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Evaluation of Acute and Chronic Anti-inflammatory Effects and Anti-nociceptive Effect of Novel Preparation Sudarshana Suspension

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Abstract. Sudarshana Suspension (SS) is a novel Ayurveda preparation. The main aim of this study was to evaluate the acute and chronic anti-inflammatory and anti-nociceptive potential of Sudarshana suspension in Wistar rats. The acute anti-inflammatory potential of the Sudarshana suspension was evaluated using the carrageenan-induced paw oedema method and the chronic anti-inflammatory activity of SS was tested using the adjuvant-induced arthritis rats' model. Anti-nociceptive activity was tested using the model of acetic acid-induced writhing in rats. SS significantly inhibited carrageenan-induced paw oedema, comparable with the known anti-inflammatory drug, Indomethacin. The maximum inhibitions of test drugs, SS were 88.5%, at the 5th hour following drug administration, whereas indomethacin produced 96.2 % inhibition at the same hour. Induction of arthritis significantly increased footpad thickness (FPT), hind paw ankle joint thickness (AJT), and loss of Body Weight (BW). Treatment with SS and standard drug Celecoxib in the arthritic animals produced significant reductions ($p < 0.001$) in AJT, and FPT, reducing erythema and oedema in the ankle joints and footpad of the adjuvant-induced arthritis rats and normalized BW. When compared to the negative control by acetic acid-induced writhing in rats, the SS showed statistically significant ($p< 0.05$) inhibition of writhing (34.15%) while the standard drug diclofenac showed 40.98% inhibition of writhing in experimental animals. Sudarshana Suspension possesses statistically significant acute and chronic anti-inflammatory effects comparable to Indomethacin and Celecoxib. Moreover, it possesses statistically significant anti-nociceptive effects that are closely similar to diclofenac sodium.

Keywords: Acute and Chronic Anti-inflammatory effect**,** anti-nociceptive effect, Wistar Rats, Sudarshana Suspension

Introduction

Sudarshana powder (SP) is the most effective and available anti-pyretic Ayurveda preparation, widely used in Sri Lanka as well as India from the very early beginning of Ayurveda treatment. It is recommended for all types of fever including bone-associated fever and common cold (Anonymous, 2003). Further, it is used traditionally as an antimalarial, antipyretic, and antiviral formulation. Sudarshana Powder contains 53 bitter ingredients which can treat fever, cough, asthmatic conditions, anorexia, anaemia, heart disorders, and pain associated with arthritis (Shri Sharangadara Samhita, 2001).

According to the Ayurveda concepts, Sudarshana powder has been used with bees' honey to mask its bitter taste, but there is no ready-to-use product with the correct amount of bees'

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honey in current dosage forms. Therefore, this powder was developed into user-friendly ready-to-use standard Sudarshana suspension (SS) using bees honey (Weerakoon, et al., 2020). In vitro and in vivo antioxidant potentials of SP (Weerakoon, et al., 2018). and Acute and Subchronic oral safety profiles of the Sudarshana Suspension (Weerakoon, et al., 2020) were established in our recent experimental studies. In the present study, the acute and chronic anti-inflammatory effects and anti-nociceptive effects of the Sudarshana suspension (SS) were evaluated in Wistar rats.

Materials and Methods

Sudarshana Power and Sudarshana Suspension

Hot water extract of SP and Prepared Sudarshana suspension was directly used for the study.

Animals

Healthy, unused male Wistar rats (200-250 g) were purchased from the Medical Research Institute (MRI), Colombo, Sri Lanka. The animals were kept in plastic cages (two per cage) under standardized animal house conditions (temperature, 28–31^{0}C; photoperiod, approximately 12 h natural light “per day” relative humidity, 50–55%) at the animal house, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. A period of one week was given for acclimatization to animal house conditions before the commencement of the experiments.

Ethical Approval for Animal Studies

Ethical clearance was obtained for the animal studies from the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. all the rats' studies were carried out under the recommendation of the guidelines for the care and use of laboratory animals.

Dosage of the Drug to Animal Models

The drug was administered orally to the test rats via Sondi needles and oral tubes. These doses corresponded to the normal therapeutic dose administered to adult humans as calculated, based on the relative surface areas of humans and each animal. The dosage was calculated based on its weight and then it was multiplied by 6 [(conversion factor km)

Acute Anti-Inflammatory Effect

The Anti-inflammatory activity of SP and SS was determined using the carrageenan-induced paw oedema model (Winter, et al., 1962).

Healthy, unused twenty-four male Wistar rats were selected and the volume of the left hind paw of each rat was measured using a plethysmometer (V0) (Panlab s.I., Barcelona, Spain). Rats were randomly assigned to 4 groups of rats, six in each. Group I was treated with distilled water, as the negative control, Group II (positive control) was treated orally with the reference drug indomethacin (10 mg/kg), Group III received SP (0.5g/kg) and Group IV received SS (4mLkg). One hour after administration of the drug, 0.1 ml of 1% w/v carrageenan (Himedia, India) suspension in Normal Sterile Saline (NSS) was injected subcutaneously into the plantar surface of the left hind paw of rats under mild ether anaesthesia. The left hind paw volumes were measured using a digital plethysmometer at hourly intervals up to the 5th hour, following administration; the degree of swelling was calculated by the paw volume increase (V_t–V_0) where V_t and V_0 are the volume of the left hind paw after and before the carrageenan

injection, respectively. The percent inhibition of inflammation at each hour compared to the controls was calculated for each group (Bhadoriya, et al., 2012) as follows,

$$\% \text{ Inhibition} = \frac{(V_t - V_0)_{\text{control rats}} - (V_t - V_0)_{\text{treated rats}}}{(V_t - V_0)_{\text{control rats}}} \times 100$$

Chronic Anti-Inflammatory Effect

Chronic anti-inflammatory activity of SS was analyzed using the adjuvant-induced arthritis (AIA) rats' model. Arthritis was induced by a single intra-dermal injection of 0.1 ml of Freund's Complete Adjuvant (FCA) containing 0.05% w/v Mycobacterium butyricum suspension in sterile paraffin oil into a foot pad (Suke, et al., 2013) of the left hind paw of all rats with help of glass syringe and 26 G needles light diethyl ether anaesthesia. The healthy control group was injected with a single dose of 0.1 ml of NSS into a foot pad. The animals were randomly divided to five groups of 6 rats each.

There were five experimental groups. Group I was healthy animals as control, Group II was arthritic animals received distilled water, Group III was arthritic animals treated with a standard non-steroidal anti-inflammatory drug Celecoxib (5 mg/kg), Group IV was arthritic animals treated with SP (0.5g/kg) and Group V was arthritic animals received SS (4ml/kg).

From the 14th day onwards, each group was treated appropriately by intragastric administration once daily for the next 14 days. All the rats were orally treated for 14 consecutive days from the 14th day to till 28th day, after induction of arthritis. Body weight (BW) and hind paw ankle joint thickness (AJT), foot pad thickness (FPT) of all animals were measured using a dial caliper on Day 0 (before injection of FCA emulsion) and Day 3, 7, 10, 14, 17, 21, 24 and 28 after the injection of adjuvant. All animals were sacrificed on day 29th and blood was collected to analyze of Full Blood Count (FBC)

Anti-Nociceptive Effects

The anti-nociceptive activity of SP, SS and BH was determined using the acetic acid-induced writhing response (Bhadoriya, et al., 2012, Adzu, et al., 2003). Healthy unused male Wistar rats were randomly assigned into 4 groups (n=6 in each). Group I was served as a negative control with Distilled Water, Group II, positive control, served with reference drug diclofenac sodium (25 mg/kg), Group III received SP (0.5g/kg) and Group IV received SS (4 mL/kg). Abdominal muscle contractions were induced in rats by intraperitoneal injection of 0.6% solution of acetic acid (10 mL/kg) to all the groups after 30 minutes of the oral treatments. The number of writings occurring between 5 and 20 minutes after acetic acid injection was counted. A significant reduction in the number of abdominal contractions (treated animals) compared to the control group (given only distilled water) was considered as anti-nociceptive response. The calculation is done as follows.

$$\% \text{ Inhibition} = \frac{\text{Writhing of the control group} - \text{Writhing of test group}}{\text{Writhing of the control group}} \times 100$$

Statistical Analysis

Acute anti-inflammatory effect

The data were expressed as arithmetic mean ± standard error of the mean (S.E.M). The student's t-test was used for statistical comparison of data between groups. Statistical significance was determined at $p<0.05$.

Chronic anti-inflammatory effect

The data were expressed as arithmetic mean ± standard error of the mean (S.E.M). untreated Arthritis group was compared with healthy control animals and the treated arthritic

groups were compared with untreated arthritic animals. The significance level was determined using the student's t-test and considered extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Anti-nociceptive Effects

The result of the experiments was expressed as mean ± S.E.M. Statistical significance was determined by the Student's T-test. (Statistical significance was determined at $p<0.05$).

Results

Acute Anti-Inflammatory Effect

The results obtained are summarized in Table No.1. As shown, SS significantly inhibited carrageenan-induced paw oedema which was comparable with known anti-inflammatory drugs. The maximum inhibitions of test drugs, SS and SP were 88.5% and 84.6% respectively at the 5th hour following drug administration, whereas indomethacin produced 96.2 % inhibition at the same hour. With SS and SP, a statistically significant reduction ($p<0.05$) in oedema was noted at every hour when compared to the negative control group.

There was no statistically significant difference between the inhibition of SS and SP. Although an overall inhibition of rat paw oedema formation was observed in SS and SP at every hour, the most significant ($p < 0.001$) effect was noted in the 5th hour after carrageenan injection. With indomethacin (10 mg/kg), the maximum inhibition was 96.2 % noted at the 5th hour and statistically significant reductions in oedema formation were observed only at the 1st, 4^{th,} and 5th hours **(Table 1).**

Table 1: Percentage inhibition of paw oedema at different time intervals

Animal Group	h1	h2	h3	h4	h5
Indomethacin	42.65**	31.09	40.30	65.79*	96.15***
SP	37.84**	41.18**	43.28*	60.53*	84.62***
SS	39.18**	43.69*	52.23**	71.05**	88.46***

Note: Values carrying different superscripts are significantly different *$p< 0.05$, **$p<0.01$ and ***$p<0.001$ with respect to the controls. n = 6 per group.

Chronic Anti-Inflammatory Effect

Body weight of the rats

Following the FCA emulsion injection, there were no significant changes in the body weight as observed on days 0 and 7 in arthritic rats. The body weight of negative control rats was significantly ($p<0.001$) decreased compared with healthy control animals. However, the arthritic rats treated with standard drug celecoxib, SP, and the SS showed significant weight gain after day 14 ($p<0.001$), as compared to negative control animals. Animals treated with SS, SP, and celecoxib did not show significant weight loss after starting the treatment. After 14 days, the weight gain of rats was gradually increased in treated groups (Figure 1).

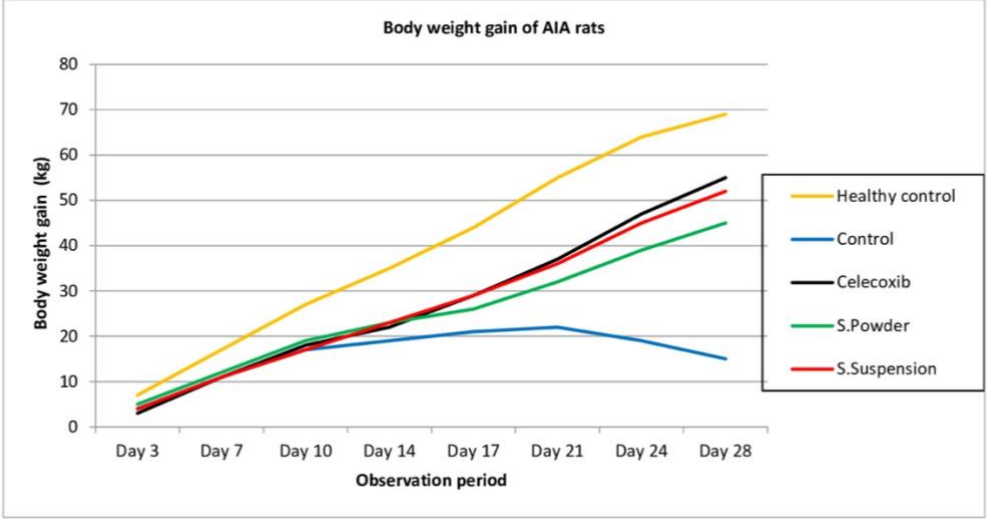

Figure 1: Effect of drugs on body weight gain of AIA rats

Effect on Ankle Joint Thickness (AJT)

At Day 0, no significant differences were found among the rats' AJT of all studied groups. A significantly enhanced in AJT was found in the FCA-injected group of animals on Day 3 (first swelling phase). Thereafter swelling slowly subsided until the seventh day and then began to increase again when disseminated arthritis appeared. After the initiation of drug administration on Day 14, significant reductions of AJT in AIA group of animals on Day 17, 21, 24 and 28 were observed as compared to the arthritic control rats. The test groups SS and SP exhibited extremely significant ($p < 0.001$) reductions of the AJT that was comparable with the celecoxib group on Day 17 and 21. Celecoxib and SS showed extremely significant ($p < 0.001$) reductions of AJT on Day 28 as compared to the arthritic control rats. (**Figure 2)**

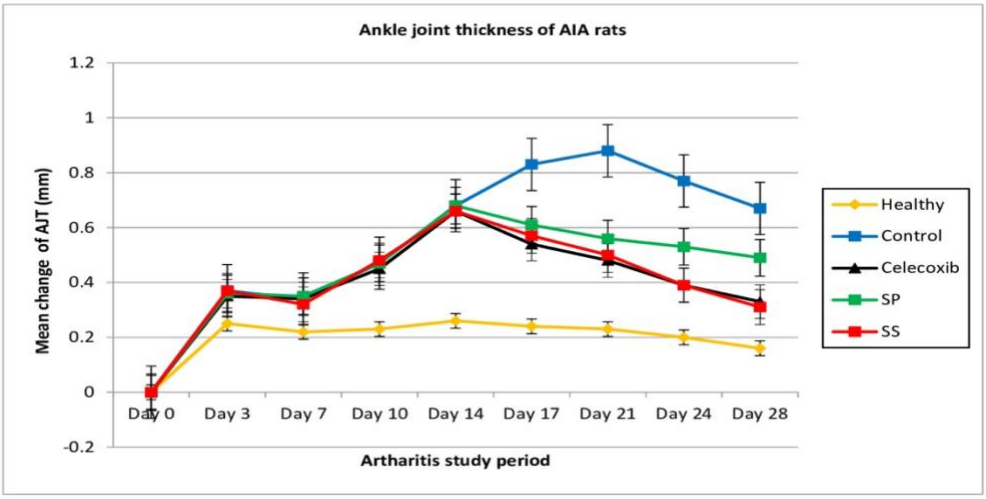

Figure 2: Effect of drugs on ankle joint thickness (AJT)

Effect on Foot Pad Thickness (FPT)

At Day 0, no significant differences were found among the rats' FPT of all the studied groups. A significant increase in FPT was observed for the adjuvant-injected group on Day 3. Swelling and redness developed over 24 hours in the hind paw injected with CFA and reached maximum intensity on day 3 (first swelling phase). Thereafter, swelling slowly subsided until

the seventh day, and then the FPT began to increase again when disseminated arthritis appeared (second swelling phase, which was greater than the first one and peaked on days 21–24. Footpad thickness of AIA rats was measured on Day 7, 10, 14, 17, 21, 24, and 28 as compared to the healthy control rats and showed statistically reduction ($p < 0.001$). There were no significant differences ($p > 0.05$) between AIA rats on Day 4, 7, and Day 14.

After day 14, treatments were started with the standard drug celecoxib, SS, SP, and BH. Significantly reduced of foot pad thicknesses were observed on Day 17, 21, 24, and 28. Extremely significant effects ($p < 0.001$) were observed in the celecoxib group on Days 21, 24, and 28; whereas in the SS-treated group, extremely significant reduction of FPT was shown on days 24 and 28 when compared with the control arthritis group. Significant differences in FPT were observed after the 14th day on SP as well. The reduction of foot pad thickness taken as a marker of disease recovery in AIA rats is shown in **(Figure 3).**

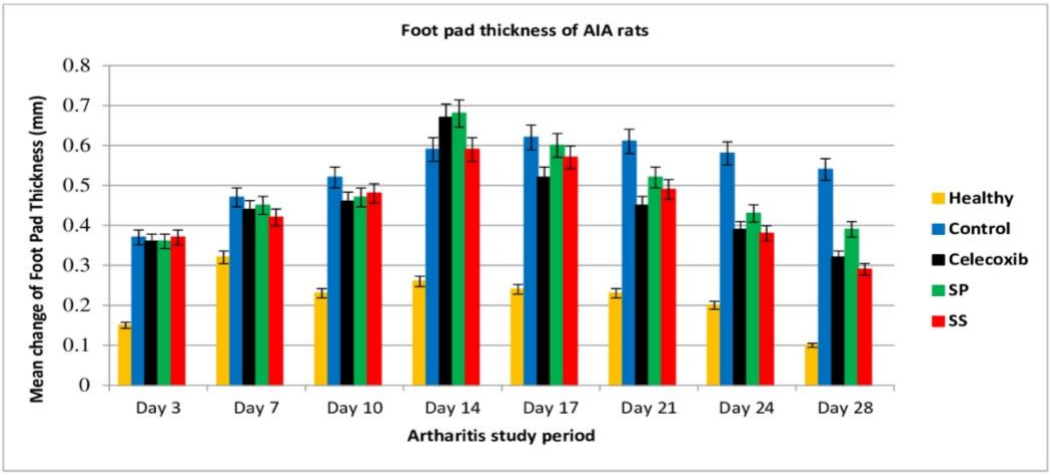

Figure 3: Effect of foot pad thickness in adjuvant-induced arthritis model

Effect of Drugs on Haematological Indices of AIA Rats

There was a significant ($p<0.01$) decrease observed in Hb and RBC concentrations in the AIA control group when compared to the healthy control rats. Moreover, WBC, neutrophils, and lymphocytes also showed significant differences when compared to the healthy control group.

In the AIA rats treated with celecoxib and SS, there was significant ($p < 0.001$) reduction in the WBC and lymphocytes and a significant ($p< 0.01$) increase in RBC **(**Table 2**).** In the group given celecoxib, there was a significant ($p<0.001$) increase in PLT when compared with the AIA control group. Sudarshana powder also showed significant differences similar to that of SS **(Table 2).**

Table 2: Effect of drugs treatment on haematological indices in arthritic rats

Blood parameters	Healthy	Control	Celecoxib	SP	SS
White blood cells (WBC) (109/L)	8.2 ± 0.2	18.2 ± 0.2	10.6±0.3***	13.8±0.4***	11.8± 0.2***
Neutrophil (%)	31.9 ± 3.6	13.1± 1.4	22.3±0.9***	19.3±2.4*	36.3 ± 3.6***
Lymphocytes (%)	55.8 ± 2.0	81.2 ± 1.3	71.1±0.8***	72.6 ±2.1*	57.6 ± 3.8***
Monocyte (%)	4.9 ± 1.7	5.8 ± 0.8	6,6 ± 0.6	8.0 ± 0.8	6.0 ± 0.6
Red blood cells (RBC), (109/L)	8.1 ± 0.5	6.3 ± 0.4	8.3 ± 0.5**	8.3 ± 0.4**	8.55 ± 0.4**

Hemoglobin (Hb) (g/dl)	14.9 ± 0.3	13.3 ± 0.3	14.2 ± 0.3	14.0 ± 0.1*	14.4 ± 0.2**
Packed cell volume (PCV) (%)	51.3 ± 2.3	31.7± 3.0	37.7 ± 1.8	40.9 ± 1.5*	43.1± 1.3*
Platelets (109/L)	836.6± 3.5	832.6 ±9.2	866.6±11.0*	822.6 ± 25.1	862.6 ± 11.0

Note: The data are expressed as mean ± SEM (n=6 per group). Symbols represent statistical significance: extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Anti-Nociceptive (Analgesic) Effect

The number of writhes and the percentage inhibition of acetic acid induce writhing of Wister rats are presented in **Table 3.** All test groups showed a statistically significant reduction($p<0.05$) in the number of writhes as compared with control rats

The acetic acid-induced writhing model showed a significant anti-nociceptive effect with the reference drug ($p<0.001$). SS ($p<0.001$) and SP ($p<0.01$). Similarly, the percentage inhibition of the writhing was high in the group given diclofenac sodium (41.0%). *Sudarshana* suspension and SP showed 37% and 31% inhibition respectively.

Table 3: Anti-nociceptive effect on acetic acid-induced writhing model of rats

Treatment groups	No. of Writhing	% Inhibition
Control	61 ± 2.67	0
Diclofenac sodium	36 ± 4.00***	40.9%
SS	38 ± 3.04***	37.1%
SP	41± 4.87**	31.9%

Note: All the values are mean ± SEM (n=6 per group) with values carrying different superscripts being significantly different (*$p < 0.05$, **$p <0.01$ and ***$p <0.001$)

Discussion

The anti-inflammatory effect of the developed drug was tested in Wistar rats. Acute inflammatory effect is considered by an upsurge in vascular penetrability and cellular infiltration causing to oedema formation, as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site. Carrageenan-induced paw oedema of rat paw is used widely as a working model of inflammation in the search for new anti-inflammatory agents (Valencia, et al., 1994) and it assisted in the development of indomethacin as an anti-inflammatory drug (Winter, et al., 1962). The oedema that develops in rats' paws after carrageenan injection is a biphasic event.

Statistically significant reductions ($p<0.05$) in oedema due to SP and SS were noted at every hour when compared to the negative control group. There was no significant difference between SS and SP. The most significant ($p < 0.001$) effect was noted at the 5^{th} hour after carrageenan injection.

Adjuvant-induced arthritis (AIA) in rats is a useful tool for studying the pathophysiology of rheumatoid arthritis (RA), especially because the experimental model and the human disease share various signs and symptoms (Pearson, et al., 1963). Therefore, in the present study, an intra-dermal injection Freund's Complete Adjuvant (FCA) containing 0.05% w/v Mycobacterium but yricum suspension was used to induce arthritic lesions and inflammation in the animals.

The effect of oral administration of SS and SP on arthritic (FPT, BW, and AJT) and inflammatory parameters (PGE_2) in the experimental animals was determined. The SS and SP showed significant therapeutic efficacy similar to the standard drug celecoxib. Anti-arthritic

potency of drugs was shown by the reversal of the altered arthritic parameters. The result of Figure 3 shows that the foot pad thickness (FPT) increased in adjuvant-challenged animals. Drug administration suppressed the severity of clinical arthritis, as demonstrated by decreased FPT in rats. The maximum inhibition of FPT was given by the new drug SS on the 28th day and significant ($p< 0.001$) effects were exerted by celecoxib on Day 21 and Day 24.

A previous report shows that there was significant body weight loss, the day following injection of the adjuvant (Yoshikawa, et al., 1985). The result of the present study also indicates that there is a close relationship between the extent of inflammation and loss of body weight. The negative control rats' body weight of was significantly decreased when compared with healthy control rats. The data suggested that oral treatment of celecoxib, SS, and SP assisted in recovering the inflammatory body weight loss in arthritic animals.

Similarly, body weight in arthritic animals was enhanced by celecoxib as well as by SS and SP administration as shown in Figure 1. The observed changes in ankle joint thickness of the experimental groups of animals are shown in Figure 2. The percentage of ankle joint thickness was significantly enhanced in the negative control group of arthritic animals as compared to healthy control rats. Decrease in ankle joint thickness in adjuvant-injected rats who were treated with SS indicated better results similar to that of the standard drug celecoxib.

Adjuvant-induced arthritis study was carried out to investigate the chronic anti-inflammatory activity of SS as well as SP in rats induced with rheumatoid arthritis. In this study, adjuvant injected paw was illustrated by a rapid onset of inflammation evident within 24 h of adjuvant injection and remained for 28 days.

The state of anemia, measured as RBC and Hb concentrations observed in arthritic conditions could be due to erythrocyte deformity that leads to a shortening of the life span of RBC (Ekambaram, et al., 2010). Low RBC and Hb concentrations of blood in the arthritic group compare well with a previous study that mentions anemia is associated with arthritis-induced rats and patients with RA (Kothavade, et al., 2015). An anemic state was observed in the AIA rats in the present study and the SS was able to increase significantly the levels of RBC and Hb to normal values, thus reversing the anemic state of arthritic rats.

Administration of FCA led to an increase in the total blood leucocyte count suggesting the involvement of WBCs in response to antigen-mediated arthritis (Kothavade, et al., 2015). WBC count was significantly increased in the AIA control rats when compared to the healthy control rats. However, following the treatment with the SS and SP; the drugs significantly ($p < 0.001$) reduced the WBC levels, which can be attributed to the resolution of the inflammatory response of herbal formulation.

The acetic acid-induced writhing test is normally used to evaluate the peripheral analgesic effects of drugs. Although this test is nonspecific, it is widely used for analgesic screening (Shibata, et al., 1989). This study, found that Sudarshana suspension and Sudarshana powder exhibited an anti-nociceptive effect in acetic acid-induced writhing response. This effect may be due to inhibition of the synthesis of the arachidonic acid metabolites (Franzotti, et al., 2000).

Conclusion

These studies reveal the promising acute and chronic anti-inflammatory effects and anti-nociceptive effects of Sudarshana suspension in Wistar rats which are reported scientifically for the first time. As found in present studies, Sudarshana Suspension possesses statistically significant anti-inflammatory effects and Anti-nociceptive effects comparable to allopathic drugs. These findings warrant further studies with human subjects.

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Conflict of Interest

The authors declare that they have no any conflict of interest regarding the publication of this manuscript. There is no conflict of interest with the granting body.

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