory activities of polymeric microparticles. *In vitro* antibacterial activity was performed for prepared microparticles followed by *in vivo* anti-inflammatory activity on rats. From the present study, it was observed that the microparticles developed were appropriate in their shape and uniform size. The topography of SEM studies revealed that, the microparticles were smooth-surfaced. The result of antibacterial activity indicated that the formulation has not exhibited any zone of inhibition against the various strains of bacteria used for this study. The result of anti-inflammatory activity (Dextran induced paw edema) exhibits that, the formulations possess the inhibitory potential for various inflammatory mediators thereby reduces the inflammation in paws. From the results, it can be concluded that *Acacia arabica* microparticles is showing significant Anti-inflammatory activity after 6 h than conventional dosage form.

Keywords: *Acacia arabica*, Dextran, Ethyl cellulose, Microparticle, Paw edema

Microparticles are the controlled drug delivery system to accomplish oral and parenteral delivery of drugs. The solvent evaporation method has gained much attention for the development of microparticles due to ease of fabrication without losing drugs activity. Medicinal plants possess several bioactive compounds responsible for biological activity[1-7]. *Acacia arabica* is a versatile tree of *Fabaceae* family extensively dispersed in tropical and subtropical countries[8]. It is utilized by conventional healers in various areas for the treatment of asthma, inflammation, cough, flu[9]. Inflammation occurs due to pathogen attack and in response to other harmful stimuli[10]. It leads to the induction of severe chemical reactions in the body due to the release of inflammatory mediators[11]. Anti-inflammatory drugs are used to relieve from inflammatory reactions, however, search for safe and effective anti-inflammatory drugs is still going on because of ulceration and bleeding consequences of existing drugs. Hence herbal remedies came into continuation as a substitute therapy to overcome the disadvantages of such drugs. However, herbal drugs must be standardized for its effective use and to avoid any toxicity[12-17]. Among the natural herbal drugs, *Acacia arabica* is used by numerous researchers for effectual treatment[18]. In the recent research work, an effort was initiated to formulate, characterize, and evaluate the improved therapeutic influence of *Acacia arabica* incorporated into microparticles[19]. It is expected that this piece of research will pave the way for further drug development & provide benefits to society.

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Materials and Methods

Drug and chemical reagents

Acacia arabica herbal extract was received as a gift from SUNPURE Pvt. Ltd New Delhi (India). Acetone and liquid paraffin were purchased from Merck Ltd Mumbai(India), n-hexane was procured from Triveni Chemicals, Imran Nagar, Vapi, (Dist.Valsad), Picric Acid was purchased from Fizmerk India Chemical, Uttar Pradesh (India), Halothane was procured from Korten Pharmaceuticals Pvt. Ltd., Maharashtra, (India), Mercury was purchased from Antares chem. Pvt. Ltd., Ghatkopar East, Mumbai, Maharashtra

(India), HCL was purchased from Paradise acid and chemicals, Thane Mumbai, (India), Ethylcellulose was purchased from EC, S.D. Fine Chemicals, Mumbai, (India), Dextran was procured from Himedia Pvt. Ltd. Thane, Mumbai, (India), Petroleum ether was purchased from Avi Chem. Industries, Thane West Mumbai, (India). All other chemicals and deionized water used were of analytical grade.

Experimental animals

Healthy adult male Albino Wistar rats weighing 180-200 g were obtained from the institute having certificate number CIP/IAEC/2017/103. The animals were acclimatized with laboratory conditions before the commencement of the experiment. Animals were maintained on a pelleted diet and water *ad libitum*.

Antibacterial activity using the agar diffusion method

The bacterial culture was maintained at 37°C and allowed to inoculate in broth media for 18 h. The media was prepared in agar plates followed by making wells on agar plates. The wells were inoculated with cultures (100 µL, 10-4 cfu), it was spread evenly on plates. The wells were then filled with test drugs after 20 min and incubated at 37°C for 24 h. The zone of inhibition was determined and *Staphylococcus aureus*, *Escherichia Coli*, MRSA bacteria were analyzed[5].

Anti-inflammatory activity of microparticles using dextran induced paw edema rat model

Albino Wistar rats weighing 80-200 g were selected for the study. Animals were deprived of food for 24 h before starting the experiment. The animals were divided into four groups and there were six animals in each group. Control group distilled water, ethylcellulose micro particles containing *Acacia Arabica* was mixed with 1% CMC solution Test-1 (formulation treated low concentration) group, Test-2 (formulation treated higher concentration) group and marketed preparation treated group were administered orally in rats. After administration of doses, inflammation in rats was induced by 1.5% of Dextran injected into sub plantar region of left hind paws of rats and edema volume was measured by the help of Plethysmometer and examined the difference between the paw volume in mL, before and 0 h, 30 min, 1 h, 2 h, 4 h and 6 h, respectively after the administration of dextran. Reduction in edema after treatment was determined using the following formula:

$$\text{Percentage reduction} = \frac{\text{Vo - Vt}}{\text{Vo}} \times 100$$

where, 0 V = volume of the paw of control at a time '*t*', Vt = volume of the paw of the test at a time '*t*'. Mean value was determined for every group and statistical analysis was performed between the control and the treated groups the results were subjected to statistical analysis ANOVA followed by Dunnet's '*t*' test[6].

Statistical analysis

Data are represented as mean ± SEM. Statistical analysis was performed using ANOVA by Graph pad prim software, significantly different at $P< 0.05$ in comparison to control group.

Result

***In vitro* antibacterial activity**

The results were shown in (Table 1) as the diameter of inhibition Zones in mm. In the anti-bacterial analysis the sample has not shown any zone of inhibition against *S. aureus* (AAMP) shown in (Fig. 1A) and *E. coli* and standard antibiotic ciprofloxacin were used to show (Table 2) NF-MIC

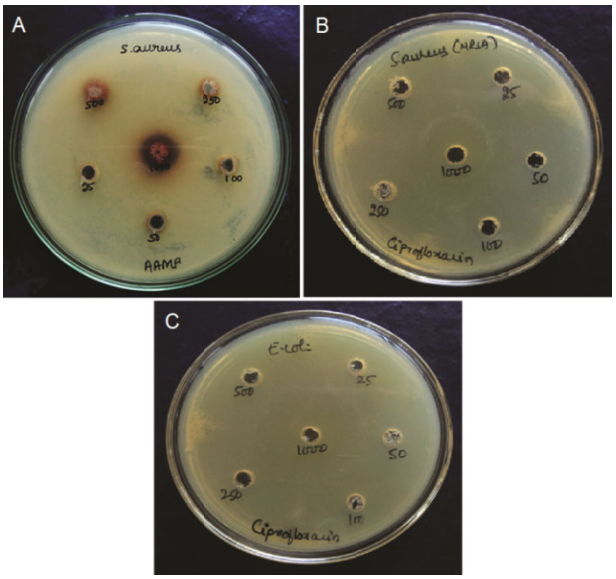

Fig. 1 — Result of anti-bacterial analysis and zone of inhibition against (A) *S. aureus* (AAMP); (B) *S. aureus*; and (C) *E. coli*

Table 1 — Anti-bacterial analysis (*S. aureus*) of prepared microparticles

Sample	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
AAMP	0	0	0	3	8	500

Table 2 — Standard antibiotics (Ciprofloxacin)

Organism	25 µg	50µg	100µg	200µg	400µg	800µg	MIC µg
E. coli	18	20	23	26	28	31	25
S. aureus	13	18	21	25	27	34	25
MRSA	7	15	20	24	25	27	25

are also shown in (Fig. 1B) was not found. Zones could not be measured due to margining. *S. aureus* (MRSA), standard antibiotic ciprofloxacin was used and also shown in (Fig. 1C) the sample has not shown any zone of inhibition.

Anti-inflammatory activity of prepared microparticles

Acacia Arabica microparticles containing 1:1 of ethyl cellulose were screened for the anti-inflammatory activity in rats. Figure 2 shows the measurement of paw edema using of plethysmograph apparatus. Anti-inflammatory activity of any compound is its ability to reduce local edema produced by the toxic irritant agents. One experiment at six different time intervals was carried out for these activities (*i.e.* 0 h, 30 min., 1 h, 2 h, 4 h, and 6 h) (Figs. 3-6). Inhibitions of paw edema were calculated using formula[7]. After inducing of Dextran in animals of control group inflammation was formed in paw (Fig. 7). Percentage inhibition of Dextran induced inflammation by the control group was calculated (Table 3).

Fig. 2 — Measurement of paw edema in plethysmograph apparatus

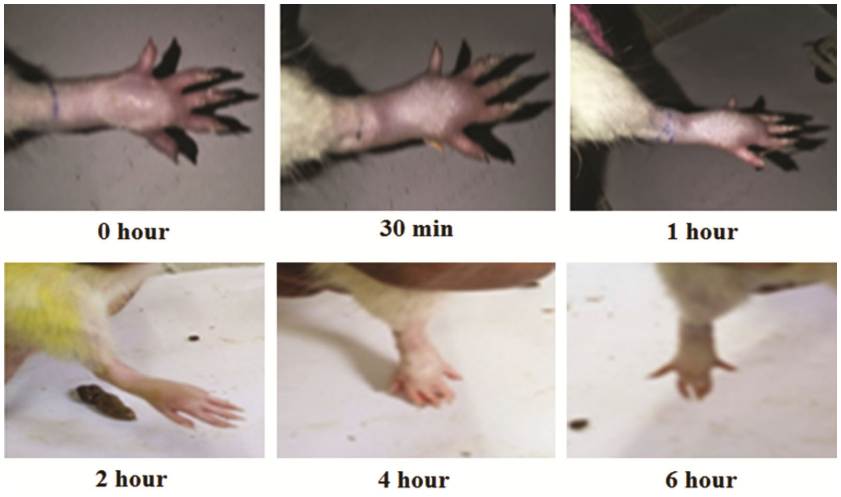

Fig 3 — Photographs of paw edema after distilled water administration in control groups of animal rats

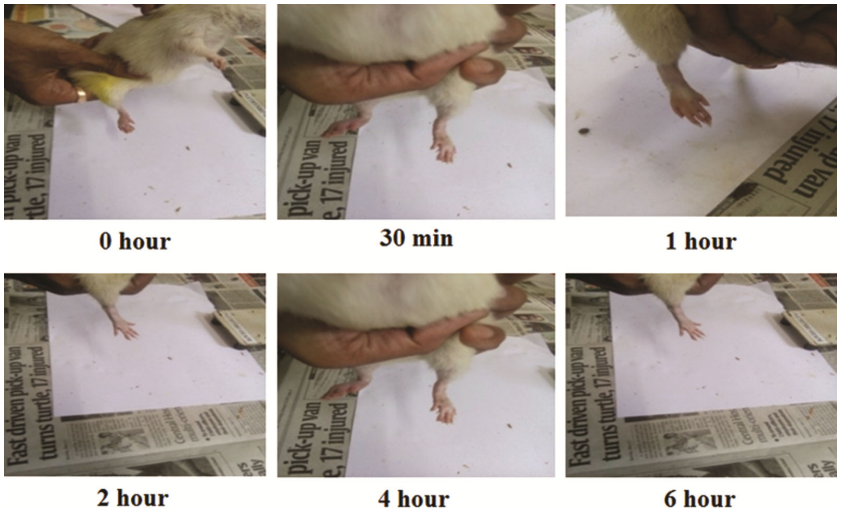

Fig 4 — Photographs of paw edema after formulated Microparticles 1:1 administration in Test-1 groups of animal rats

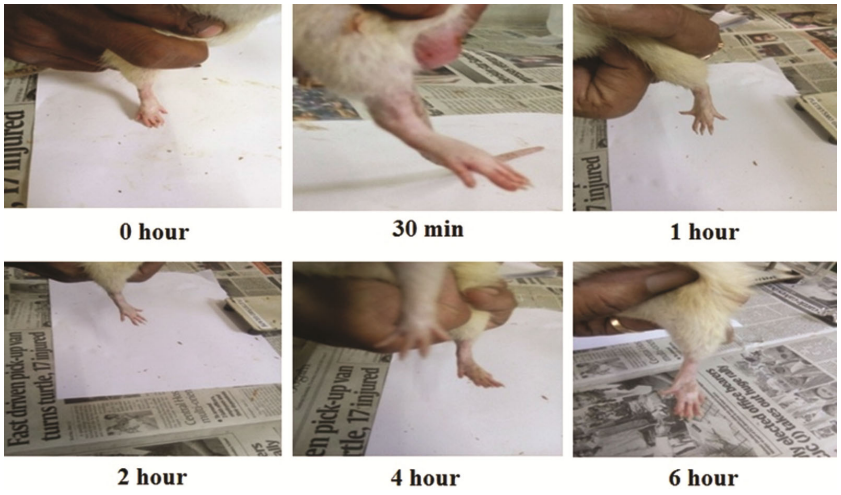

Fig 5 — Photographs of paw edema after formulated Microparticles 1:2 administrations in Test-2 groups of animal rats

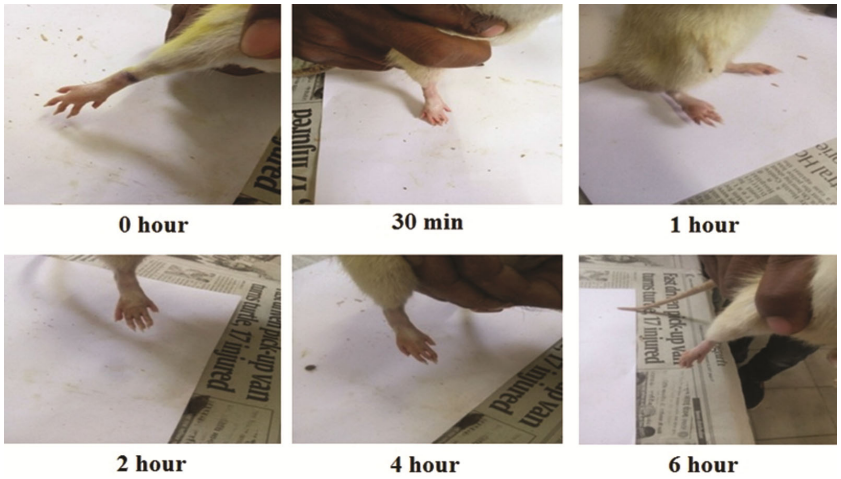

Fig 6 — Photographs of paw edema after standard drug (Ibuprofen) administration in standard groups of animal rats

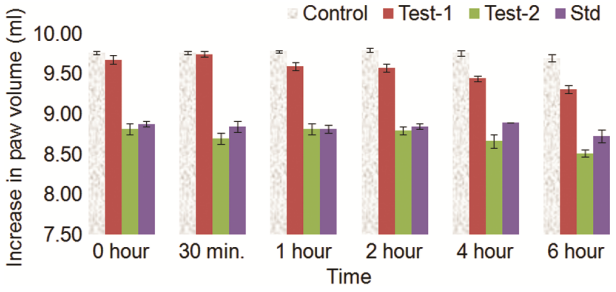

Fig 7 — Graphical representation of Dextran induced paw edema in different group of animals

Discussion

Inflammation is the defensive mechanisms of the body against harmful stimuli like irritants, microbes, and damaged cells[20]. During vascular injury the cell components get exposed that leads to blood coagulation, haemostasis and defense mechanism[21]. Treatment of inflammation is done with many classes of drugs include analgesics. Salicylates, propionic acid derivative, acetic acid derivative, preferential COX-2 inhibitors, selective COX-2 inhibitors. Natural herbal drugs have been used for the treatment of several bacterial infections since ancient times[22-26]. Antimicrobial agents derived from plant sources have been gaining attention to present researchers[27]. The microparticles containing herbal extract of *Acacia arabica* was used as the core material. The microparticles developed were appropriate in their shape and a uniform size ranging between the size ranges of 500-800 µM were measured by optical microscopy. The result of SEM studies revealed that the micro particles were smooth-surfaced and uniform size range. *In vitro* studies of microparticles of antibacterial analysis results indicated that the sample has not shown any zone of inhibition against *S. aureus* (AAMP), from

Table 3 — Results of anti-inflammatory activity of different *Acacia arabica* formulations in Dextran induced paw edema rat model

S. No	Time period	Control (mL)	Test-1 (1:1) (mL)	Test-2 (1:2) (mL)	Standard (mL)
1.	0 h	9.76±0.021	9.68±0.054**	8.81±0.070**	8.88±0.030**
2.	30 min	9.76±0.021	9.75±0.034**	8.7±0.068**	8.85±0.067**
3.	1 h	9.78±0.016	9.6±0.044**	8.81±0.070**	8.81±0.047**
4.	2 h	9.8±0.025	9.58±0.047**	8.8±0.051**	8.85±0.035**
5.	4 h	9.76±0.033	9.45±0.034**	8.66±0.080**	8.9±0.00**
6.	6 h	9.7±0.044	9.31±0.047**	8.51±0.047**	8.73±0.080**

Data are represented as mean ± SEM

in vivo Dextran induced rat paw edema model, percent edema inhibition (PEI) was determined and *Acacia arabica* microparticles containing 1:1, 1:2 ethyl cellulose was evaluated for paw edema in rats. Researchers have also prepared nanoparticles by using bacteria for better results[28]. Inflammation is effectively produced in rat's paw by induction of Dextran for 0-6 h. It was observed that 0-6 h after the induction inflammations were found in the left hind paw. This indicated that inflammation produced at this time[29-30]. Hence, administration of *Acacia arabica* containing microparticles inhibited the inflammatory mediators such as: (Prostaglandin, leukotriene, histamine, and bradykinin) and prevent the paw edema thus it shows anti-inflammatory activity.

Conclusion

From the results, it can be concluded that, *Acacia Arabica* microparticles were effective in reducing inflammation and may be used as an alternative drug in the treatment of inflammation caused by toxic agents.

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Conflict of interest

All authors declare no conflict of interest.

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