



Research Article

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Phytochemistry and evaluation of *in vivo* antioxidant and anti-inflammatory activities of *Oroxylum indicum* Vent. root bark

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ABSTRACT

Oroxylum indicum Vent., (Bignoniaceae) tree roots form ingredients of Ayurvedic formulation, Dasamoola. Methanolic extract of the root bark was tested for its *in vivo* antioxidant and anti-inflammatory activities, followed by phytochemical analyses. Antioxidant effect of *O. indicum* root bark extract was studied on sodium fluoride mediated oxidative stress-induced animals. Animals were pre-treated orally with the extract at dosages of 200 and 400 mg/kg b.wt. Standard group received 3mM/L/day of ascorbic acid orally. From 8th day onwards, oxidative stress induction was carried out by the administration of 600 ppm/day of sodium fluoride orally which continued upto 14th day, along with extract treatment. Antioxidant profile of blood, measured as superoxide dismutase activity (SOD) and glutathione level were found to be enhanced in *O. indicum* extract treated group (OIM) and standard group, when compared to untreated control. Similar trend was noticed in SOD, GSH and GPx levels in liver tissue, even after sodium fluoride challenge. Extract treatment significantly reduced the extent of lipid peroxidation in liver tissue. In acute and chronic paw oedema models treated with dosages of the extract at 150 and 300 mg/kg b.wt., significant reduction in inflammation was observed, when compared to the control animals. The extent of inhibition of oedema in carrageenan induced acute models and formalin induced chronic models were 41.28 and 48.57% respectively, for the high dosage groups. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins and terpenoids in the extract.

Key words: *Oroxylum indicum*, Antioxidant, Anti-inflammatory

INTRODUCTION

Dasamoola refers to a medical formulation consisting of the roots of ten medicinal plants, used in the indigenous system of Indian medicine namely *Ayurveda*. All the ten plants are endowed with credible medicinal properties and they act synergistically in combination too. *Oroxylum indicum* Vent., the plant in the present study, is a member of family Bignoniaceae, medium-sized, deciduous tree in habit, found in the Eastern and Western Ghats and North-East regions of India. The medicinal preparations of the plant find use in the pacification of rheumatic disorders, diarrhoea, cough, diabetes, cystitis as well as a number of skin ailments [1].

Different parts of *Oroxylum indicum* viz., leaves, seeds, pods, stem and roots have also been individually studied for their biological activities and have been screened for the presence of phytochemical constituents. The ethyl acetate, methanolic, and aqueous extracts of the leaves are reported to have *in vitro* antioxidant properties [2]. The stem bark extract of the plant has also been evaluated to possess anti microbial properties against the strains of *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* [3]. By subjecting the dichloromethane and hexane

fractions of *O. indicum* stem bark to column chromatography and preparative TLC, four compounds viz., baicalein, oroxyline, pinostrobin and stigmast-7-en-3-ol have been isolated [4]. The preparations of the stem bark powder extracted using petroleum ether, benzene, chloroform, ethanol and water have been analysed for their anti oxidant properties through the β -carotene bleaching assay [5] and have produced significant results. Five flavonoids have been isolated from the seeds of *Oroxylum indicum* by high speed counter current chromatography and identified four of them as baicalein-7-O-glucoside, baicalein-7-O-diglucoside, baicalein and chrysin, using high-performance liquid chromatography–mass spectrometry and nuclear magnetic resonance [6]. Methanolic extract of the fruits revealed notable antimutagenicity towards the food derived mutagen Trp-P-1, determined by Ames's pre-incubation method using *Salmonella typhimurium* TA98 [7]. Chrysin, isolated from the roots of *Oroxylum indicum* showed significant nephroprotective activity in cisplatin induced animal models by the reduction of glomerular congestion, as revealed by histopathological analysis. The treated animals exhibited nearer normalcy in urinary functional parameters too [8].

Besides the plant parts of *Oroxylum indicum* discussed above, endowed with outstanding medicinal properties, there is scope for further exploration of the biological attributes conceived by its root bark. Moreover, the roots form one among the ten ground materials of *Dasamoolapreparations*, the *in vitro* and *in vivo* properties of root bark need detailed investigation. Tree barks are limitless sources of pharmacognostically important phytochemicals; preliminary phytochemical screening indicated that the root bark of *Oroxylum indicum* was rich in flavonoids [9] and had earlier been reported to contain compounds like chrysin, baicalein, biochanin-A, and ellagic acid through TLC and RP-HPLC [10]. Among these, baicalein has already been demonstrated to inhibit growth of human prostate cancer cell lines through apoptotic induction, and to reduce the tumour volume in *in vivo* murine models. The anti-angiogenic nature of this compound has been proved to be high using the sprout formation assay [11].

Regarding these worth-mentioning attributes of the root bark, the current attempt is an investigation of the *in vitro* cytotoxic, *in vitro* and *in vivo* antioxidant and *in vivo* anti-inflammatory activities of the methanolic extract of *O. indicum* root bark. Cytotoxicity was determined through the trypan blue exclusion method, antioxidant activities through free radical scavenging assays and anti-inflammation using carrageenan and formalin induced acute and chronic paw oedema models.

EXPERIMENTAL SECTION

1.1. Collection of plant material and preparation of the extract

The roots of *Oroxylum indicum* were collected from the Ayurvedic Garden maintained in the Amala Cancer Hospital campus and were authenticated by Dr. Sujanalal., Taxonomist, KFRI, Peechi, Kerala. The bark was isolated by peeling, and then dried at 45°C and was ground to fine powder for soxhlet extraction using methanol as solvent. The extract was filtered, concentrated and evaporated to dryness and the residue (OIM) re-dissolved in distilled water for further studies.

1.2. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and carrageenan were purchased from Sigma-Aldrich, India. Thiobarbituric acid was purchased from Himedia Laboratories. All the other reagents and chemicals used were of analytical grade.

1.3. Animals

Female Swiss albino mice of body weight ranging from 25-30 g were purchased from the Small Animal Breeding station, Agricultural University, Mannuthy, Kerala, India. The animals were lodged in the animal house at Amala Cancer Research Centre, under a standardized environmental temperature range of 22-28°C, relative humidity of 60-70% and exposure to 12 hour dark-light cycle and were brought up with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. Prior approval was procured from the Institutional Animal Ethics Committee for the *in vivo* experiments conducted, obliging strictly to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

1.4. Preliminary in vitro antioxidant assays

As a preliminary step before *in vivo* analysis, *in vitro* antioxidant assays were conducted using the extract to check its ability to scavenge superoxide, 2-diphenyl-1-picrylhydrazyl(DPPH), ABTS and hydroxyl radicals, as well as its reducing power using ferric reducing antioxidant power (FRAP) assay [12,13,14,15].

1.5. Acute toxicity studies for the determination of LD₅₀

The acute toxicity of OIM extract was performed as per OECD – 423 guidelines (Acute oral toxicity). For each dosage tested, a sample size of 5 female Swiss albino mice of body weight ranging from 25-30 g was used for the study. The mice were kept under starvation for 3 hours prior to drug administration, with only food withheld, but not water. The animal weight was estimated, followed by oral administration of the extract at a single dosage of 500 mg, 1 g and 2 g per kg body weight, for 3 different groups using oral gavage. One group was maintained as untreated control, administered with distilled water alone. The treated mice were individually observed for the next 14 days for death, behavioural changes such as increased motor activity, tremors, lacrimation, muscle spasm etc., or diarrhoea. Determination of body weight was done on the 2nd, 7th and 14th days and food intake on each day was also calculated. Lethal dose₅₀ (LD₅₀) value was determined on the basis of mortality of the treated animals.

1.6. In vivo antioxidant studies

The anti-oxidant activity of OIM extract was determined using sodium fluoride mediated oxidative stress induced female Swiss albino mice models.

1.6.1. Drug treatment

Thirty mice were divided into five groups- normal, control, standard, OIML (low dose) and OIMH (high dose), each with six animals. For the first 7 days of the study, the following treatment protocol was maintained.

Normal and control - kept untreated
Standard - Ascorbic acid, 3 mM/L/day through drinking water
OIML and OIMH- OIM extract, 200 and 400 mg/kg b.wt respectively (*p.o*)

From 8th day to 14th, all groups except normal, received sodium fluoride (NaF) through drinking water at a concentration of 600 ppm/day. All animals were sacrificed on the 15th day following overnight fasting. Change in body weight of all animals over the period of sodium fluoride induction was determined, prior to sacrifice.

1.6.2. Sample collection

Blood was collected in heparinised tubes. Liver weight was determined for calculating the organosomatic index [(organ weight/body weight) x 100]. Afterwards, 25% homogenates of liver tissue were prepared in ice cold 0.1 M Tris buffer (pH 7).

1.6.3. Evaluation of lipid peroxidation level as well as blood and liver tissue antioxidant status

The anti-oxidant profile of blood and liver tissue was checked through determination of reduced glutathione (GSH) and superoxide dismutase (SOD) using standard methods[16]and [12]. Liver tissue was also subjected to the estimation of glutathione peroxidase (GPx) activity [17] and the extent of lipid peroxidation in terms of n mols of malondialdehyde (MDA) generated/mg protein[18].

1.7. Anti-inflammatory studies

The anti-inflammatory activity of OIM extract was evaluated on the basis of its ability to bring down acute and chronic paw oedema respectively in carrageenan and dextran injected female Swiss albino mice models.

1.7.1. Drug treatment

Animals of body weight ranging from 25-30 g were divided into the following 4 groups, each with sample size 6 according to the following treatment protocol.

I. Untreated control:- 100 μ l distilled water, *p.o*.
II. Standard reference drug:- 100 μ l Diclofenac (10 mg/kg b.w.), *i.p*.
III. OIM 150 mg/kg b.w.:- 100 μ l of *Oroxylum indicum* extract (150 mg/kg body weight), *p.o*.
IV. OIM 300 mg/kg b.w.:- 100 μ l of *Oroxylum indicum* extract (300 mg/kg body weight), *p.o*.

1.7.2. Carrageenan induced acute inflammation

One hour prior to the injection of carrageenan, the paw thickness was read using vernier callipers and were given drug treatment. Acute inflammation was induced in all groups by the sub-plantar injection of 0.02 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hand paw. The paw thickness was then measured up to the 6th hour of induction at one hour intervals.

1.7.3. Formalin induced chronic inflammation

The sub-plantar injection of 0.02 ml of 1% solution of formalin into the left hand paw of mice induced chronic inflammation in female Swiss albino mice models. Prior to induction, the animals were subjected to drug treatment, which was continued for the next 6 consecutive days in the same time regime. The paw readings were taken one hour before and one hour after formalin injection. Thereafter, the measurements were continued in an interval of 24 hours up to day 6. Mean \pm SD values were determined for the 6 animals in each group.

The percentage of inhibition of acute and chronic paw oedema for the standard, OIM low dose group and OIM high dose group were calculated using the method described in [19].

1.8. Phytochemical analyses

Preliminary phytochemical analyses were carried out to detect the presence of alkaloids, flavonoids, tannins, phenols, terpenoids, saponins and phytosterols using standard protocols [20, 21, 22].

1.9. Statistical analysis

The values were expressed in mean \pm SD of 3 independent experiments (for *in vitro* studies) or 6 animals per group (for *in vivo* studies). All groups were analysed for one way ANOVA by Dunnett's test using Graph Pad Instat software. The groups with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Tree barks are outstanding sources for a broad spectrum of compounds investigated for their phyto-pharmacognostic properties; noteworthy examples are those of *Cinchona*, *Premnaserratifolia*, *Moringaoleifera* and *Azadirachtaindica* [23]. Many studies have been conducted in plant bark derived compounds to divulge their antioxidant and anti-inflammatory properties along with limited toxicity posed to the tested subjects. In the *in vitro* system containing superoxide radicals generated by photo-reduced riboflavin, an extract concentration of 63.32 $\mu\text{g/ml}$ of OIM was found to cause 50% inhibition. Moreover, at 150 $\mu\text{g/ml}$, the extract led to 97.77% inhibition of superoxide radicals (Fig. 1a). An extract concentration of 60.28 $\mu\text{g/ml}$ showed 50% inhibition of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the assay system (Fig. 1b). The OIM extract exhibited significant activity in scavenging hydroxyl radicals generated by the Fe^{3+} /ascorbate /EDTA/ H_2O_2 system, with IC_{50} value of 95.78 $\mu\text{g/ml}$ (Fig. 1c). The extract effectively scavenged the ABTS radicals of the assay system in a concentration-dependent manner. The percentages of inhibition attained at 9, 12 and 15 $\mu\text{g/ml}$ were 74.35, 83.11 and 85.34% respectively. The extract showed IC_{50} value at 6.1 $\mu\text{g/ml}$ (Fig. 1 d). In the assay for determining the ferric reducing power of OIM extract, the reducing equivalents of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ profoundly increased with increase in concentration of the extract. At concentrations of 4, 12 and 20 $\mu\text{g/ml}$ of OIM extract, the reducing equivalents were determined as 2.27, 2.96 and 3.15 mmoles, respectively.

Overproduction of free radicals in cells can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans. Consequently, a correlation has been derived between the anti oxidant nature of a wide array of biologically obtained compounds and their efficacy in cancer therapy. A number of plants with characteristic anti-cancer effects, have also been proved to yield compounds with remarkably high anti-oxidant properties. *Catheranthusroseus* yielding anti-cancer phytochemicals such as vincristin and vinblastin has been demonstrated to exhibit maximum anti oxidant activity, as revealed through DPPH radical scavenging assay and inhibition of peroxidation in linoleic acid system [24]. By mitigating the oxidative stress, anti oxidant compounds slow down the degenerative tendency of tissues. In the current study, significant scavenging of free radicals occurred in the presence of *Oroxylum indicum* root bark extract, determined through superoxide, DPPH, hydroxyl and ABTS scavenging assays, and also through the FRAP assay, exploring the scope of using this plant extract in therapeutic purposes.

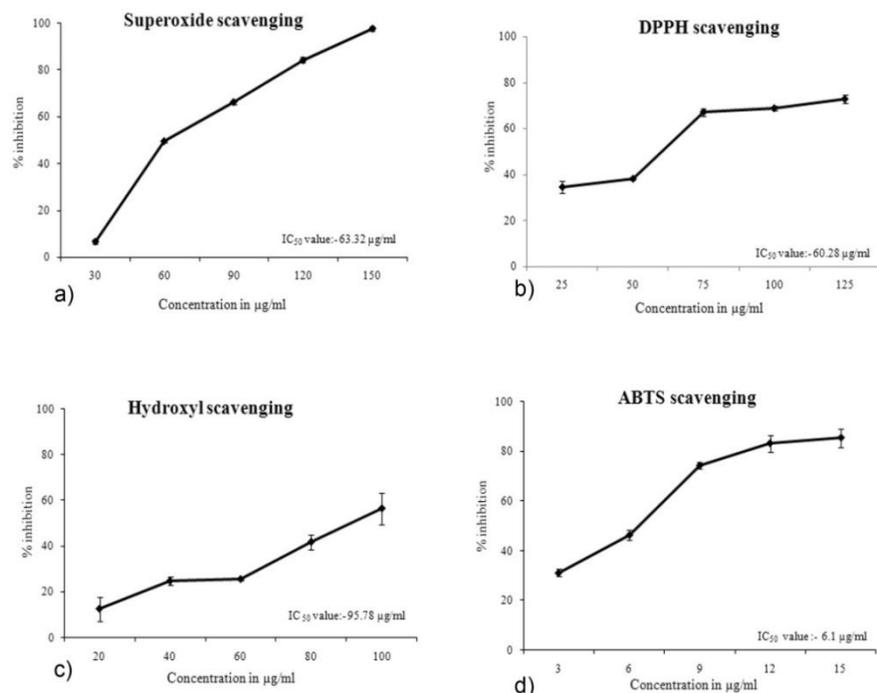


Figure 1.a-d. Free radical scavenging activities of *Oroxyllum indicum* root bark extract
Values plotted are mean \pm SD of experiments run in triplicate

The acute toxicity studies conducted in female Swiss albino mice indicated neither signs of toxicity nor mortality, concluding that the LD₅₀ value of OIM extract was higher than a dosage of 2 g per kg b.wt. The food intake was also found to be normal for all days throughout the experiment.

The present study was also focussed on the evaluation of in vivo anti-oxidant properties of methanol extract of *O. indicum* root bark.

Table 1. Effect on body weight-gain and organosomatic index of liver in sodium fluoride induced oxidative stress

Values are expressed as mean \pm SD for 6 animals; ns- not significant, when control is compared to normal, and std, OIML and OIMH compared to control

Animal groups	Body weight-gain in gm	Organosomatic index of liver (%)
Normal	17.25 \pm 1.08 ^{ns}	33.56 \pm 3.14 ^{ns}
Control	17.00 \pm 0.86 ^{ns}	34.12 \pm 4.08 ^{ns}
Standard	16.50 \pm 1.69 ^{ns}	31.26 \pm 2.98 ^{ns}
OIML	17.75 \pm 0.87 ^{ns}	32.47 \pm 1.98 ^{ns}
OIMH	16.75 \pm 0.93 ^{ns}	32.41 \pm 2.98 ^{ns}

As evident from Tab 1, no significant change was observed in body weight gain or the organo somatic index of liver, on comparison between normal, control or treated groups. In blood of untreated control mice induced with sodium fluoride alone, there was significant ($p \leq 0.01$) reduction of superoxide dismutase activity (15.13 \pm 3.1 U/g Hb) and glutathione level (8.13 \pm 0.81 nmoles/g Hb), when compared to normal group. Meanwhile, in pre-treated groups subjected to OIM and NaF, these anti-oxidant markers in blood showed significant elevation in a dose dependent manner, compared to the stress induced control (Tab 2).

Table 2. Effect on blood antioxidant status in sodium fluoride induced stress

Values are expressed as mean \pm SD for 6 animals; ^a $p < 0.01$, ^b $p < 0.05$ compared to normal; ^c $p < 0.01$, ^d $p < 0.05$ compared to control

Animal groups	SOD (U/g Hb)	GSH (nmoles/g Hb)
Normal	32.13 \pm 2.99	12.13 \pm 0.99
Control	25.13 \pm 3.1 ^a	8.13 \pm 0.81 ^a
Standard	32.13 \pm 3.7 ^c	11.73 \pm 0.72 ^c
OIML	28.13 \pm 3.6 ^d	12.09 \pm 0.6 ^c
OIMH	31.13 \pm 4.1 ^c	12.16 \pm 0.51 ^c

The effects of pre-treatment were similar to those shown by the ascorbic acid treated standard animals. Sodium fluoride induction had significant ($p \leq 0.01$) reducing effect on antioxidant profile of liver (Tab 3), as evident from the decrease in reduced glutathione level as well as SOD and glutathione peroxidase activities in control animals. But, OIM pre-treated groups apparently restored near normal values of these parameters, even after sodium fluoride exposure through drinking water.

Table 3. Effect on liver lipid peroxidation level and tissue antioxidant status in sodium fluoride induced rats

Values are expressed as mean \pm SD for 6 animals; ^a $p < 0.01$, ^b $p < 0.05$ compared to normal; ^c $p < 0.01$, ^d $p < 0.05$ compared to control

Animal groups	LPO (nmoles of MDA/mg protein)	SOD(U/mg protein)	GPx(U/mg protein)	GSH(nmoles/mg protein)
Normal	0.185 \pm 0.005	0.213 \pm 0.012	8.15 \pm 0.39	9.92 \pm 0.133
Control	0.427 \pm 0.006 ^a	0.123 \pm 0.010 ^a	3.93 \pm 0.30 ^a	5.11 \pm 0.102 ^a
Standard	0.398 \pm 0.008 ^c	0.201 \pm 0.007 ^c	7.86 \pm 0.73 ^c	8.99 \pm 0.110 ^c
OIML	0.407 \pm 0.016 ^c	0.165 \pm 0.014 ^d	8.14 \pm 0.93 ^c	7.36 \pm 0.096 ^d
OIMH	0.413 \pm 0.012 ^c	0.197 \pm 0.009 ^c	8.31 \pm 1.01 ^c	8.67 \pm 0.167 ^c

There was notable decrease in the extent of lipid peroxidation (measured as nmols of MDA/mg protein) in extract treated animals given low and high doses, (0.407 \pm 0.016 and 0.413 \pm 0.012 nmoles/mg protein, respectively) when compared to untreated control group. The above results obtained in OIM treated animals were parallel to those obtained in the standard animals treated with ascorbic acid.

Similar enhancement of blood and hepatic tissue anti-oxidant profiles were reported in oxidative stress induced animals treated with various plant extracts [25, 26, 27, 28]. Oxidative stress was induced in rats by the administration of sodium fluoride through drinking water. Sodium fluoride intoxication is reported to generate free radicals in the body, thereby leading to inhibition of enzyme systems as well as hepatic, cardiac, renal and testicular toxicities [29, 30]. In a study conducted to evaluate the protective effect of *Embilica officinalis* against toxicity induced by sodium fluoride (100 ppm) for four weeks, significant loss in the body and liver weights were observed, associated with increase in plasma glucose levels, hepatic G-6-Pase activity, reduction in hepatic glycogen content and hepatic hexokinase activities. This may be due to the similar diabetic effects caused by fluoride leading to unavailability of carbohydrates for energy assimilation [31]. However, in another study, rats exposed to 100 ppm NaF for a period of thirty days, showed no changes in body weight or relative weights of liver, kidneys, testis, epididymis, ventral prostate and seminal vesicle [32]. In the present study, comparison of body weight-gain and relative weight of liver in normal, untreated and treated groups showed insignificant changes after sodium fluoride challenge for 7 days at 600 ppm. It is possible that the experimental animals survived the effects of sodium fluoride on metabolism and thereby, body weight due to the short period (7 day) of exposure to fluoride, as evident from the results.

The effect of methanolic extract of *Oroxylum indicum* root bark in reducing paw oedema on carrageenan induced acute inflammation models and formalin induced chronic inflammation models are depicted in figures 2a and 2b. In acute paw oedema carrying mice treated with OIM extract at a dosage of 300 mg/kg body weight, a notable reduction of 41.28% occurred, in comparison to the control. Meanwhile, a reduction of 35.47% was obtained in 150 mg/kg b.w. treated group. The activity of the former is comparable to the standard reference group, where diclofenac treatment caused 49.42% inhibition of paw oedema (Tab.4).

Table 4. Effect of OIM extract on carrageenan induced acute paw inflammation

Group	Initial paw thickness(cm)	Paw thickness in the 3 rd hour(cm)	Increase in paw thickness (cm)	% inhibition
Control	0.183 \pm 0.002	0.355 \pm 0.004	0.172 \pm 0.006	-
Diclofenac(10 mg/kg)	0.195 \pm 0.004	0.282 \pm 0.005	0.087 \pm 0.007 ^a	49.42%
OIM (150 mg/kg)	0.204 \pm 0.005	0.315 \pm 0.009	0.111 \pm 0.009 ^a	41.28%
OIM (300 mg/kg)	0.203 \pm 0.009	0.304 \pm 0.009	0.101 \pm 0.009 ^a	35.47%

Values are expressed as mean \pm SD for 6 animals; ^a $p < 0.01$ and ^b $p < 0.05$ compared to control

The effectiveness of OIM extract in reducing chronic inflammation was studied in formalin induced paw oedema models. Compared to the untreated control group, the mice treated with OIM extract showed remarkable reduction in paw thickness, during the six consecutive days of drug treatment and observation. At dosages of 150 and 300 mg/kg b.wt, the percentage inhibition was determined to be 41.14 and 48.57% respectively, evidently showing its efficacy in reducing paw oedema in a dosage dependent manner (Tab. 5).

In the study, the standard reference drug caused 54.29% inhibition of chronic paw oedema in the animal models. The inter-relationship between inflammation and cancer has been recognised for long, by experimental and statistical data. Inflammation has invariable role in the establishment, progression and aggressiveness of many malignancies. During inflammation, leukocytes secrete a variety of proliferative cytokines and angiogenic factors to the site of tissue damage for the mediation of proper wound healing, stimulation epithelial cell proliferation etc. However, if these exaggerated they could lead to dysplasia and ultimately cancer. Tumour cells themselves also produce various cytokines and chemokines that attract leukocytes, which in turn produce cytokines and chemokines that stimulate further tumour cell proliferation [33]. In the current investigation, *Oroxylum indicum* root bark has been found to mitigate the extent of both acute and chronic inflammation in carrageenan and formalin induced murine models.

Table 5. Effect of OIM extract on formalin induced chronic paw inflammation

Group	Initial paw thickness(cm)	Paw thickness on the 6 th day (cm)	Increase in paw thickness (cm)	% inhibition
Control	0.206 ± 0.010	0.381 ± 0.010	0.176 ± 0.011	-
Diclofenac(10 mg/kg)	0.208 ± 0.002	0.288 ± 0.003	0.080 ± 0.003 ^a	54.29%
OIM (150 mg/kg)	0.208 ± 0.003	0.311 ± 0.022	0.103 ± 0.022 ^a	41.14%
OIM (300 mg/kg)	0.205 ± 0.009	0.295 ± 0.003	0.091 ± 0.005 ^a	48.57%

Values are expressed as mean ± SD for 6 animals; ^a p < 0.01 and ^b p < 0.05 compared to control

A study previously done on the petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions of the root bark of this plant has discussed its anti inflammatory activity, in terms of its effect on cotton pellet induced granuloma in mice. The present study, therefore offers the scope of elucidating the mechanism of anti inflammatory activity, by assessment of molecular markers involved in the inflammatory process.

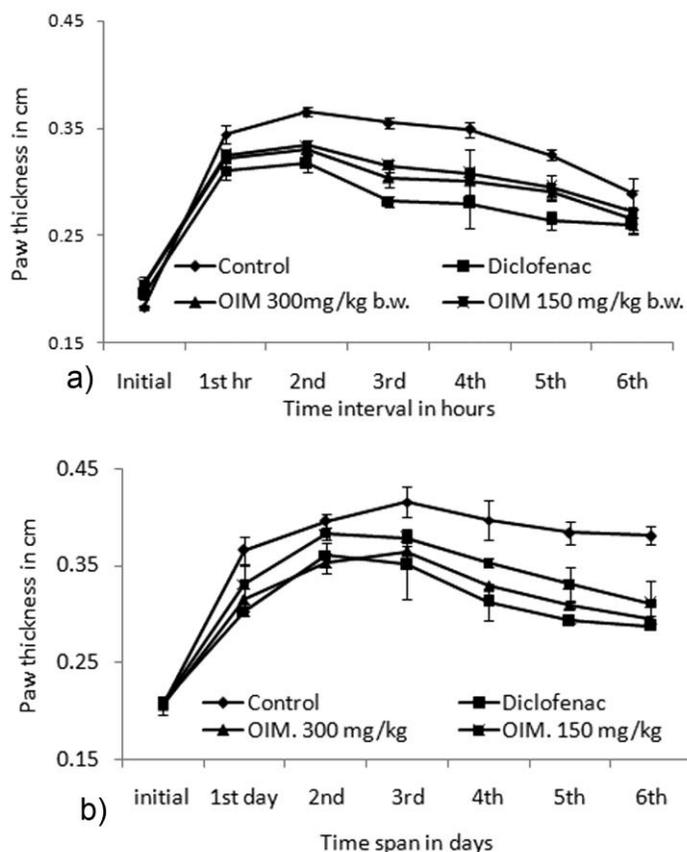


Figure 2.a-b. Effect of administration of Oroxylum indicum root bark extract on carrageenan induced acute inflammation (a) and formalin induced chronic inflammation (b) in mice

Values are mean ± SD; n=6.

Preliminary phytochemical analyses revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins and terpenoids in 70% methanol extract of *O. indicum*. There is mounting evidence of the role of phytochemicals as

potent anti oxidants and anti inflammatory agents [34, 35, 36, 37]. The results of the present study demonstrated that the extract showed significant *in vivo* anti oxidant and anti inflammatory activities in stress induced models, which may be attributed to the synergistic effect of the various bioactive compounds present in it. The study, therefore poses a scope of identification and isolation of the chemical compounds in the root bark of the plant as well as elucidation of the molecular mechanism of their biological action.

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