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# EFFECT OF *SOLANUM TORVUM* SW. METHANOLIC EXTRACT ON *IN-VITRO* AND *IN-VIVO* MODELS OF INFLAMMATION

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**Keywords:**

Inflammation, *Solanum torvum* Sw., Tiwa tribe, Morigaon, Paw edema model, RBC membrane stabilising activity

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**ABSTRACT:** The word inflammation comes from the latin word called "inflmmare" which means "To set on fire". It is a complex biological process that is mediated by several chemicals and biological mediators like pollens, irritants, pathogens and damaged cells. The body's inflammatory reaction destroys such chemical and biological mediators and gets rid of injured tissues. But in chronic state this inflammation seems to produce symptoms that are quite serious and may be life-threatening. *Solanum torvum* Sw. is a normal weed found in road side and abandoned field very often in Assam, but it's berries are used by the Tiwa tribe of Morigaon district, Assam, to treat painful inflammation caused due to trauma or any kind of infection. It is quite common among the tribe to coat the inflamed area with fresh raw berry paste of *Solanum torvum* Sw. for 2 to 3 days, they take the bitter juice of berries as drink. The author have carried out both *in-vitro* and *in-vivo* experiment on the anti inflammatory properties of this berry methanolic extract. The study showed a strong efficacy of *Solanum torvum* Sw. berry extract in the bothmodels of inflammation in compared with standard drug. A preliminary phytochemical analysis was also conducted to make sure the presence of effective phytochemicals which have anti inflammatory properties.

**INTRODUCTION:** Plants have been widely recognized as an important source of novel therapeutic compounds since ancient times for treating various diseases and were reported in traditional medicine systems such as Siddha and Ayurveda [1]. Inflammation is an adaptive response triggered by noxious stimuli and conditions such as infection and tissue injury [2]. It has extensively been demonstrated that strong and complex interconnections occur between oxidative stress and the inflammatory response [3].

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Alteration of the endocellular redox state plays a key role in the activation and dysfunction of immune cells. Here the plant kingdom contains an immense variety of secondary metabolites named phytochemicals, with significant redox-modulating properties that have been recently shown to modulate the inflammatory response 4 effectively. First considered 'health promoting' by virtue of their radical-scavenging activity of cellular biomolecule [5].

Such compounds are now believed to interfere with cell functions by intercepting reactive species at the level of critical cell signalling pathways. In addition, knowledge of the interaction of these molecules with enzymes, receptors and transcription factors has recently emerged [6]. However, the properties of natural-based mixtures need to be carefully investigated to determine their

pharmacological effects on biological system [7]. Near about every commercially available medicine of the world depend on plant-based bioactive components for curing various diseases [8].

The drugs commonly used for managing inflammatory conditions are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) which have various adverse effects, especially gastric irritation, leading to the formation of gastric ulcers. The rich wealth of plant kingdom represents a novel compound with significant anti-inflammatory properties [9]. However, most of these plant resources have not been undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound [10]. The phytochemicals such as alkaloids, flavonoids, saponin, steroids, tannins, amino acids, and trigonillin were responsible for their biological activity. The leaves and seed have been widely used to prepare concentrate and powders for restorative uses [11].

Tiwa tribes are one of the ethnic tribe of Assam, India, rich in their own culture and known for their great knowledge about medicinal plants. The authors has conducted an extensive field study and found that the tribe has used a fresh paste of *Solanum torvum* Sw. (berries) over infammed body parts due to trauma of infection or taken orally with food as an anti-inflammatory medicine. The present study was designed to investigate the anti-inflammatory effects of *Solanum torvum* Sw. in both *in-vitro* and *in-vivo* models of inflammation.

## MATERIAL AND METHODS:

**Plant Materials:** The plant material (Berries) were collected from random roadsides of Morigaon district, Assam, India. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, India (Acc. no: 46 Herbarium no: 16, Submitted on 20/1/2011). One voucher specimen was deposited there for future reference. The whole plant material was shade dried and extracted in methanol by soxhlet apparatus (MLE, yield 5%). The extracts were suspended in 0.5% Tween-80 in saline water and administered p.o.

**Experimental Animals:** For *in-vivo* experiments, Wister rats of both sexes were used. All the animals required for experiments were obtained from the Animal House Facilities of the Department of Zoology, Gauhati University, India. Experiments on animals were approved by the Institutional Animal Ethics Committee (IAEC/PER/2017/RF/BBC/AS/2016-30) and accepted according to veterinary medical practice. Animal of same age group *i.e.*, adult approximately 3 months of age weighing about 190-180 gm were taken for different experiments per the respective protocol. Animals were housed in wire mesh plastic cages with solid bottom containing sawdust, maintained under uniform condition of natural photoperiod (12 hr light/dark cycle), relative humidity ( 50-85%) and temperature (25±2°C). A complete hygienic condition of the animal house was ensured before and during experiments. All the rats had free access to water and commercially available animal diet with vitamins and minerals supplements (purchased from Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India) and were fed *ad libitum*. Body weight and clinical sign were recorded, and also a regular health check up of the animals was performed on a daily basis throughout the experimental period.

**Chemicals:** Pure sample of Indomethacene was obtained from sigma chemicals, USA. Tween-80 (Polyoxy methylene sorbitan monoleate), and Formaldehyde solution were obtained from Merck India Ltd.

Alsever solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.0% citric acid and 0.42% of sodium chloride in distilled water, followed by sterilization. Local North-East Chemicals Pvt. Ltd. supplied all chemicals to Guwahati, Assam, whenever required.

**Phytochemical Analysis:** A qualitative phytochemical test was carried out to detect the presence of volatile oils, alkaloids, tannins, saponins, flavonoids, glycosides, steroids, terpenoids and phenols utilizing standard methods of analysis [12, 13, 14]. The intensity of the colouration determines the abundance of the compound present. Qualitative phytochemical analysis of the powder of the study plant material determined as follows:

**Tannin:** For tannins, one gm plant grinded, then sample was boiled in 20 ml ethanol 70% for 2 min on a hot plate. The mixture was filtered and a

portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. Blue-black precipitate indicated the presence of tannins.

**Phenol:** For phenol 2 ml of extract was added to 2 ml of ferric chloride solution ($FeCl_3$); a deep bluish-green solution was formed with the presence of phenols.

**Alkaloid:** The test for alkaloids was carried out by subjecting 5 gm ground plant material extracted with 10 ml ammoniacal chloroform and 5 ml chloroform. After filtration, the solution was shaken with 10 drops aqueous sulphuric acid 0.5 M. Creamish precipitate indicated the presence of respective alkaloids.

**Steroid and Terpenoids:** For steroids, the Liebermann-Burchard reaction was applied. Two hundred mg plant material was boiled in 10 ml chloroform, and the mixture was filtered; a 2 ml filtrate was added to 2 ml acetic anhydride and concentrated $H_2SO_4$. The blue-green ring indicated the presence of steroids, and the red indicated terpenoids.

**Flavonoids:** The alcoholic extract (15 ml, corresponding to 3 gm of plant material) was treated with a few drops of concentrated HCl and magnesium Ribbon (0.5 gm). Pink-tomato red colour indicated the presence of flavonoids.

**Saponin:** The test for saponin was carried out by subjecting 5 gm of the plant powder extracted with 15 ml methanol. After evaporation, the residue was shaken vigorously with ethyl ether and 5 ml HCl 2N. Precipitate indicated the presence of saponin.

**Volatile Oil:** For detection of volatile oils, 1 gm fresh plant sample was boiled in 10 ml petroleum ether, filtered and then 2.0 ml of extract solution was shaken with 0.1 ml dilute sodium hydroxide and a small quantity of dilute hydrochloric acid. A white precipitate indicated the presence of volatile oils

**Glycoside:** The extract was also tested for free glycoside. Fehling`s solution (A and B) was added to the extract and the solution was heated on a hot plate and brick-red precipitate indicated the presence of glycosides.

**Toxicity Studies ($LD_{50}$):** Wistar rats of both sexes were taken for this experiment. Animals were divided in six groups (n=6) and were given different doses of plant extract (p.o.) (150, 300, 500, 1000, 2000, 3000mg/kg, b.w.) for four consecutive days, and their mortality, loss of body wt. and general behaviour was recorded from the first dose up to 72 hours after the last administration of plant extract. One group was taken as control group and was administered with normal saline (p.o.) [15].

***In-vivo*** **Anti-inflammatory Activity (Formaldehyde-induced Rat Paw Oedema):** In this method, rats were again divided into four groups of four each. The animals of each group were pre-treated with only vehicle, drug (Indomethacene 10mg/kg b.w.) and methanolic extracts in the concentration of (MLE-300mg/kg, and 600mg/kg b.w.) were given by (p.o.) one hour before formaldehyde injection. 0.2ml of 1% w/v formaldehyde was injected into the sub-plantar tissue of the left hind paw of each rat. Swellings of formaldehyde-injected foot were measured once at 0 hr, 1 hr, 2 hr and 3 hr for acute study and another in day 1, day 2, day 3, day 4 and day 15 for chronic study by using cotton [16, 17, 18, 19].

**Statistical Analysis of the Data:** Values are expressed as Mean ± SEM & significance within the group was analyzed using Student's t test and $P < 0.05$ and $P < 0.01$ was considered to be significant [20].

**Calculation of Inhibition (%):** The percentage of inhibition of pow volume for each biological parameter was calculated using the following formula:

$$\text{Inhibition rate I \%} = (Vt-Vo)\,C-(Vt-Vo)\,E\,/\,(Vt-Vo)C$$

Where, Vt =left hind pow volume at time t, Vo=left hind pow volume before sub plantar injection, C= control group, E= experimental group [21].

***In-vitro*** **Anti inflammatory Activity:**

***In-vitro*** **Anti-inflammatory Study:**

**Collection of Blood:** Blood was collected from healthy rat by using cardiac puncture method. Blood was obtained from the heart of a deeply anesthetized rat in a terminal procedure. Rat was laid on its back and a 25 to 30 gauge needle

attached to a 3-5 ml syringe was inserted just behind the xiphoid cartilage and slightly left of the middle. The needle was introduced at 10-30 degrees from the horizontal axis of the sternum in order to enter the heart. The blood was collected slowly to avoid heart collapse.

**Membrane Stabilizing Activity of Rat RBC:** The blood collected from rat was mixed equal volumes of Alsevers solution. The blood was centrifuged at 3000 rpm, the packed cells were washed with isosaline and 10% (v/v) suspension was made. Different concentrations of plant extract (50, 100, 150, 200,250, 500 and 1000 µg/ml) were prepared by suspending them in hot water. The assay mixture contained the extract, 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml RBC suspension. Hydrocortisone sodium was used as the reference drug and 2 ml of distilled water as Control. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm [22].

**Calculation:**

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) × 100

The percentage of Rat RBC membrane stabilization can be calculated as follows:

% Protection = 100 – (Optical density of Test sample / Optical density of Control) × 100 [23].

**RESULT:**

**Preliminary Phytochemical Screening of Plant Sample:** Preliminary qualitative phytochemical screening of plant sample was done using standard methods of analysis, where the abundance of the compound present was determined by observing the intensity of the colouration. The results of the preliminary phytochemical screening are presented in **Table 1**. The result shows a strong indication for the presence of tannin, phenol, alkaloid, saponin and flavonoid, was very low indication for the presence of steroids and glycosides and no indication for the presence of terpenoid and volatile oil.

**TABLE 1: RESULTS OF PRELIMINARY PHYTO-CHEMICAL ANALYSIS OF PLANT SAMPLE**

| Phytochemicals | Indication |
|---|---|
| Tannin | +++ |
| Phenol | +++ |
| Alkaloid | +++ |
| Flavonoid | +++ |
| Steroid | + |
| Saponin | +++ |
| Glycosides | + |
| Terpenoid | – |
| Volatile oil | – |

+++: strong indication of presence, ++: medium indication, presence, +: very low indication of presence, -: absent.

**Acute Toxicity Study of the Plant Extract (LD-50 Study):** A number of doses of the methanol extract of *Solanum torvum Sw.* (berries) were administered to rat in the (LD-50) study.

Rat were subjected to different doses of MLE (150, 300, 500, 1000, 2000, 3000, 5000, 8000 and 10,000 mg/kg) (p.o.) for four consecutive days and their mortality, loss of body weight and general behaviour recorded from the first dose up to 72 hours after the first administration. As in **Table 2,** no mortality (0%) was recorded.

**TABLE 2: TREATMENT SCHEDULE OF ADMINISTRATION OF PLANT EXTRACT FOR ACUTE TOXICITY STUDY IN RAT**

| **Group** | **Doses (mg/kg)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Normal Control** | **Not significant** | | | | | | | | |
| **Mortality** | **0%** | | | | | | | | |
| *Solanum torvum* (berries) | 150 | 300 | 500 | 1000 | 2000 | 3000 | 5000 | 8000 | 10,000 |
| Mortality | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

***In-vivo* Anti-inflammatory Study:** Edema was induced in left hind paw on each rat by injecting 1% formaldehyde solution in all the 4 groups. All the animals were pre-treated with vehicle (group I), 300mg/kg body weight concentration of plant extract (group II), 600 mg/kg body weight concentration of plant extract(group III) and 10 mg/kg body weight concentration of Indomethacene as a positive control (group IV) before giving the formaldehyde injection. At the acute phase of the experiment the result showed a significant decrease in the paw volume ($P<0.05$) at

the first hour, second hour and third hour after an injection of formaldehyde in positive control groups. But in the experimental groups (group II and group III) no significant result was seen at the acute phase of the experiment **Table 3.**

**TABLE 3: EFFECT OF METHANOLIC EXTRACT OF *SOLANUM TORVUM* SW. AND REFERENCE DRUG ON THE SWELLING OF RAT HIND PAW INDUCED BY FORMALDEHYDE (ACUTE PHASE)**

| Groups | Treatment | 0hr | 1hr | 2hr | 3hr |
|---|---|---|---|---|---|
| I | Vehicle treated as negative Control | 2.63±0.048 | 3.55±0.029 | 3.5±0.025 | 3.45±0.029 |
| II | Extract treated (300mg/kg body weight) | 2.67±0.025 $^{NS}$ | 3.53±0.048 $^{NS}$ | 3.4±0.04 $^{NS}$ | 3.3±0.092 $^{NS}$ |
| III | Extract treated (600mg/kg body weight) | 2.63±0.048 $^{NS}$ | 3.47±0.063 $^{NS}$ | 3.35±0.065 $^{NS}$ | 3.33±0.048 $^{NS}$ |
| IV | Indomethacene treated as positive control(10mg/kg body weight) | 2.6±0.041 $^{NS}$ | 3.23±0.111* | 3.23±0.095* | 3.15±0.087* |

Student's t-test: *P< 0.05; NS: Not Significant.

**Percentage of Inhibition in Paw Volume (Acute Phase):** The result of the percentage of inhibition in paw volume of rat at formaldehyde induced rat paw edema model showed not a very satisfactory result at acute phase. Both the experimental group displayed neck-to-neck results, where the positive control group showed a higher percent of inhibition compared to both group II and group III **Fig. 1.** At the chronic phase of the experiment, the result showed a significant decrease in the paw volume (P<0.01) in both the experimental group from second to 15$^{th}$ day after giving formaldehyde injection. The positive control group was also showing significant results from day 1(P<0.05) to day15 (P<0.01) **Table 4.**

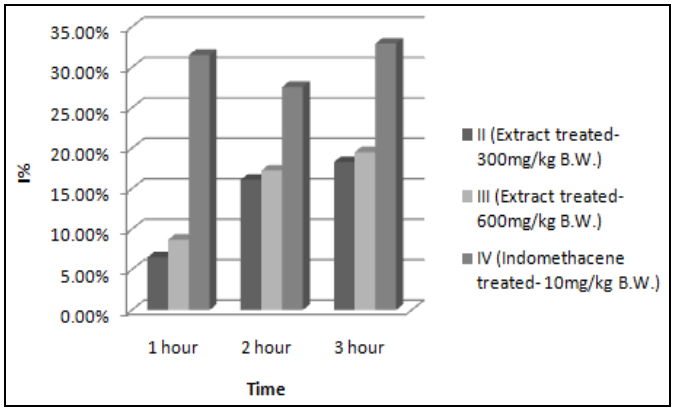


**FIG. 1: FIGURE SHOWING PERCENTAGE OF INHIBITION AGAINST SWELLING OF RAT HIND PAW INDUCED BY FORMALDEHYDE (ACUTE PHASE)**

**TABLE 4: EFFECT OF METHANOLIC EXTRACT OF *SOLANUM TORVUM* SW. AND REFERENCE DRUG ON THE SWELLING OF RAT HIND PAW INDUCED BY FORMALDEHYDE (CHRONIC PHASE)**

| Groups | Treatment | Day 1 | Day2 | Day 3 | Day4 | Day15 |
|---|---|---|---|---|---|---|
| I | Vehicle treated as negative Control | 3.45±0.029 | 3.37±0.025 | 3.35±0.029 | 3.33±0.025 | 3.27±0.048 |
| II | Extract treated (300mg/kg body weight) | 3.33±0.092$^{NS}$ | 3.15±0.029** | 2.97±0.058** | 2.97±0.048** | 2.93±0.025** |
| III | Extract treated (600mg/kg body weight) | 3.30±0.048 $^{NS}$ | 3.07±0.029** | 2.85±0.029** | 2.83±0.025** | 2.75±0.029** |
| IV | Indomethacene treated as positive Control (10mg/kg body weight) | 3.15±0.087* | 3.03±0.025** | 2.83±0.025** | 2.77±0.063** | 2.67±0.048** |

Mean± SEM. n=4, Student's *t*-test: *$P$<0.05;**P<0.01; $^{NS}$: Not Significant.

**Percentage of Inhibition in Paw Volume (Chronic Phase):** Calculation of percentage of inhibition in chronic phase of formaldehyde induced modal of rat revealed that the 3$^{rd}$ experimental group showed neck to neck result in percentage of inhibition in paw volume with the positive control group, where the result of 2$^{nd}$ experimental group displayed lower percentage of inhibition comparing to the 3$^{rd}$ experimental group

and the positive control group from 1st day to 15th day of experiment **Fig. 2.**

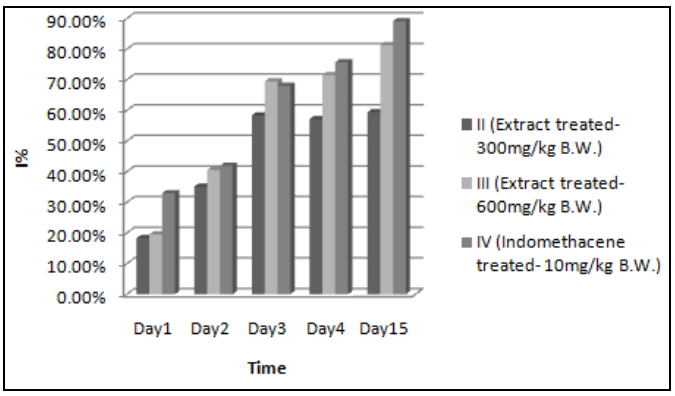


**FIG. 2: FIGURE SHOWING PERCENTAGE OF INHIBITION AGAINST SWELLING OF RAT HIND PAW INDUCED BY FORMALDEHYDE (CHRONIC PHASE)**

***In-vitro* Anti-inflammatory Study:** The inhibition of hypotonicity-induced rat RBC membrane lysis *i.e,* stabilization of RBC membrane was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilisation for methanolic extracts and Hydrocortisone sodium were done at 50, 100,150,200, 250, 500, 1000 µg/ml. Methanolic extracts of *Solanum torvum* Sw. are effective in inhibiting the heat-induced hemolysis of rat RBC at different concentrations (50-2000µg/ml) as shown in **Table 5.** It showed a maximum inhibition 94.97% at 1000µg/ml. With the increasing concentration, the membrane hemolysis is decreased, as shown in **Fig. 3** and membrane stabilization/protection is increased, as shown in **Fig. 4.**

**TABLE 5: RESULTS OF *IN-VITRO* ANTI-INFLAMMATORY EFFECT OF *SOLANUM TORVUM* SW. SHOWING PERCENTAGE OF HEMOLYSIS AND PROTECTION OF RAT RBC MEMBRANE WHEN TREATED WITH PLANT EXTRACT AND HYDROCORTISONE SODIUM**

| Conc. (µg/ml) | % Hemolysis of *S. Torvum* MLE | % Protection of *S. Torvum* MLE | % Hemolysis of Hydrocortisone sodium | % Protection of Hydrocortisone sodium |
|---|---|---|---|---|
| 50 | 32.27 | 67.72 | 48.32 | 51.67 |
| 100 | 21.65 | 78.34 | 25.36 | 74.63 |
| 150 | 18.12 | 81.87 | 18.68 | 81.31 |
| 200 | 13.23 | 86.76 | 14.38 | 85.61 |
| 250 | 9.11 | 90.88 | 8.28 | 91.71 |
| 500 | 7.34 | 92.65 | 5.13 | 94.86 |
| 1000 | 5.02 | 94.97 | 1.29 | 98.70 |

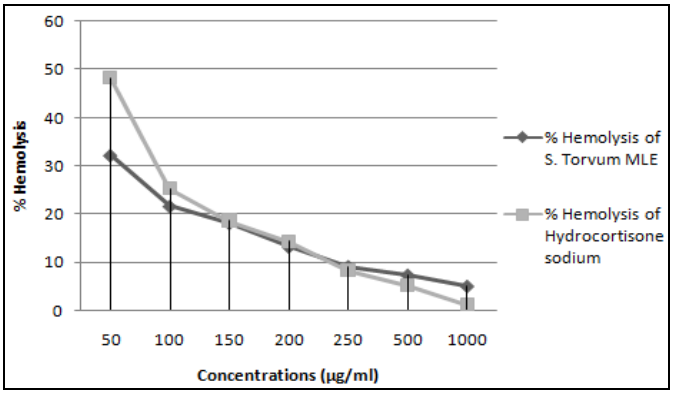


**FIG. 3: EFFECT OF *SOLANUM TORVUM* SW. ON RAT RBC MEMBRANE HEMOLYSIS**

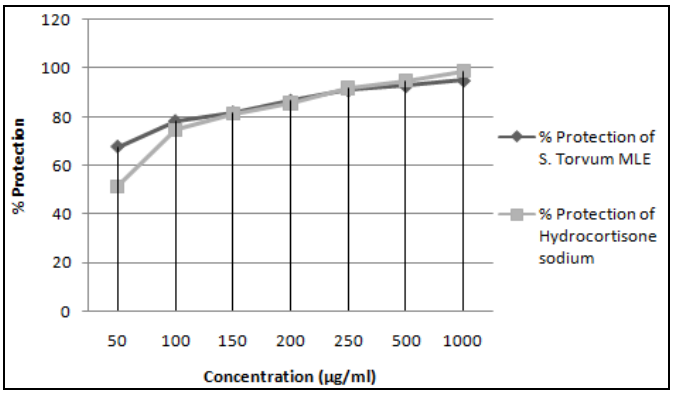


**FIG. 4: EFFECT OF *SOLANUM TORVUM* SW. ON RAT RBC MEMBRANE STABILISATION**

**DISCUSSION:** Aromatic and medicinal plants are very important sources of secondary metabolites, which have a range of applications in the Control of human and plant disease, the pharmaceutical industry and cosmetics [24]. Polyphenolic compounds are secondary plant metabolites that exist as monomers, oligomers, and polymers. They are presented as simple phenols, flavonoids, lignans and their derivatives, tannins etc. various studies determined that plant polyphenols have a wide range of pharmacological activities, including anti-inflammatory action [25]. In the present study, authors found the presence of Tannins, Phenol, alkaloids, flavonoids, and saponins in the plant sample. In the anti-inflammatory study, experiment on the *in-vivo* model, the higher dose 600mg/kg B.W. shows a neck-to-neck percentage of edema inhibition like the standard indomethacin drug.

At *in-vitro* model also, the authors found promising anti-inflammatory effect by the plant extract. The results were compared with the standard hydrocortisone sodium which showed a 98.70 % protection1.29% haemolysis, where our plant extract showed 94.97% protection and 5.02% haemolysis in higher concentration. The extract exhibited membrane stabilisation by inhibiting hypotonicity-induced lyses of the erythrocyte membrane [26] and its stabilisation implies that the extract may stabilise lysosomal membrane. Stabilisation of lisosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage by extracellular release [27]. The extract may inhibit these processes, stimulating or enhancing the efflux of these intracellular components [28].

**CONCLUSION:** *Solanum torvum* Sw. is found widespread in India. This plant has potential anti-inflammatory activity, which confirms its therapeutic potential. This plant is an important source of phytochemicals with pharmaceutical potential; therefore, this plant could lead to the isolation of novel agents with good efficacy in treating inflammation.

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