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Anti-Inflammatory and Antioxidant Properties of the *Mucuna sanjappae* Seeds in the Rat Model and *In Vitro* Assays

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The *Fabaceae* (*Leguminosae*) plant family contains several species of the *Mucuna* Adans. genus possessing therapeutic potential and growing widely in tropical and sub-tropical regions. In this research, we investigated the anti-inflammatory and antioxidant properties of the extract from the *Mucuna sanjappae* Aitawade & S.R.Yadav seeds. Initially, we conducted an *in vitro* anti-inflammatory activity test using the bovine serum albumin anti-denaturation assay and found promising dose-dependent activity. Subsequently, we performed an *in vivo* anti-inflammatory and antioxidant study on a rat paw edema model induced by carrageenan. Three different doses of *M. sanjappae* seed water extract (50, 100 and 200mg/kg B/W) were used for the study (Oral administration). Edema measurement was carried out at 0, 2, 4 and 6 hr intervals. Dose dependent inhibition in edema in the *M. sanjappae* seed extract treatment group was observed with maximum activity for 200mg/kg B/W dose at 4 hr (53.49%). Standard drug showed maximum edema inhibition (54.94%) at 6hr. Our results also showed that, *M. sanjappae* seed extract inhibited pro-inflammatory cytokine TNF-á and increases anti-inflammatory cytokine IL-10 with increased level of blood serum antioxidants. Phytochemical analysis for secondary metabolites including polyphenol, flavonoids, phytic acid, proanthocyanidin, tannin and saponin was also quantified which might be the responsible component for biological activities under study.

Keywords: Antioxidant, Anti-inflammatory drug, Carrageenan, *C*ytokine, Inflammation, *Mucuna*.

Inflammation can be defined as essential response exhibited by host after tissue injury or infection. However, its longstanding persistence may result in chronic diseases like cancer, cardiovascular diseases, neurological disorders, diabetes, pulmonary diseases, and arthritis[1-7]. Inflammation is characterized by pro-inflammatory enzymes, chemokines, cytokines, and certain signal proteins generated in response to infection or injury. Management of inflammatory diseases today represents an important medical problem since currently used non-steroidal anti-inflammatory drugs (NSAIDs) has several other adverse effects commonly known as gastroenteropathy[1,7]. Hence,

identification of novel and effective therapies for safer management of inflammatory diseases remains an urgent priority. Natural plant wealth is continually being investigated for novel bioactive molecules with therapeutic properties. In contrast to modern synthetic drugs, natural bioactive are cost effective and provides significant protection from diseases without secondary adverse complications. Therefore, researchers are investigating plant-based drugs which can provide promising anti-inflammatory activity with lower secondary complications[8]. Numerous reports endorse the use of various plants for treating inflammation[1,9].

Genus *Mucuna* belongs to family fabaceae, popularly known as cowitch, kapikachu and atmagupta. *Mucuna* species are recognized for their itching properties due to hairs present on the pods. Since ancient time, Indian ayurveda system of medicine uses seeds of *Mucuna* for the management of different types of diseases and disorders. Remarkable research has been carried out on *Mucuna pruriens* particularly for its anti-Parkinson's, anti-infertility, anti-venom, anti-inflammatory and anti-bacterial activity[7,10-16]. *Mucuna* seed powder is very common ingredient in various ayurvedic formulations marked in India and across the world. However, exploitation of *M. pruriens* at large scale may affect ecosystem adversely and due to limited availability, final product cost may also increase. To avoid this, there is increasing interest in investigating hidden potential of other underutilized *Mucuna* spp. as a promising alternative therapeutic agent. Previously, our research group have successfully reported *Mucuna* species including *M. macrocarpa, M. bracteata*, M. *imbricata* and *M. sanjappae* etc. for their nutritional and medicinal benefits[15-24]. In 2019, we have reported L-DOPA (L-3,4-dihydroxyphenylalanine), an FDA approved anti-Parkinson's drug in seeds of different *Mucuna* species found in Indian contingent and proved that number of *Mucuna* species possesses higher level of L-DOPA as compared to the commonly used *M. pruriens*[25].

M. sanjappae is endemic plant species found in Western Ghats region of Maharashtra, India which belongs to genus *Mucuna*[26]. In previous studies we have demonstrated that *M. sanjappae* seeds possess promising level of nutritional components with gross energy 383 kcal and around 5.43 g of protein. Moreover, it contains about 7.3 % L-DOPA and other important primary and secondary metabolites, minerals, important phenolics etc. Furthermore, anti-Parkinson's activity of *M. sanjappae* seed extract in Parkinson's disease (PD) mice model intoxicated by MPTP is reported[18,19]. *M. sanjappae* seed extract could successfully ameliorate PD symptoms developed by MPTP toxicity. However, till date, there is no data available on the effect of *M. sanjappae* on inflammatory diseases and oxidative stress using *in vitro* or *in vivo* model. Carrageenan induced rat paw edema model is popular method of anti-inflammatory studies of natural as well as synthetic drugs[1]. Hence, present efforts have been made to examine anti-inflammatory and antioxidant properties of *M. sanjappae* seed on carrageenan induced rat paw edema model.

MATERIAL AND METHODS

Chemicals and reagents

Analytical grade solvents and chemicals were used the study. Carrageenan and enzyme-linked immunosorbent assay (ELISA) cytokine kits for TNF-á and IL-10 measurement were obtained from Sigma-Aldrich, USA. Diclofenac (Standard anti-inflammatory drug) was obtained from Recon, Bangalore, India respectively.

Plant material and preparation of drugs for administration

The pods of *M. sanjappae* were collected from its original location (Pune district, Western Ghats, Maharashtra, India). The herbarium was carefully prepared and stored at the herbarium center of the Botany department, Shivaji University, Kolhapur under the guidance of taxonomist Prof S. R. Yadav.

After removing the healthy seeds from the pods, fine powder was prepared. Extract of seed was produced by adding and macerating 1g *M. sanjappae* seed powder in 100 ml D/W in the mortar and pestle. Further, sonication for 15min and centrifugation at 10000 rpm for 10 min was carried out. Supernatant was carefully separated and stored for further use. Effective yield was calculated by evaporating water. Importantly, seed extract and standard drug diclofenac were prepared freshly at the time of dosing.

***In vitro* anti-inflammatory activity**

Bovine serum albumin (BSA) anti-denaturation assay

This test was performed using a method defined earlier with slight modifications[27]. Various concentrations of seed extract and standard drug diclofenac were reacted with 1ml of 1% BSA solution prepared in 50mM Tris buffer (pH 6.5). Incubation was carried out at 37°C for 20 min and further heating at 64°C in water bath till mixture get turbid (around 5 to 10 min). Finally, tubes were cooled, and absorbance of generated turbidity was measured at 660 nm. D/W was used as a control. Following formula was used to calculate denaturation inhibition percentage:

$$\% \text{ denaturation inhibition} = A \text{ (control)} - A \text{ (sample)} / A \text{ (control)} \times 100$$

Where, A (control): Absorbance of the control; A (sample): Absorbance of samples.

Hypotonicity-induced HRBC membrane stabilization method

Capacity of *M. sanjappae* seed to protect hypotonicity encouraged human red blood cell membrane protection have been determined[28]. Various concentrations of *M. sanjappae* seed extract were made into 1 mL using distilled water in a tube. Initially, 0.5ml of 10% HRBC suspension and 0.5 mL of 0.25% hyposaline were added to each tube. Then, mixture was incubated at static condition for 30 mins at 37°C and centrifuged at 3000 rpm for 20 mins at 4°C. The amount of hemoglobin in the supernatant was performed at a wavelength of 560 nm. Working solution of standard drug aspirin was prepared in 0.2 M phosphate buffer (1 mL) at various concentration ranging from 100 µg to 500 µg. To induce complete hemolysis (100%) without any sample or drug, a control was prepared using distilled water as a replacement for hyposaline. To calculate the percentage of HRBC hemolysis and evaluate the degree of membrane stabilization or protection, below given formula was employed:

$$\% \text{ of hemolysis} = \text{Absorbance of test} / \text{Absorbance of control} \times 100$$

$$\% \text{ protection} = 100 - (\% \text{ of hemolysis})$$

***In vivo* anti-inflammatory activity**

Rats were divided into seven groups (6 rats per polypropylene cage) housed under controlled conditions (Relative humidity 44–56 %, temperature of 25±2 °C, and 12 h light/ dark cycles). Standard diet and water *ad libitum* was provided to the experimental animals. The experiment was started after proper acclimatization of animals in the laboratory environment after a week. Details of randomization, grouping and drug dosing are given in table 1. Seven days prior dosing was carried out by *M. sanjappae* seed extract in group IV, V, VI and VII. On the 8th day rats were kept fasted but water was provided *ad libitum*. Extract was administered 2hr before inducing the inflammation by carrageenan (0.9%) through sub plantar way and further investigation was accomplished.

Rat paw edema measurement

The inflammation in terms of swelling of carrageenan induced foot of animal was measured using plethysmometer (UGO Basile, Italy) as a water displacement in ml. The measurement was done at 0, 2, 4 and 6 hr. The decrease in paw volume was compared to the vehicle control. The percentage of inhibition in seed water extract and diclofenac treated group was compared with carrageenan induced group.

Inflammatory biomarkers and Oxygen radical absorbance capacity (ORAC) assay

TNF-á and IL-10 concentration in serum of control and drugs treated respective groups was determined using ELISA kit. The experiment was performed as per instructions of manufacturer. TNF-á and IL-10 level was expressed as picogram per milligram (pg/mg).

Antioxidant level of serum samples in treated and control animal group was carried out by ORAC method[29]. Shortly, 25 ìl of serum sample added in fresh 150 ìl of 10nM fluorescein solution and allowed to stand at 37 ^{0}C for 30 min. After incubation, 25 ml of AAPH substrate (500mM) mixed and the fluorescence was calculated for 150 min at 485 and 520nm (Excitation and emission wavelengths respectively) using microplate reader. Standard trolox was used as a and results were expressed as micromoles of Trolox equivalents (TE) per liter of sample.

Phytochemical analysis

The total polyphenol level of *M. sanjappae* seeds was determined spectrophotometrically[30] and represented as mg of gallic acid equivalent per gram (mg GAE g^{-1}) of dry mass. The flavonoids

content was analyzed[31] and results represented as milligram of quercetin equivalents per gram (mg QUE g^{-1}) of dry weight. Proanthocyanidin examined and reported as catechin equivalents per gram (mg CAE g^{-1}) of dry weight[32]. The phytic acid was determined and absorbance was measured at 500 nm[33]. Tannin and saponin level also studied according to methods reported earlier[34,35].

Statistical Analysis

GraphPad Prism 5 is used for data analysis. All the results were represented Mean ± SEM. P-values of less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Nature has gifted us with medicinally important, inexhaustible sources of secondary metabolites, including alkaloids, terpenoids, phenolics, saponins, and other classes of organic compounds. These phytometabolites have tremendous health benefits for the overall growth and the management of diseases. Over time, experimental procedures and tools for the isolation, characterization, validation, and development of drugs for disease have been well established[36]. The present attempt was aimed to find out the anti-inflammatory and antioxidant properties of *M. sanjappae* seeds for future natural drug development.

In vitro anti-inflammatory activity

Preliminary screening of anti-inflammatory potential was performed using table assay as given below:

Bovine serum albumin (BSA) anti-denaturation potential

Denaturation of proteins causes several inflammatory responses in the body. Many synthetic drug molecules show promising anti-inflammatory activity[37] but are known to cause secondary complications after long-term use[38]. Hence, the use of plant-mediated drugs may prove more advantageous than their synthetic counterparts. *M. sanjappae* seed extract has shown inhibition of heat-induced albumin denaturation activity at different concentrations, as shown in Fig. 1. *M. sanjappae* extracts showed strong inhibition of albumin denaturation (87.73±3.81%) at 500ìg/ml concentration. The standard drug diclofenac showed 94.82± 1.79% inhibition at 500 ìg concentration. The results suggested good anti-inflammatory activity of *M. sanjappae* seed extract.

HRBC Membrane stability potential

Stabilisation of lysosomal membrane is an essential process in the regulation of inflammation process. It occurs by preventing the release of lysosomal components of active neutrophils including bactericidal enzymes and proteases. Red blood cell membrane bears resemblance to lysosomal membrane[28], therefore understanding the stability of RBC membrane by our drug of interest gives idea about its potential to protect lysosomal membrane to prevent release of inflammatory response markers. Haemoglobin released in the supernatant in the control tube (without std drug or plant extract) due to bursting of red blood cells, while the yellow supernatant in the plant

Table 1. Randomization and grouping of animals for *in vivo* study

Group number	Group name	Dose content	Number of animals
I	Normal control	Vehicle solvent (D/W)	6
II	Carrageenan control	100 µl (0.9% carrageenan)#	6
III	Diclofenac	10mg/kg BW	6
IV	Test Dose-1 50mg/kg BW*	50mg/kg BW	6
V	Test Dose-2 100mg/kg BW	100mg/kg BW	6
VI	Test Dose-3 200mg/kg BW	200mg/kg BW	6
VII	Sham Control	200mg/kg BW	6

BW* - Body Weight of animals.

- 0.9% carrageenan were prepared in saline solution

extract tube indicates the stabilisation of HRBCs. Our results demonstrated that *M. sanjappae* seed extract could protect the HRBC membrane with maximum protection at 500ug *M. sanjappae* seed extract (60.47±2.1 %) as depicted in Fig. 2. As concentration of *M. sanjappae* seed extract was increased, HRBC membrane stability was also increased suggesting concentration dependent stabilization activity by *M. sanjappae* seed. Standard drug aspirin showed superior HRBC membrane protection capacity as compared to *M. sanjappae* seed extract. The maximum protection by aspirin at 500ug concentration was 99.56±1.01 and it also represented dose dependent increase in activity.

***In vivo* anti-inflammatory activity**

Carrageenan is a sulfonated polysaccharide widely used in food industries. It is extracted from red seaweed algae. It does not have any nutritional potential but is preferentially used as thickening, gelling and emulsifying agent[39]. Several studies have successfully reported use of carrageenan as

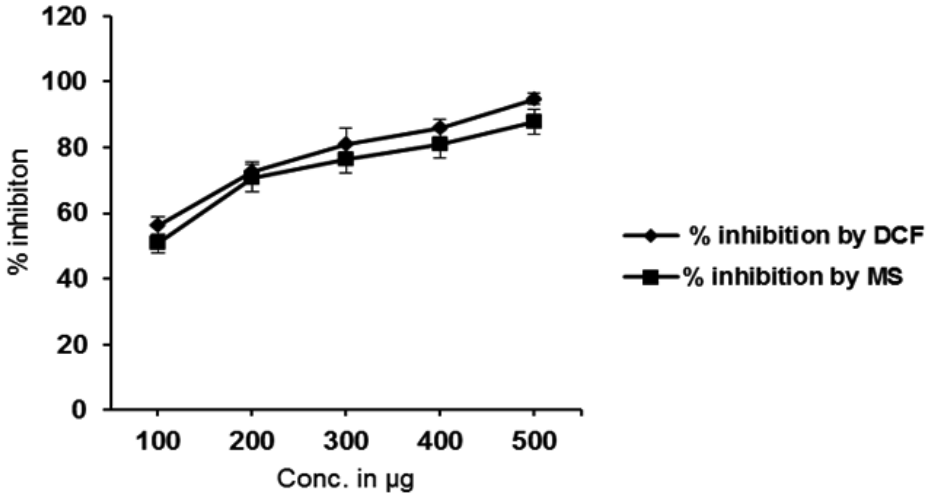

Fig. 1. Heat induced BSA anti-denaturation activity *M. sanjappae* seed water extract in % comparison with standard drug Diclofenac (Mean ± D, n=3).

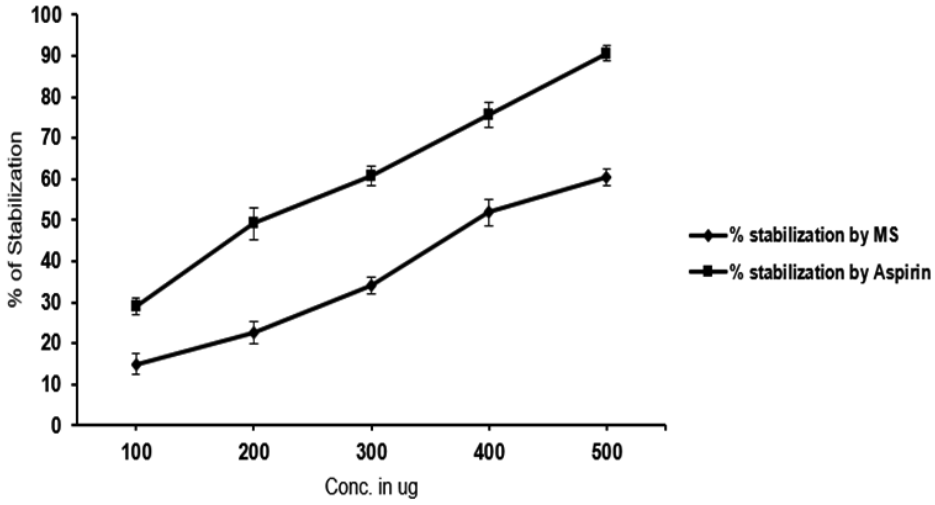

Fig. 2. HRBC membrane stability assay of different concentration of *M. sanjappae* seed water extract in comparison with standard drug Aspirin (Mean ± D, n=3)

an inflammatory agent to induce acute paw edema[1]. It is a simple and effective mean of assessing anti-inflammatory properties of drug.

Paw edema measurement

Determination of paw edema level after carrageenan toxicity and its furth treatment using oral dose of *M. sanjappae* seed extract was evaluated. Carrageenan generates acute paw edema which can be seen as redness and swelling at the site of injection. In the present study, paw edema was induced by using carrageenan and measured at 0hr, 2hr, 4hr and 6hr post injection. Carrageenan toxicity successfully developed inflammation in the rat paw which was evident from redness

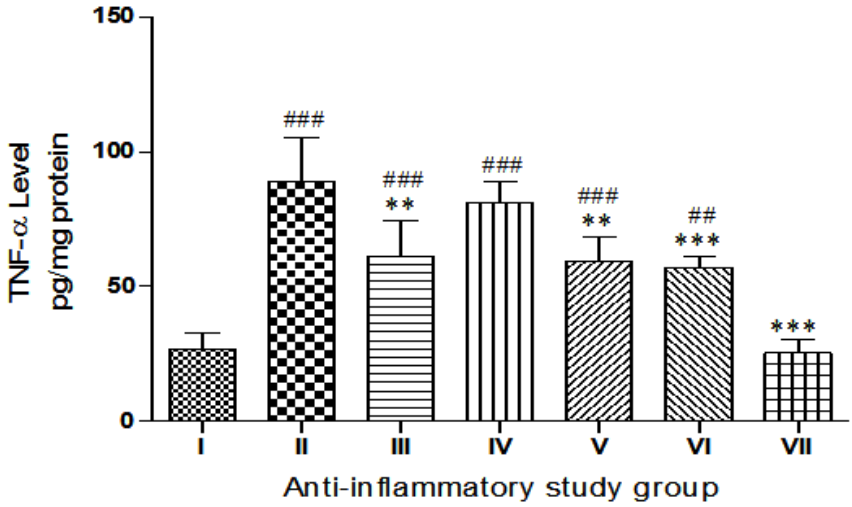

Fig. 3a. Effect of *M. sanjappae* seed extract on TNF- á level in serum of carrageenan induced rat at 6hr. Values are represented as Mean±SEM (n=6). **p<0.01, **p<0.01, ***p<0.001 when compared with carrageenan induced group. ##p<0.01, ###<0.001 when compared with vehicle control group (X axis indicates group number which are shown in detail in Table no. 1)

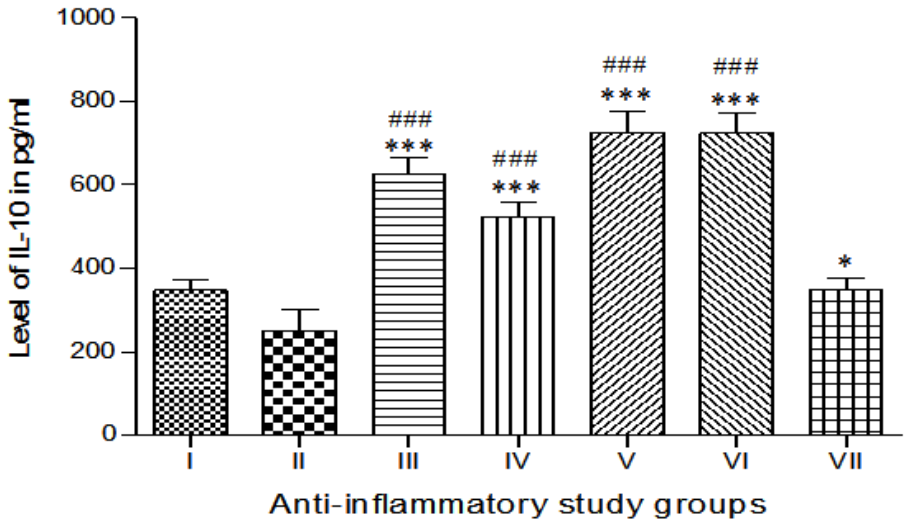

Fig. 3b. Effect of *M. sanjappae* seed extract on IL-10 level in serum of carrageenan induced rat at 6hr. Results are represented as Mean±SEM (n=6). ***p<0.001 when compared with carrageenan induced group. ###p<0.001 in comparison with vehicle control group. (X axis indicates group number which are shown in detail in Table no. 1).

and swelling at the site of induction with highest paw thickness at 4hr (9.44±0.25). Vehicle control animal group does not show edema. Animal group treated with different doses of *M. sanjappae* seed extract exhibited significant reduction in the paw edema with maximum result at 200mg/kg body weight (Table 2). It showed 53.49% of edema inhibition after 4 hr treatment. Standard drug showed maximum edema inhibition (54.94%) at 6hr.The anti-inflammatory potential of *M. sanjappae* seed extract was observed very close to the standard drug Diclofenac control group. Dose dependent increase in the anti-inflammatory activity of *M. sanjappae* seed extract in respect to edema reduction was clear from the study. Sham control group was given the highest dose of *M. sanjappae* seed extract (200mg/kg body weight) and does not show any inflammatory symptoms during the experiments. Our finding supports anti-inflammatory potential of *M. sanjappae* seed and its traditional use in the management of inflammation related disorders.

Table 2. Effect of *M. sanjappae* on carrageenan induced rat paw edema.

	0hr	2hr	4hr	6hr
Group I: Vehicle control	4.11±0.04	4.16±0.05	3.97±0.06	4.05±0.04
Group II: Carrageenan Control	4.41±0.08	8.23±0.07	9.44±0.25	8.9±0.39
Group III: Diclofenac control	3.89±0.04	4.51±0.06*** (47.0)	4.26±0.21*** (54.87)	4.01±0.18*** (54.94)
Group IV: 50mg test drug$	3.91±0.14	6.02±0.36*** (26.85)	5.24±0.19*** (44.49)	5.04±0.2*** (43.37)
Group V: 100mg test drug	4.28±0.09	5.99±0.25*** (27.21)	5.27±0.33*** (44.17)	5.11±0.29*** (42.58)
Group VI: 200mg test drug	3.81±0.02	4.53±0.15*** (44.95)	4.39±0.22*** (53.49)	4.18±0.31*** (53.03)
Group VII: SHAM control	4.49±0.05	4.47±0.08	4.52±0.1	4.36±0.11

$: *M. sanjappae* seed water extract. Values have been represented as mean±SEM, n=6 in each group. * p<0.05; ** p<0.01; *** p<0.001 when compared with carrageenan induced control. Values shown in parentheses represent the percent (%) reduction in paw edema in comparison with carrageenan induced control.

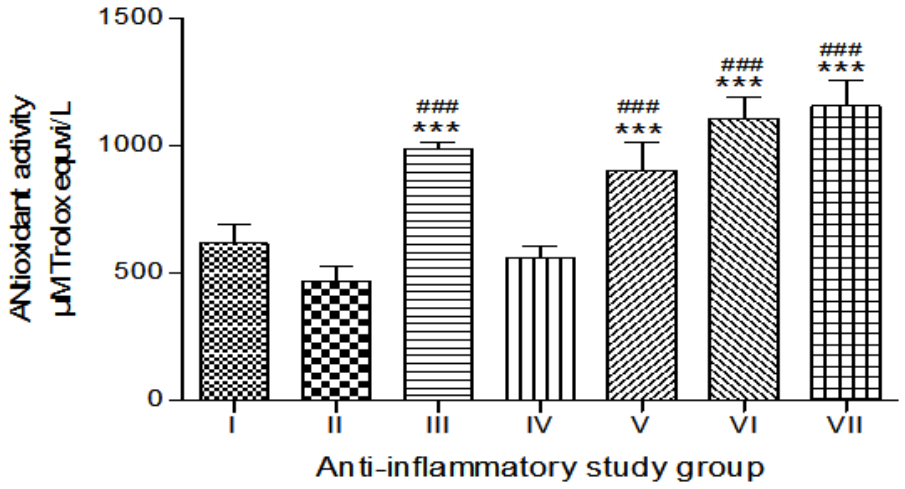

Fig. 4. Effect of *M. sanjappae* seed extract on serum oxygen radical absorbance capacity in carrageenan induced rat at 6hr. Results are represented as Mean±SEM (n=6). ***p<0.001 when compared with carrageenan induced group. ###p<0.001 when compared with vehicle control group

Effect of *M. sanjappae* treatment on pro-inflammatory cytokine TNF-á

TNF-á is a mediator of inflammatory responses playing a key role in the development of innate immune system via activating macrophages, T cells and secretion of other inflammatory cytokines. In the carrageenan induced acute inflammatory model, complement system stimulation along with inflammatory mediators' synthesis are major events [40]. Serum TNF-á level was determined after 6hr post carrageenan treatment (Fig 3a). There was considerable increase in TNF-á (89±8.13 pg) in serum due to inflammatory response after carrageenan injection confirming its pro-inflammatory properties (Group II). However, treatment of *M. sanjappae* seed extract at various doses significantly decreased TNF-á level. Among tested doses, 100 and 200mg dose significantly reduced ($p<0.01$) TNF-á level (59.5±4.48 pg and 57±1.96 pg respectively) in animal (Fig 3a) and we found superior results as compared to standard drug diclofenac (61.3±6.61 pg). Vehicle control and Sham control represented normal TNF- á level. According to the results, it can be concluded that, *M. sanjappae* has potential of reducing the inflammation caused by external toxic compounds and possesses potential to use for managing inflammatory diseases.

Effect of *M. sanjappae* treatment on anti-inflammatory cytokine IL-10

IL-10 is an important anti-inflammatory cytokine which attenuates the activity of pro-inflammatory markers including TNF- á[41]. In the present study, in contrast to TNF- á, IL-10 level was increased in Diclofenac and *M. sanjappae* seed extract treated animal groups (Fig. 3b). Reduction in IL-10 in carrageenan induced group (II), suggests, carrageenan exerts its anti-inflammatory response through suppression of anti-inflammatory cytokines. Positive effect of *M. sanjappae* seed extract was validated from considerable augmented IL-10 level ($p<0.001$) with maximum activity at 100 and 200mg *M. sanjappae* seed extract dose. Vehicle control (I) and SHAM control (VII) exhibited normal level of IL-10 which was higher than carrageenan induced group II. Overall, our study signifies *M. sanjappae* exerts its anti-inflammatory action by suppressing pro-inflammatory cytokines and expressing anti-inflammatory cytokines in the serum of carrageenan toxicated rat.

Oxygen radical absorbance capability (ORAC) assay

Serum antioxidant levels of different animal groups is studied by oxygen radical absorbance capacity method[1]. Serum antioxidant activity was significantly ($p<0.001$) elevated in *M. sanjappae* seed extract treated animal group. The higher activity was found for 100 and 200mg/kg B/W dose. There was also significant increase in serum antioxidant capacity in sham control animal suggesting *M. sanjappae* seed possesses secondary metabolites which are enhancing antioxidant properties in the animal. Carrageenan control showed lower level of serum antioxidant activity (468±31.6µM Trolox equiv/L) suggesting oxidative stress is get generated due to toxicity of carrageenan. Different doses of *M. sanjappae* seed extract showed 560±22.4, 905±53.8 and 1105±42.4 µM Trolox equiv/L respectively showing concentration dependent increase in oxygen radical absorbance potential (Fig 4).

Human disorders show pathogenesis through cellular inflammation and oxidative stress mediated cell degeneration. An inbuilt antioxidant system comprising antioxidant molecules and enzymes performs a crucial role in removing free radicals[7,42]. But increased oxidative stress because of environmental toxins, genetic changes, or unknown causes results in cell components/organelle to degenerate or alter leading to apoptosis of cell. In such cases, supplementary antioxidants through food or drugs become essential way of disease management. Present study demonstrated *M. sanjappae* seeds contains vital phyto-metabolites which have capacity to induce anti-inflammatory and antioxidant properties required in the disease treatment.

Phytochemical analysis

Phenolics and flavonoids are considered as major secondary metabolites with prominent role as antioxidant and anti-inflammatory agents[20,21,22,24]. These secondary metabolites are with diverse biological activities combating diseases through multiple pathways. Their role in managing diseases including neurodegenerative diseases is via unstable superoxide radicals scavenging and cellular inflammation decreasing pathway[43]. *M. sanjappae* showed 80.78±2.56 mg GAE g^{-1} of phenolics and 419.5±7.18 mg QAE g^{-1} of flavonoids. Earlier we have analyzed gallic acid, tannic acid,

p-hydroxybenzoic acid and p-coumaric acid as major phenolic compounds present in *M. sanjappae* seeds using HPLC[20]. *M. sanjappae* seed possesses higher levels of flavonoids than polyphenols which may be attributed to the specificity, and accuracy of reaction and respective standard used for the reaction. Proanthocyanidin level was 2.14 ± 0.13 mg CAE g^{-1}. Proanthocyanidin is flavan-3-ol group having compound with strong antioxidant, anti-inflammatory, antihypertensive, antimicrobial and antiallergic activity[44-46]. *M. sanjappae* seed contains a higher concentration of phytic acid 197.23±0.11 mg g^{-1}. Phytic acid is metal chelating and anti-inflammatory in nature [47,48]. Phytic acid decreased inflammation by reducing the level of NF-êB and p-ERK in MPTP intoxicated PD mice model[48]. It is also natural iron chelating agent which prevents iron induced dopaminergic neuron degeneration in Parkinson's disease[49]. *M. sanjappae* beans showed 0.52±0.11 mg g^{-1} and 18.71 ± 0.13 mg g^{-1} of tannin and saponin content respectively. Both of these compounds possess anti-inflammatory and antioxidant activity[50, 51].

Investigation of traditional ayurveda knowledge with respect to disease management has lain to find novel drug molecules and related molecular pathways responsible for it. In modern medicine, single synthetic drug is preferred for targeted and quick action. But on the other side, those synthetic molecules usually have several side effects to the patients. In this connection, plant-based drug therapy which comprises several effective drug molecules proves to be most effective and moreover exerts minimum side effects. Based on the present results, *M. sanjappae* may prove as a promising lead for treating inflammatory diseases.

CONCLUSION

Natural drugs isolated from plants show promising anti-inflammatory properties with little to no side effects. Local indigenous peoples have been using *Mucuna* species as a staple food and medicinal purposes, mainly for Parkinson's disease, male infertility, and snake bite treatment. However, there has been no elaborative investigation of the anti-inflammatory properties of *M. sanjappae* using *in vivo* or *in vitro* model. The present study revealed the anti-inflammatory potential of *M. sanjappae* seed extract by inhibiting pro-inflammatory cytokines and upregulating anti-inflammatory cytokines. The carrageenan-induced edema was reduced after treatment with *M. sanjappae* seeds' water extract. Furthermore, the antioxidant level was elevated after the treatment by *M. sanjappae* extract. Phytochemical analysis confirmed presence of active secondary metabolites such as phenolics, flavonoids, phytic acid, saponins etc. Thus, the study strongly supported therapeutic potential of *M. sanjappae* and suggests further molecular-level investigations for its future exploration as a pharmacological agent in the management of inflammation and oxidative stress-related diseases.

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Conflict of interest statement

Authors declare no conflict of interest.

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