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PHARMACOLOGICAL ACTIVITY OF *DESMODIUM TRIFLORUM*- A REVIEW

Anu K Thankachan^{*1}, Meena Chandran¹, K. Krishnakumar²

¹Department of Pharmaceutical Analysis, St. James College of Pharmaceutical Sciences, Chalakudy, Thrissur, Kerala, India.

²St James Hospital Trust Pharmaceutical Research Centre (DSIR Recognized), Chalakudy, Thrissur, Kerala, India.

ABSTRACT

Desmodium triflorum is a plant belongs to the family Fabaceae. It is a global species native to tropical regions and introduced to subtropical regions including the southern United States. The plant is having antipyretic, antiseptic, expectorant properties. A decoction is commonly used to treat diarrhoea and dysentery; quench thirst; and as mouthwash. The crushed plant, or a poultice of the leaves, is applied externally on wounds, ulcers, and for skin problems. In general the whole plant is used medicinally for inducing sweat and promoting digestion, anti-oxidant, anti-inflammatory, anti-convulsant and anti-bacterial actions. This review explains the different pharmacological activities of *Desmodium triflorum*.

KEYWORDS

Desmodium triflorum, Anti-oxidant, Anti-inflammatory and Anti-convulsant.

Author for Correspondence:

Anu K Thankachan,
Department of Pharmaceutical Analysis,
St. James College of Pharmaceutical Sciences,
Chalakudy, Thrissur, Kerala, India.

Email: stjamespharmacyproject@gmail.com

INTRODUCTION

Plants have formed the basis for treatment of diseases in traditional medicine for thousands of years and continue to play a major role in the primary health care of about 80% of the world's inhabitants¹. It is also worth noting that (a) 35% of drugs contain 'principles' of natural origin and (b) less than 5% of the 500,000 higher plant species have undergone pharmacological screening. Each plant has potentially 10,000 different constituents². The discovery and development of efficacious therapeutic agents from natural sources provided convincing evidence that plants could be a source of novel drugs. Western medicine use many drugs

extracted from natural products: atropine, cocaine, digitoxin, ephedrine, hyoscyne, codeine, morphine, pilocarpine, quinine, reserpine, taxol, warfarin, menthol, etc. While the natural product isolated as the active compound might not always be suitable for development as an effective drug, it can provide a suitable lead for conversion into a clinically useful agent³. Plants and their secondary metabolites have a long history of use in modern 'western' medicine and in certain systems of traditional medicine. Monographs on selected herbs are available from a number of sources, including the 'European Scientific Cooperative on Phytotherapy' [ESCOP, 1999], 'Natural Medicines Comprehensive Database', 'The complete German Commission E monograph' and the 'World Health Organization' [WHO, 1999]^{4,5}. The WHO monographs, for example, describe the 'herb' itself by a number of criteria including synonyms and vernacular names and the herb part commonly used, its geographical distribution, tests used to identify and characterize the herb (including macroscopic and microscopic examination and purity testing), the active principles, dosage forms and dosing, medicinal uses, pharmacology, contra-indications and adverse reactions. Information about other available data bases has been published⁶. *Desmodium triflorum* (L.) DC. Fabaceae, is perennial herb belonging to the family papilionaceae⁷. The plant is available in all tropical countries. It contains hypaphorine (major alkaloid) N, N-dimethyltryptophan, beta in and choline. The leaves contains total alkaloid, 0.01-0.015% and rare diholosylflavone, 2-O-glucosylvitexin had been isolated from *D. triflorum*⁹. It also contains ursolic acid, vitexin, genistin, fucosterol. In traditional medicinal system, different parts of the plant have been mentioned to be useful in a variety of diseases. The leaves are used in diarrhoea, convulsions, anti-spasmodic, sympathomimetic, central nervous system stimulation, curare-mimetic activity, diuretic and as a galactagogue¹⁰. The fresh leaves of the plant are applied to wounds and abscesses that are usually difficult to heal. The paste is sometimes applied to sores and itch. The fresh juice of the plant is often

given to the children for coughs and asthma. The traditional use of the plant also recommends for use in dysentery and as a laxative⁹, in high fever¹¹ and to cure bone-fracture¹². Different extracts of *D. triflorum* exhibit analgesic and anti-inflammatory activities¹³ and also possesses antioxidative and anti-proliferative activities¹⁴. Anti-nociceptive activity of cold water extract of *D. triflorum* DC in rats^{15,16} and antioxidant activities of phenolic components from various plants of *Desmodium* species¹⁷. Anticonvulsant activity of ethanolic leaves extract of *D. triflorum*¹⁸ anthelmintic activity¹⁹ antimicrobial activity²⁰ were reported.

PHARMACOLOGICAL ACTIVITIES OF *DESMODIUM TRIFLORUM*

Anthelmintic Activity

The anthelmintic activity of the leaves and roots of the *Desmodium triflorum* (L.) was studied. The leaves and roots were extracted separately with cold water, methanol and petroleum ether by following maceration method. Various doses of cold water, methanolic and combined (cold water, Methanol and petroleum ether) extracts were evaluated for their anthelmintic activity on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being were used for this study^{12,11}. All earthworms were of approximately equal size. They were collected from local place, washed kept in water and normal saline solution to remove all faecal matter. Different concentrations of the dried extracts (10-25mg/mL in saline solution with tween 80) were prepared. 20ml of each concentration of cold water extract was delivered into a petridish. Then six worms (same type) were placed in it. Similarly for each concentration of methanolic extract six worms were used. Time taken for paralysis was noted when the worm did not revive even in normal saline solution. Time taken for the death of worms was also recorded when the worms lost their body colour (when dipped in warm water of 50°C). Albendazole (10mg/mL in vehicle tween 80) was used as

positive control and saline solution was used as negative control. Cold water, methanol and combined extracts of *Desmodium triflorum* exhibited anthelmintic activity. The cold water and methanol extracts of *Desmodium triflorum* at different concentrations produced an anthelmintic activity in a dose dependent manner. Considering time taken for paralysis and death of earthworms, the positive control (Albendazole 10mg/mL) was more potent than both the concentrations of the cold water and methanolic extracts. While the combined extract (10mg/mL) produced more potent effect than the Albendazole (positive control). The negative control (saline water with tween 80) did not show any activity against earthworms^{19,21}.

ANTIBACTERIAL ACTIVITY

The efficacy of aqueous and methanolic extracts of plant *Desmodium triflorum* for potential antibacterial activity was evaluated. The whole plant *Desmodium triflorum* was collected from the local dealer of medicinal herbs and it was washed properly to remove dirt, powdered well by grinding and avoiding the moisture contamination. This powdered drug passed through sieve for obtaining uniform particle size and prepared methanolic and aqueous extract. The extracts were investigated against (G^{+ve}) and (G^{-ve}) bacteria such as *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Escheria coli*. The Results revealed that methanolic extracts exhibit considerably increased antibacterial activity for (G^{+ve}) bacteria than aqueous extracts. Methanolic extracts of *Desmodium triflorum* possessed increased antibacterial activity for (G^{+ve}) bacteria as compared to (G^{-ve}) bacteria in dose dependent manner. The ZOI of methanolic as well as aqueous extracts were found to be 16-18 mm and 13-14 mm respectively using 100 µg/ml. The ZOI of samples were also compared with the ZOI of gentamycin 10 µg/ml which showed ZOI of 22-25 mm for (G^{+ve}) bacteria and 17-18 mm for (G^{-ve}) bacteria²⁰.

ANTICONVULSANT ACTIVITY

Anticonvulsant activity of aqueous and ethanolic extracts of *Desmodium triflorum* (L.) were studied in mice. Animal models of epilepsy namely the pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsion were used to evaluate the anticonvulsant effects of the extracts. The biochemical estimation was done by measuring the lipid per oxidation and reduced glutathione in brain. In brain glutathione, the aqueous leaf extract of *Desmodium triflorum* (L.) DC. Asteraceae, demonstrated potential anticonvulsant properties and less toxicity in the experimental animals at the doses used. However, further studies still needed to be carried on exposure of the extract to humans, and its use in folk medicine for seizure control should be accompanied by regular assessment of level of consciousness and blood pressure¹⁶.

ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES

The antioxidant and antiproliferative activities of the crude extract and fractions of *Desmodium triflorum* (L.) DC was evaluated. The total phenolic content, 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), reducing power assay and total flavonoid content of *D. triflorum* were evaluated for the exploration of its antioxidant activities. The extraction and fractionation done from the crude methanol extract (253 g) which was in turn dissolved and suspended in 500 ml of water in a separatory funnel and then partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol in sequence Under reduced pressure. The result showed that ethyl acetate fraction exhibited the best antioxidant potency in DPPH free radical scavenging activity, trolox equivalent antioxidant capacity and reducing power assays. Among all fractions, ethyl acetate fraction was the most active in scavenging DPPH and ABTS radicals. These activities approached three times the efficacy of the crude extract in scavenging DPPH and ABTS radicals. The presence of flavonoids in *D. triflorum* has been

reported and has similar efficacies as astragal in, cosmoosiin, tectorigenin, vitexin, genistin, etc. The flavonoid compounds in *D. triflorum* can scavenge excess free radicals in the human body and prevent advanced aging, cardiovascular diseases and degenerative diseases. Vitexin was used as a marker component for the standardization of flavonoid ingredients of the ethyl acetate fraction, *n*-butanol fraction and methanol extract by using HPLC. The retention time of vitexin was at 8.71 min at 336nm (Figure No.2). The presence of vitexin was found in HPLC fingerprints of the methanol extract, ethyl acetate fraction and *n*-butanol fraction, as shown in Figure No.2b, 2c and 2d. The *n*-butanol fraction had higher vitexin content.

Vitexin may also be an active constituent of antioxidant factors in *D. triflorum*. The LD50 of the methanol extract of *D. triflorum* indicated that *D. triflorum* is safe to use with low toxicity. From this study found that ethyl acetate fraction exhibited good antioxidant activities. These activities may be attributed to the high phenolic contents in the fraction^{14,17}.

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES

Analgesic effect of methanol extract from *Desmodium triflorum* (MDT) was evaluated by using animal models of acetic acid-induced writhing response and formalin test. The anti-inflammatory effect of MDT was investigated by λ -carrageenan-induced paw edema in mice. In order to study the anti-inflammatory mechanism of MDT, we detected the activities of glutathione peroxidase (GPx) and glutathione reductase (GRd) in the liver, the levels of interleukin-1 β (IL-1 β), tumor necrosis factor (TNF- α), malondialdehyde (MDA) and nitric oxide (NO) in the edema paw tissue. In the analgesic test, MDT (0.5 and 1.0 g/kg) decreased the acetic acid-induced writhing response and the licking time on the late phase in the formalin test. The vitexin was used as a marker component for the standardization of flavonoids ingredients of MDT by using HPLC. The retention time of vitexin was

found at 11.19 min in the MDT. The HPLC fingerprint of MDT is shown in Figure No.3.

Effect of MDT on Acetic Acid Induced Writhing Response

The results of acetic acid-induced writhing responses in mice which indicate the analgesic activity of the methanol extracts of MDT were presented in Figure No.4. It was found that the extract and indomethacin at the assayed doses caused a significant ($p < 0.001$) inhibition on the writhing responses induced by acetic acid when compared to the control.

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Analgesic effect by Formalin Test

MDT demonstrated a dose-dependent relationship in late phase of the formalin induced pain. In the early phase, there were no significant inhibition at the doses of 0.1, 0.5, 1.0g/kg. MDT compared to the control group (Figure No.5A). In the late phase, the doses of 0.1, 0.5 and 1.0 g/kg significantly reduced the nociception similar to indomethacin (10 mg/kg) (Figure No.5B).

Each value was represented as mean \pm SEM. *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range tests). In the anti-inflammatory test, MDT (0.5 and 1.0 g/kg) decreased the paw edema at the 3rd, 4th, 5th and 6th hour after λ -carrageenan administration. On the other hand, MDT increased the activities of SOD and GRd in liver tissues and decreased the MDA level in the edema paw at the 3rd hour after λ -carrageenan-induced inflammation. MDT also affected. The levels of interleukin-1 β , tumor necrosis factor- α , NO and MDA which were induced by λ -carrageenan. The results suggested that MDT possessed analgesic and anti-inflammatory effects. In conclusion, MDT possessed analgesic and anti-inflammatory effects. The anti-inflammatory mechanisms of MDT might be related to the decreases in the levels of MD and NO in the edema paw via increasing the activities of SOD, GPx and GR in the liver and decreasing

the levels of IL-1 β and TNF- α in the serum of mice. MDT may be used as a pharmacological agent for

preventing and treating diseases in which free radical formation is a pathogenic factor¹³.

Table No.1: Different Chemical Constituents Present in *Desmodium Triflorum* Extraction

S.No	Class of Compounds	Aqueous extract	Methanolic	Pet.ether
1	Alkaloid	+	+	+
2	Glycoside	-	+	-
3	Steroid	+	+	+
4	Saponines	+	+	-
5	flavonoids	-	+	-
6	Carobohydrate	-	-	-
7	proteins	+	+	-
8	amino acid	+	+	-
9	phenolic	-	+	-

[+] – Present; [-] – Absent



Figure No.1: *Desmodium triflorum*

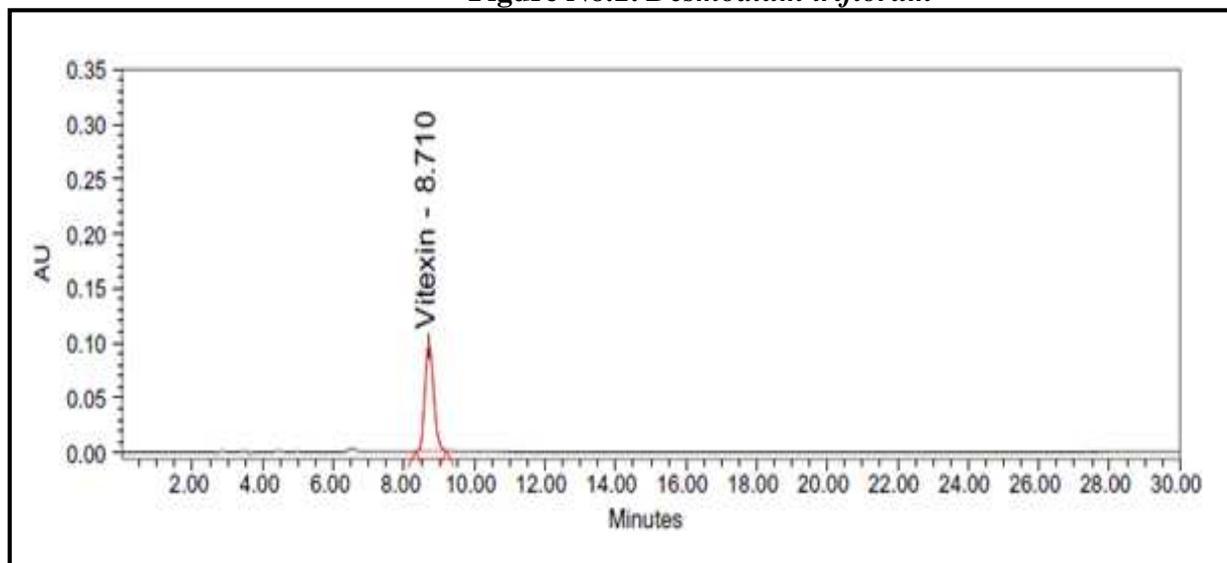


Figure No.2: Retention Time of Vitexin

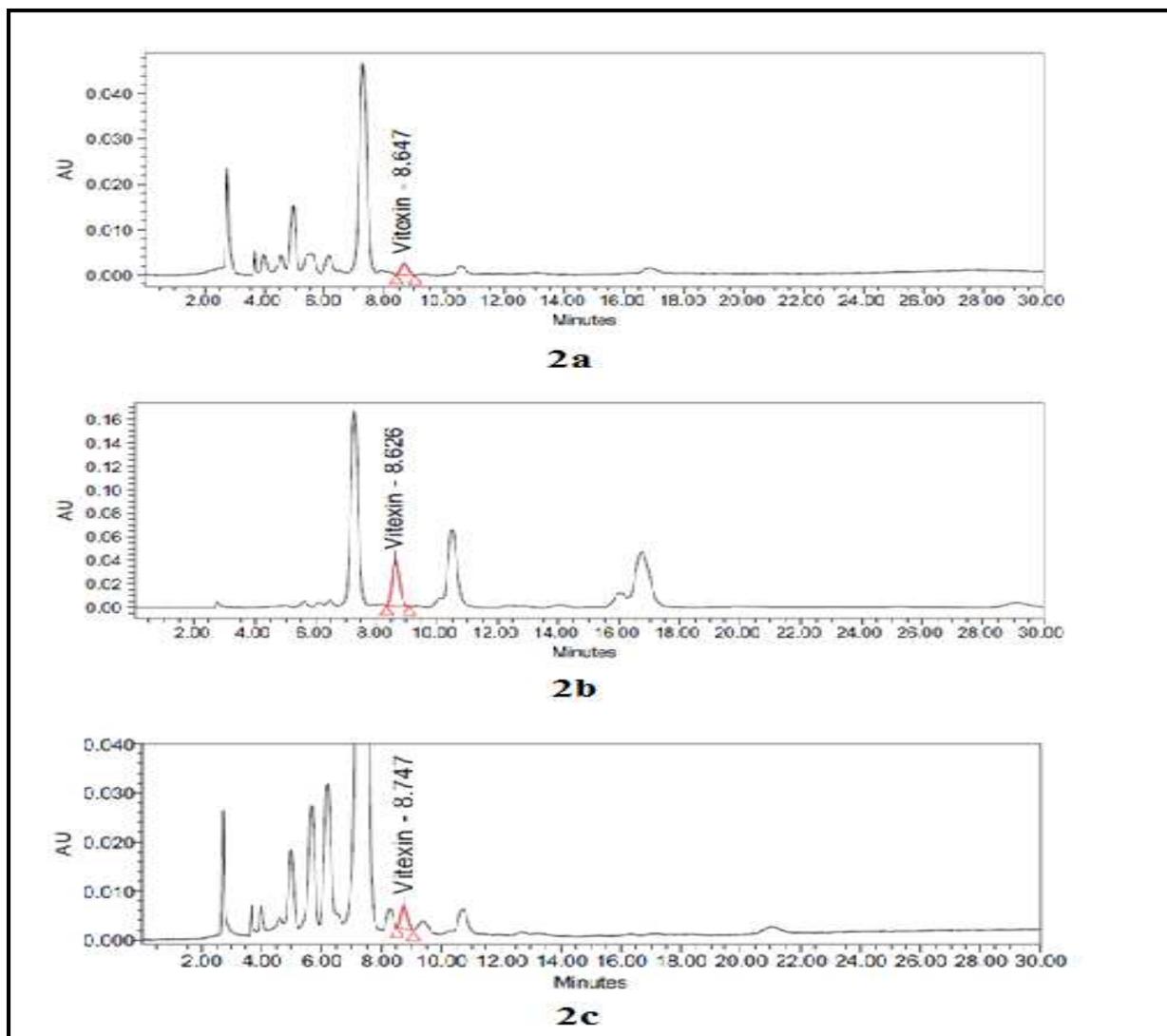


Figure No.2a, 2b, 2c: HPLC Fingerprints of the Methanol Extract, Ethyl Acetate Fraction and N-Butanol Fraction at 336nm

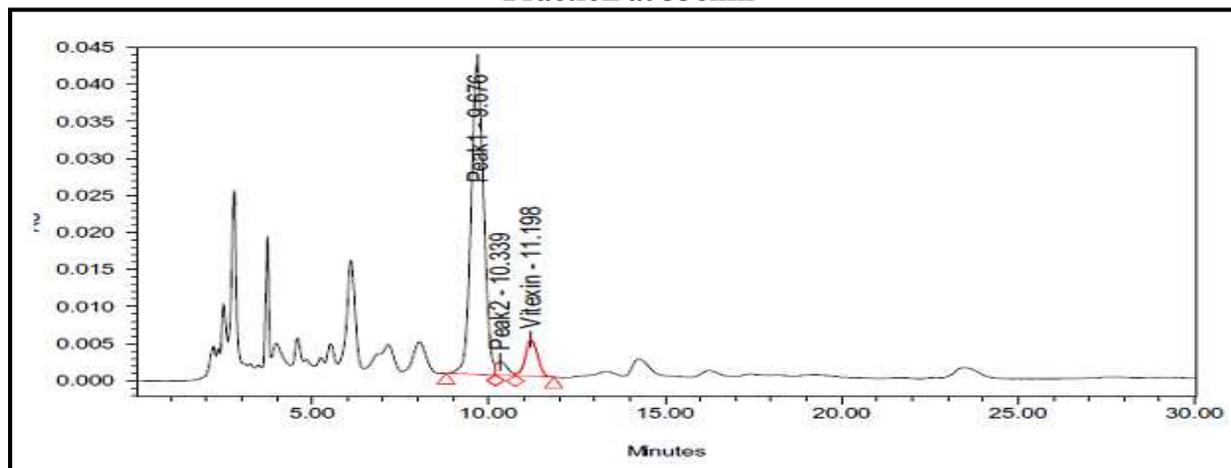


Figure No.3: HPLC fingerprint of MDT

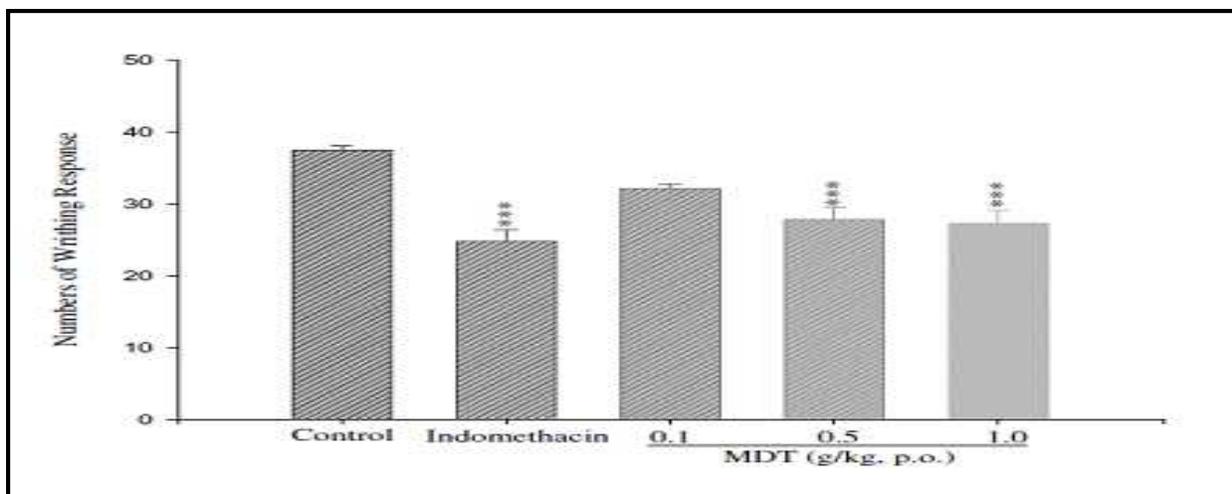


Figure No.4: Analgesic Activities of the Methanol Extracts of MDT

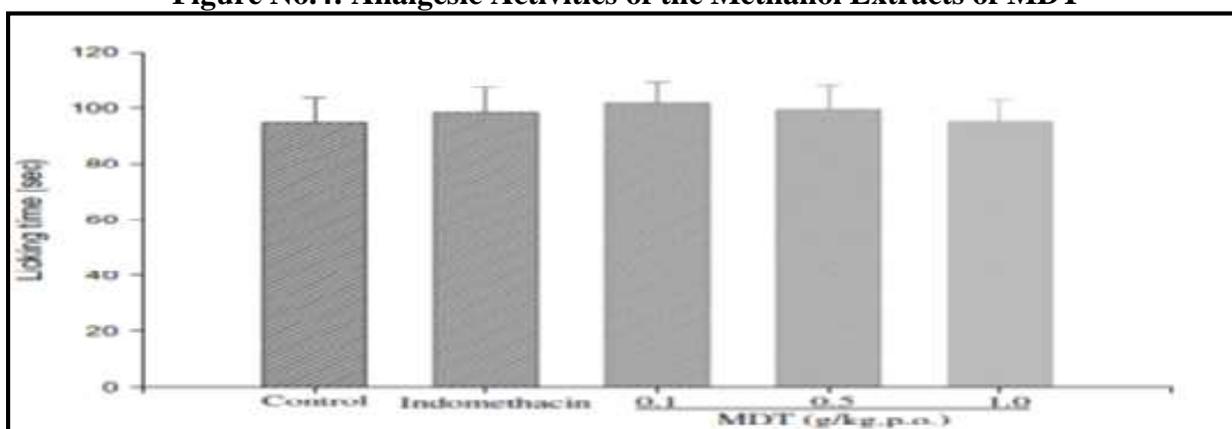


Fig. 5A

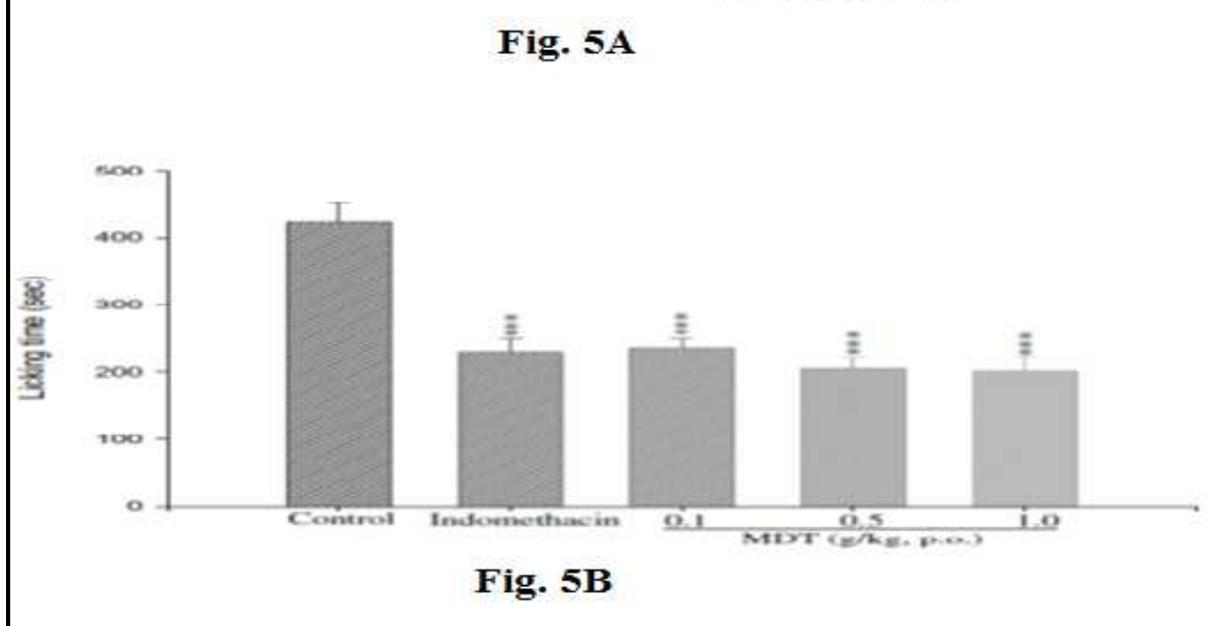


Fig. 5B

Figure No.5A and 5B: Effect of Methanol Extract of MDT and Indomethacin on the (A) Early Phase and (B) Late Phase of Formalin Test in Mice

CONCLUSION

Furthermore it is concluded that *Desmodium triflorum* has been conducted only in few activities such as anti-oxidant, anti-inflammatory, anti-convulsant, anti-bacterial studies. Various other components and activity may be present in *Desmodium triflorum* and its discovery will be beneficial for human life.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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