Biological activities of *Ocimum sanctum* L. fixed oil—An overview

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Seeds of *Ocimum sanctum* L. (Labiatae; popularly known as ‘Tulsi’ in Hindi and ‘Holy Basil’ in English) contain a pale yellow colored fixed oil. The oil possesses antiinflammatory activity due to dual inhibition of arachidonate metabolism supplemented by antihistaminic activity. The antiinflammatory activity is not dependent on the pituitary adrenal axis. The oil possesses antipyretic activity due to prostaglandin inhibition and peripherally acting analgesic activity. The oil has been found to be effective against formaldehyde or adjuvant induced arthritis and turpentine oil induced joint edema in animals. Lipooxygenase inhibitory, histamine antagonistic and antisecretory activities of the oil contribute towards antiulcer activity. The oil can inhibit enhancement of vascular capillary permeability and leucocyte migration following inflammatory stimulus. The LD$_{50}$ of the oil is 42.5 ml/kg and long-term use of oil at 3 ml/kg dose does not produce any untoward effects in rats. The oil contains α-linolenic acid, an ω-3 fatty acid, which on metabolism produces eicosapentaenoic acid and the same appears to be responsible for the biological activity. The oil has hypotensive, anticoagulant and immunomodulatory activities. Antioxidant property of the oil renders metabolic inhibition, chemoprevention and hypolipidaemic activity. Presence of linolenic acid in the oil imparts antibacterial activity against *Staphylococcus aureus*. The oil alone or in combination with cloxacillin, a β-lactamase resistant penicillin, has been found to be beneficial in bovine mastitis, an inflammatory disorder resulting from staphylococcal infection. Existence of anti-inflammatory, analgesic and antibacterial activities in single entity i.e. fixed oil appears to be unique.

**Keywords**: Antiinflammatory, Analgesic, Dual-inhibitor, Antiulcer, Anticoagulant, Antioxidant, Antibacterial, α-Linolenic acid, ω-3 Fatty acid

*Ocimum sanctum* L. (Labiatae), popularly known, as ‘Tulsi’ in Hindi and “Holy Basil” in English is a herbaceous sacred plant of the Hindus and is worshipped in both homes and temples. The plant is found throughout India up to an altitude of 1800 m in Himalayas and in Andaman and Nicobar Islands. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases. Indian Materia Medica describes the use of the plant in the treatment of number of ailments, including bronchitis, rheumatism and pyrexia$^{1,2}$. Most of the studies of *O. sanctum* have been carried out with leaves, aqueous and non-aqueous extracts of leaves or volatile oil distilled from leaves. However, in the recent past, a considerable number of studies have been reported on fixed oil extracted from *O. sanctum* seeds. An attempt has been made to present the biological activities of *O. sanctum* fixed oil in this review.

**Extraction of *O. sanctum* fixed oil**

Dried seeds of *O. sanctum* were crushed and cold macerated in petroleum ether (40°-60°C) for 3 days. The petroleum ether was evaporated from the extract and the pale yellow colored oil was filtered to clarity. The weight per ml of the oil at 25°C was 0.8750. Gas liquid chromatographic analysis of the oil revealed the presence of five fatty acids i.e. palmitic, stearic, oleic, linoleic and linolenic acid$^3$ confirming the findings of Nadkarni and Patwardhan$^4$. The contents of individual fatty acids were however, found to be varying from the previous study, which could be attributed to geographical variation. The fixed oil (i.e. nonvolatile oil) thus obtained, was used for biological evaluation.

**Biological activities**

Most of the pharmacological activities of *O. sanctum* fixed oil were evaluated following administration of oil by intraperitoneal (ip) route. The reason for selecting ip administration is that absorption from intraperitoneal route is free from the effects of gastric emptying or presystemic gastro-
Antiinflammatory activity

Antiinflammatory activity of *O. sanctum* fixed oil was evaluated on carrageenan-induced paw edema in rats. The fixed oil administered intraperitoneally (ip) significantly inhibited carrageenan-induced paw edema in rats. The edema inhibition is due to true antiinflammatory effect of the oil and not due to any counter irritant property. The antiinflammatory activity of a number of drugs has been reported to be partially dependent on pituitary adrenal axis. Evaluation of antiinflammatory activity of *O. sanctum* fixed oil in adrenalectomised and nonadrenalectomised rats showed similar results, which suggest that antiinflammatory activity of the oil, is not dependent on pituitary adrenal axis. Carrageenan-induced paw edema development involves three distinct phases of mediator release. The early phase is attributed to the release of histamine and serotonin, an intermediate phase mediated by kinin like substances and the next phase due to the release of prostaglandin like substances. To ascertain the inhibitory effect of *O. sanctum* fixed oil on various mediators of inflammation, the antiinflammatory effect of the oil was evaluated against histamine, serotonin, bradykinin, and PGE2-induced paw edema in rats. The oil at 3 ml/kg dose (ip) significantly inhibited edema formation induced by the inflammatory mediators. Castor oil-induced diarrhoea in rats has been reported to be mediated by prostaglandin. *O. sanctum* fixed oil inhibited castor oil-induced diarrhoea. Inhibition of carrageenan-induced paw edema and castor oil-induced diarrhoea by *O. sanctum* fixed oil suggests the probable inhibitory effect of oil on prostaglandin (or cyclooxygenase pathway of arachidonic acid metabolism). However, lipoxygenase inhibitors also could inhibit carrageenan-induced paw edema. Thus, inhibition of carrageenan-induced paw edema by *O. sanctum* fixed oil could also result from its inhibitory effect on lipoxygenase (the enzyme involved in lipoxygenase pathway of inflammation). To explore the same the antiinflammatory activity of fixed oil was evaluated against leukotriene (LTB4 methyl ester)-induced paw edema. The oil at 3 ml/kg dose (ip) significantly inhibited edema formation indicating its capacity to inhibit lipoxygenase pathway of arachidonic acid metabolism. Arachidonic acid (5,8,11,14 eicosatetraenoic acid) is a component of plasma membrane phospholipid. Phospholipase A2 cleaves the phospholipid and liberates arachidonic acid. Cyclooxygenase oxidizes/metabolizes arachidonic acid and produces prostaglandin e.g. PGE2 which is proinflammatory. Similarly metabolism of arachidonic acid by lipoxygenase yields leukotriene e.g. LTB4 which could cause inflammation. Arachidonic acid-induced paw edema in rats is an *in vivo* model to distinguish between cyclooxygenase and lipoxygenase inhibitors. Accordingly, antinflammatory activity of *O.sanctum* fixed oil was evaluated against arachidonic acid-induced paw edema in rats. The oil at 3 ml/kg dose (ip) significantly inhibited the edema formation. Chlorpheniramine maleate (antihistaminic) and cyproheptadine (antihistaminic/antiserotonin agent) also showed edema inhibition which appears to be due to inhibition of mast cell mediator release suggesting that mast cell mediator release may partly contribute towards arachidonic acid-induced paw edema. In order to evaluate the relative contribution of cyclooxygenase inhibition, lipoxygenase inhibition and inhibitory effect of antihistaminic towards the inhibition of arachidonic acid-induced paw edema in rats; series of studies were carried out. The results indicated that edema inhibition of oil was higher compared with either indomethacin (cyclooxygenase inhibitor) or caffeic acid (lipoxygenase inhibitor) and was comparable to that produced by combination of indomethacin and caffeic acid (cyclooxygenase inhibitor + lipoxygenase inhibitor). Combination of indomethacin, caffeic acid and chlorpheniramine maleate, however showed slightly higher edema inhibition compared to *O. sanctum* oil. The results suggest *O. sanctum* fixed oil may have the potential to inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (dual inhibitor property) which may be supplemented by antihistaminic property of the fixed oil and the contribution of lipoxygenase inhibitory activity appears to be more than the rest.

Antipyretic activity

Drugs having antiinflammatory activity generally possess antipyretic activity e.g. non-steroidal antiinflammatory drugs (NSAIDs). The mode of antipyretic action of NSAIDs is not fully clear. It has been suggested that prostaglandin (PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit
The antipyretic activity of prostaglandin synthesis could help to explain their antipyretic activity\textsuperscript{12}. The antipyretic activity of *O. sanctum* fixed oil was evaluated by testing it against typhoid-paratyphoid A/B vaccine-induced pyrexia in rats. The oil on ip administration considerably reduced the febrile response in rats indicating its antipyretic activity. At a dose level of 3 ml/kg, the antipyretic activity of the oil was comparable to aspirin. The fixed oil possesses prostaglandin inhibitory activity and the same could explain its antipyretic activity\textsuperscript{5}.

**Analgesic activity**

Pain is one of the cardinal signs of inflammation. Hence, it is necessary to evaluate whether any new drug modify the inflammatory pain which appears to be the most relevant test because this type of pain is prevalent in most of the conditions for which antiinflammatory drugs are prescribed. Analgesic activity of *O. sanctum* fixed oil was evaluated using tail flick, tail clip and tail immersion method. In tail flick or tail immersion method the reaction time of a rat or mouse to withdraw its tail from a hot wire or hot water (55°C) is measured while in tail clip method, reaction time to dislodge the clip is noted. A longer reaction time indicates a positive analgesic response. The oil on ip administration showed insignificant analgesic activity compared to morphine. It is known that centrally acting analgesic drugs elevate the pain threshold of animals towards heat and pressure. The results showed the inability of *O. sanctum* fixed oil to raise the pain threshold indicating the oil is not centrally acting. In order to distinguish between central and peripheral analgesic action of *O. sanctum* fixed oil, acetic acid-induced writhing response in mouse was used. The fixed oil administered by ip or oral route significantly inhibited acetic acid-induced writhing. The oil effectively reduced the wave of constriction, and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb in mice due to nociceptive property of acetic acid. The percentage inhibition of writhing with 3 ml/kg dose of oil was close to that observed with 100 mg/kg of aspirin. Analgesic activity of the oil appears to be peripherally mediated. It has been reported that in addition to NSAIDs, antihistaminics and anticholinergics can inhibit writhing response\textsuperscript{13}. *O. sanctum* fixed oil possesses significant antiinflammatory property against phlogistic agents like carrageenan, prostaglandin (PGE\textsubscript{2}) and histamine\textsuperscript{11}. Anticholinergic property of the oil against acetylcholine-induced contraction of rat ileum has been reported\textsuperscript{14}. Thus, the analgesic activity of *O. sanctum* fixed oil is peripherally mediated and could result from the combined inhibitory effects of prostaglandin, histamine and acetylcholine\textsuperscript{15}.

**Antiarthritic activity**

Freund’s complete adjuvant-induced arthritis in rats is probably the most widely used model, as it resembles human rheumatoid disease. Shortly after administration of adjuvant into one hind paw of rat, a pronounced swelling appears in the injected paw, which persists for weeks. This is usually considered as a primary reaction. After a few days the contralateral paw as well as the front paws also becomes swollen and arthritic nodules appear in the ear and tail (delayed systemic response). For evaluation of antiarthritic activity of *O. sanctum* fixed oil, the magnitude of swelling on the hind paws (in both adjuvant injected and noninjected paw) was taken into consideration. The edema of the adjuvant-injected paw was used for evaluating the inflammatory response following adjuvant injection while the increase in the size of noninjected paw was measured to estimate the delayed immunologically mediated component of the disease. *O. sanctum* fixed oil, at 3 ml/kg dose (ip) showed significant edema inhibition comparable to aspirin (100mg/kg, ip). Significant decrease in inflammation and arthritic nodes was also observed.

Antiarthritic activity of *O. sanctum* fixed oil was also evaluated against formaldehyde-induced arthritis in rats. The fixed oil significantly reduced the diameter of the inflamed paw. On administration of the fixed oil daily for 10 days, (ip) there was marked improvement in the arthritic conditions in rats. The antiarthritic effect at 3 ml/kg dose was comparable to aspirin (100 mg/kg, ip).

In order to correlate the antiinflammatory activity with biochemical changes, the effect of *O. sanctum* fixed oil on transaminase activity was studied in normal and arthritic rats. It has been proposed, that many antiinflammatory drugs inhibit enzyme system like transaminases\textsuperscript{16}. It was observed that serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels increased in control and formaldehyde-induced arthritic rats. *O. sanctum* fixed oil partially prevented the rise in the enzymatic activity associated with the inflammatory reactions.
Effect of *O. sanctum* fixed oil was also evaluated against turpentine oil-induced joint edema in rats. The fixed oil (3 ml/kg, ip) significantly inhibited turpentine oil-induced joint edema in rats at 1, 2, 3, 4 and 5 hr after treatment. There are some sequential releases of the mediators in turpentine oil-induced joint edema i.e. histamine and serotonin in the early phase, kinin like substances in the intermediate phase and prostaglandin in the late phase. It may be noted that carrageenan induced paw edema also involves similar sequence of mediator release. Since *O. sanctum* fixed oil inhibited carrageenan and inflammatory mediator like histamine, serotonin, bradykinin and PGE₂-induced inflammation, it is natural that the oil could inhibit any inflammatory response involving these mediators. The results suggest potentially useful antiarthritic activity of the fixed oil since it was active in all the inflammation models including adjuvant as well as turpentine oil-induced joint edema in rats.

**Antagonism of increased vascular permeability and leucocyte migration**

An important characteristic of the inflammatory reaction is a sustained elevation in the permeability of small blood vessels to protein and cells, which occur sometime after an initial transient increase in permeability. The initial phase involves primarily the venules whereas the delayed phase of increase in permeability involves the capillaries as well and is more relevant to chronic inflammation.

Antagonism of increased vascular permeability occurring in a variety of experimental inflammation has been the criteria for assessing antiinflammatory activity. Artificial peritoneal or pleural inflammation induced in mice or rats has been utilized to test the antiinflammatory effect of drugs. As the vascular permeability increases early with inflammatory process, the amount of dye-labeled plasma protein exuding into the peritoneal fluid has been exploited to estimate intensity of inflammation. *O. sanctum* fixed oil at 3 ml/kg dose (ip) significantly inhibited the rise in protein concentration and dye leakage in peritoneal fluid, in acetic acid-induced peritoneal inflammation in mice. In carrageenan-induced pleurisy in rats, the fixed oil showed significant inhibition of leucocyte migration in the pleural exudates. The results suggest that the fixed oil can inhibit enhancement of the vascular/capillary permeability and leucocyte migration following inflammatory stimulus.

**Antiulcer activity**

The antiulcer activity of *O. sanctum* fixed oil was evaluated on experimentally induced gastro-intestinal ulcers in animals. The fixed oil on ip administration was found to possess significant antiulcer activity against aspirin, indomethacin, ethanol (50%), reserpine, serotonin or stress-induced ulcers in rats. The oil on oral administration significantly inhibited histamine-induced ulcers in guinea pigs. The oil also demonstrated antisecretory activity. NSAIDs like aspirin and indomethacin are known to induce gastric ulceration; the reason being attributed principally to inhibition of "cytoprotective prostaglandins" e.g. PGE’s and PGI₂ (by inhibition of cyclooxygenase pathway of arachidonic acid metabolism) resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway. Hence, the protective action of *O. sanctum* fixed oil against aspirin and indomethacin induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect.

Ethanol induced gastric ulcer results from stasis of gastric blood flow and hemorrhage. Leukotriene antagonist and 5-lipoxygenase inhibitors can inhibit alcohol and NSAIDs-induced gastric ulceration in rats. Thus protection afforded by *O. sanctum* fixed oil against ethanol-induced ulceration could also be due to inhibition of 5-Lipoxygenase or leukotriene antagonistic activity.

Histamine induced gastric ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine. Hence it appears that *O. sanctum* fixed oil suppresses the histamine induced vasospastic effect and gastric secretion. The antisecretory effect of oil is demonstrated in the experiment on pylorus-ligated rats, where the fixed oil has been found to inhibit both the gastric output and total acidity.

Reserpine induced gastric ulceration has been attributed to the degranulation of the gastric mast cells and consequent liberation of histamine which is believed to be cholinergically mediated. Consequently, the antiulcer effect of *O. sanctum* fixed oil could be due to histamine antagonistic and anticholinergic effects. Serotonin induced ulceration is believed to arise from a disturbance of gastric mucosal microcirculation and the oil appears to improve such a disturbance. Since the development of ulcers by serotonin and reserpine usually takes about 18 h, it may be inferred that the oil has a sustained effect.
Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucous production. Moreover, stress induced ulcers can be prevented partially or entirely by vagotomy; vagal over activity has been suggested to be principal factor in stress-induced ulceration\(^2\). Accordingly, the protective action of *O. sanctum* fixed oil against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic and antisecretory effects.

It appears from the results that *O. sanctum* fixed oil possesses significant antiulcer activity, which may be due to the lipoxygenase inhibitory, histamine antagonistic and antiseretory effects of the oil. *O. sanctum* fixed oil also possesses significant antiinflammatory activity. A drug that possesses both antiinflammatory and antiulcer activity is of great therapeutic importance as most of the antiinflammatory drugs used in modern day medicine are ulcerogenic\(^3\).

**Chemical constituent responsible for activity**

Attempts were made to identify the active principle(s) responsible for the biological activity of *O. sanctum* fixed oil. The antiinflammatory and analgesic activity of the fixed oil and triglycerides isolated from fixed oil at a dose level of 3 ml/kg (ip) were compared. Percentage inhibition of carrageenan-induced paw edema and acetic acid-induced writhing were higher with triglycerides treatment, which indicates that the pharmacological activities are due to the triglycerides fraction of the fatty acid of the fixed oil\(^3\).

In order to ascertain the contribution of various unsaturated fatty acids towards antiinflammatory activity, a number of plant lipids like linseed oil (flaxseed oil), soybean oil and sunflower oil (having varying composition of unsaturated fatty acids) along with *O. sanctum* fixed oil were tested against carrageenan-induced paw edema, at a dose level of 3 ml/kg (ip). Results indicated that only those oil, which contained linolenic acid (i.e. linseed oil, soybean oil and *O. sanctum* fixed oil), could significantly inhibit the edema and linseed oil (having maximum content of linolenic acid) showed maximum percentage edema inhibition followed by *O. sanctum* fixed oil. It would be appropriate to mention here that linseed oil has already appeared in Indian market as Volini gel (manufactured by Oscar Pharmaceutical Private Limited, a division of Ranbaxy Laboratories Limited) where it has been formulated with diclofenac diethylamine, methyl salicylate and menthol. Thus, the antiinflammatory activity of the oil has been potentiated by addition of three prostaglandin inhibitors (diclofenac diethylamine, methyl salicylate, menthol). The product is used for quick relief of pain, swelling and inflammation. In order to consolidate the finding further, the antiinflammatory activity of various unsaturated fatty acids viz oleic, linoleic and linolenic acid was evaluated against PGE\(_2\), leukotriene (LTB\(_4\)) and arachidonic acid-induced paw edema in rats, following ip administration of methyl esters of fatty acids at a dose equivalent to 3 ml/kg of fixed oil.

The results showed that linolenic acid and *O. sanctum* fixed oil could significantly inhibit the edema induced by PGE\(_2\), LTB\(_4\) and arachidonic acid.

The results indicate that linolenic acid (Fig. 1) in *O. sanctum* fixed oil could account for the antiinflammatory activity of the oil by dual inhibition of arachidonic acid metabolism. Lipids extracted from the seeds of evening prime rose and borage plants have been reported to be antiinflammatory due to the presence of relatively large amounts of gammalinolenic acid (GLA), an \(\omega-6\) (18:3, n-6) fatty acid (all cis-6, 9, 12 octadecatetraenoic acid) which contains the first double bond at 6\(^{th}\) carbon atom from the methyl (\(\omega\)) end of the fatty acid chain (refer Fig. 2). GLA is rapidly converted to dihomogamalinolenic acid (DGLA) (20:3, n-6) (a precursor of antiinflammatory prostaglandin E\(_1\)), which competes with arachidonic acid for oxidative enzymes, thereby reducing the production of cyclooxygenase products derived from arachidonic acid. In addition, DGLA is converted by 5-lipoxygenase to 15-hydroxy DGLA (15-HETE) (20:4, n-6) (a precursor of prostanoid prostaglandin F\(_2\))\(^\text{28}\). Studies on *O. sanctum* fixed oil show that (\(\omega\)-linolenic acid also, could inhibit both cyclooxygenase and lipoxygenase pathways of inflammation. Linolenic acid, an \(\omega-3\) (18:3, n-3) fatty acid (all cis-9, 12, 15 octadecatetraenoic acid), is progressively metabolized in the body to 6, 9, 12, 15-octadecatetraenoic acid (18:4, n-3), stearadonic acid (20:4, n-3) and eicosapentaenoic acid (20:5, n-3)\(^\text{29}\). The end product eicosapentaenoic acid has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes derived from

![Fig. 1—Linolenic acid](image)
arachidonic acid while serving as a substrate for prostaglandins with three double bonds and leukotrienes with five double bonds, which could be one possible mechanism for the dual inhibition and antiinflammatory activity of linolenic acid. Octadecatetraenoic acid and stearadonic acid possessing four double bonds could also act in the same way. Furthermore, linolenic acid containing three double bonds could compete with arachidonic acid and offer itself as a substrate for cyclooxygenase and reduce the formation of prostaglandins. Polyunsaturated fatty acids having two cis double bonds separated by methylene group is a substrate for lipoxygenase. Since, linolenic acid contains three double bonds in this configuration; it could also act as a substrate for lipoxygenase and inhibit the formation of leukotrienes. Thus, linolenic acid and its metabolites could competitively inhibit the formation of prostaglandins and leukotrienes from arachidonic acid and thereby produce antiinflammatory effect. However further studies are needed to know the exact mechanism of action.

Hypotensive effect

\( O. sanctum \) fixed oil (3 ml/kg, ip) prolonged blood-clotting time and the response was comparable to that obtained with aspirin (100 mg/kg). The effect appears to be due to the antiaggregator action of oil on platelets. Linolenic acid contained in the oil can be metabolized to EPA, which can inhibit the formation of TXA2 through cyclooxygenase and produces PGI3 and TXA3. Like PGI2, PGI3 also possesses antiaggregatory property and while TXA3 has much less proaggregatory property towards platelets compared with TXA2. Thus combined antiaggregatory effects of PGI2 and PGI3 supplemented by the inhibition of TXA2 could contribute towards the anticoagulant effect of \( O. sanctum \) fixed oil. The oil could therefore be used as an anticoagulant like aspirin.

Anticoagulant effect

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Potentiation of sedation

\( O. sanctum \) fixed oil (2-3 ml/kg, ip) increased pentobarbitone-induced sleeping time in rats. When the oil is given alone it does not produce any sedation indicating no CNS depressant activity of its own, but when given along with pentobarbitone, it increased the sleeping time. Lipids containing linoleic and linolenic acids absorb oxygen on exposure to air and are called drying oil. \( O.sanctum \) fixed oil contains both linoleic and linolenic acid, and hence would also have affinity for oxygen. Pentobarbitone is metabolized in the liver by oxidative pathway involving cytochrome p450, NADPH and molecular oxygen. \( O.sanctum \) fixed oil may absorb the oxygen and get itself oxidized/metabolized thereby preventing the metabolism of pentobarbitone. In addition, the oil,
being a prostaglandin inhibitor, may also inhibit renal vasodilatory prostaglandins resulting in reduced blood flow to kidney and reduced clearance of pentobarbitone. Thus, inhibition of hepatic metabolism of pentobarbitone/renal clearance by *O. sanctum* fixed oil could be responsible for potentiation of pentobarbitone induced sleeping time\(^35\). Therefore, the oil can be used as an adjunct in the therapies requiring sedation utilizing the medicinal compounds that are metabolized by oxidative pathway and consequently lowering the dose that is required to achieve the same level of sedation as is being achieved by medicinal compound alone.

**Chemopreventive activity**

The seed oil of *O. sanctum* at 100 µl/kg dose significantly reduced 20-methylcholanthrene induced tumor incidence and tumor volume. Subcutaneous injection of 20-methylcholanthrene in the thigh region of Swiss albino mice induces-fibro sarcoma tumors. Liver enzymatic (superoxide dismutase, catalase, glutathione-S-transferase), non-enzymatic antioxidants (reduced glutathione) and lipid peroxidation end product and malondialdehyde levels were significantly modulated with oil treatment as compared to untreated 20-methylcholanthrene injected mice. The study suggests the potential chemopreventive activity of the oil, which is partly attributable to its antioxidant properties and is comparable to that of 80 mg/kg of vitamin E\(^36\). *O. sanctum* fixed oil, contains polyunsaturated fatty acids like linoleic and linolenic acids, which have affinity for oxygen and could be responsible for its antioxidant activity\(^35\). ω-3 Fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can effectively reduce the risk of skin cancer whereas ω-6 fatty acids such as arachidonic acid reportedly promote risk. The effects of ω-3 fatty acid (e.g. EPA and DHA) and ω-6 fatty acid (e.g. arachidonic acid) on tumor genesis are more significant for DHA than for EPA due to inhibition of AP-1\(^37\). This further supports the chemopreventive effect of *O. sanctum* fixed oil containing linolenic acid, which is mainly responsible for the production of DHA and EPA. Apoptotic pathways are frequently disrupted in cancers. Malfunction in the apoptosis pathway increases COX-2 expression, over-the-top angiogenesis and activation of the NF-kB pathways, all of which make the cells more resistant to death signals, as well as increase the rate at which the cancer cells proliferate. ω-3 Fatty acid blocks NF-kB and COX-2 activation, which in turn would restore apoptosis and reduce proliferation. DHA has been reported to inactivate Bcl-2 (an antiapoptic gene) and increase apoptosis\(^38\).

**Antihypercholesterolaemic activity**

A recent study has described antihypercholesterolaemic activity of *O. sanctum* seed oil. Oral administration of oil (0.8 g/kg/day) for 4 weeks in cholesterol (100 mg/kg/day) fed rabbits, significantly reduced serum cholesterol, triacylglycerol and LDL- +VLDL-cholesterol compared with untreated cholesterol fed group. There was significant fall in atherogenic index in the oil treated group. Oil treatment decreased lipid peroxidation and increased reduced glutathione content in blood\(^39\). It has already been stated earlier that lipids containing linoleic and linolenic acids absorb oxygen on exposure to air and they are called drying oil. *O. sanctum* oil, since contains both linoleic and linolenic acid, could be considered as a drying oil and is expected to behave similarly. Accordingly, *O. sanctum* oil may absorb the oxygen and get itself preferentially oxidized/metabolized thereby inhibiting the oxidation/metabolism of cholesterol. The increased level of reduced glutathione in blood following oil treatment also supports reduced oxidative stress. Thus the hypolipidaemic activity of *O. sanctum* oil appears to originate from the reducing/antioxidant effect of the oil.

**Immunomodulatory activity**

The effect of *O. sanctum* seed oil (OSSO) on some immunological parameters in both non-stressed and stressed animals has been studied. A significant increase in anti-sheep red blood cells (SRBC) antibody titre and a decrease in percentage histamine release from peritoneal mast cells of sensitised rats (humoral immune responses), and decrease in footpad
thickness and percentage leucocyte migration inhibition (LMI) (cell-mediated immune responses) was observed with OSSO when given in a dose of 3 ml/kg, ip.

Restraint stress (RS) produced a significant reduction in the anti-SRBC antibody titre, footpad thickness and percentage LMI (% LMI). Co-administration of diazepam (1 mg/kg, sc), a benzodiazepine (BZD), with OSSO (1 ml/kg, ip) enhanced the effect of OSSO on RS-induced changes in both humoral and cell-mediated immune responses. Flumazenil (5 mg/kg, ip), a central BZD receptor antagonist inhibited the immunomodulatory action of OSSO on RS-induced immune responsiveness. Thus, OSSO appears to modulate both humoral and cell-mediated immune responsiveness and GABAergic pathways may mediate these immunomodulatory effects40.

Polyunsaturated fatty acids are acting like proton-ionophores on the vesicular uptake of GABA and glutamate neurotransmitters. The cis-polyunsaturated fatty acid arachidonic acid (20:4), eicosapentanoic acid (20:5) and linolenic acid (18:3) at 150-nmol/mg protein (50 microM) inhibited the vesicular uptake of glutamate and GABA more than 70%41. This further confirms the OSSO containing linolenic acid can inhibit the vesicular uptake of GABA, thus increasing the availability of the neurotransmitter.

Toxicity studies
Mean lethal dose or LD50 was calculated after ip administration of O. sanctum fixed oil in mice. The fixed oil was well tolerated up to 30 ml/kg while 100% mortality was recorded with a dose of 55 ml/kg. The calculated LD50 of oil was 42.5 ml/kg.

Subacute toxicity studies were conducted following ip administration of O. sanctum fixed oil in rats at a dose of 3 ml/kg/day for 14 days. The results of the subacute toxicity studies, following administration of the oil for a longer period did not reveal any untoward effect on behavioral response, body weight, normal reflexes and visceral appearance in rats. The oil did not produce any ulcerogenic effect nor any histopathological changes5,42.

Effect of route of administration on antiinflammatory activity
The anti-inflammatory activity of O. sanctum fixed oil was evaluated against carrageenan and PGE2-induced paw edema in rats after administration of oil (3 ml/kg) by intraperitoneal or intramuscular or oral route. In the carrageenan-induced paw edema model percentage inhibition of edema after 3 hr was similar for all the route of administration. The carrageenan-induced edema development takes about 3 hr during which time the extent of absorption of oil from different routes approaches completion, as a result no difference in edema inhibition could be observed. In the PGE2-induced edema model, the percentage inhibition of edema observed after half an hour, following intraperitoneal, intramuscular and oral administration of fixed oil were 49.10%, 42.00% and 24.53% respectively suggesting fastest absorption by intraperitoneal route. Lower percentage inhibition, observed after oral administration, appears to be due to delay in gastric emptying. The results indicate that administration of O. sanctum fixed oil by ip, im or oral route produces anti-inflammatory effect and ip route appears to provide quicker effect due to faster absorption43.

Antibacterial activity
O. sanctum fixed oil showed good antibacterial activity against Staphylococcus aureus, Bacillus pumilus and Pseudomonas aeruginosa, where S. aureus was the most sensitive organism. O. sanctum fixed oil showed maximum zone of inhibition with S. aureus followed by sesame and soybean oil. Higher content of linolenic acid in O. sanctum fixed oil could contribute towards its antibacterial activity44.

Malondialdehyde is an aldehyde formed as a breakdown product of peroxidized polyunsaturated lipids45. Therefore, linoleic acid and linolenic acid on oxidation could give malondialdehyde. Malondialdehyde is a cross linker and initiates oxidation reactions in which undesirable bonds form between nucleic acids. (RNA and DNA, the genetic blueprint material)46. The probable result is inhibition of DNA replication. In addition, malondialdehyde could also crosslink amino group of bacterial enzymes and thereby inhibit the growth.

Efficacy of O.sanctum fixed oil in bovine mastitis
Bovine mastitis is a disease, which is characterized by inflammation and bacterial infection of the udder, mainly by Staphylococcus aureus and physical factors like cold and mechanical injuries have been reported to act as predisposing factors. A number of antibiotics either alone or in combination with steroid are available in the market as intramammary injection for the treatment of bovine mastitis. O.sanctum fixed oil possesses antiinflammatory and analgesic activity; in addition the oil possesses antibacterial activity against...
Considerably reduced the udder inflammation after the 2nd day of treatment and complete cure was observed after 5th day. Combination of fixed oil and cloxacillin cured the disease after 3rd day of the treatment while cloxacillin alone cured the condition after 4th day of the treatment. Thus O. sanctum fixed oil alone or in combination with cloxacillin cured the disease with in 3-5 days. Currently bovine mastitis is being treated with an antibiotic either alone or in combination with a steroid like prednisolone or dexamethasone. The therapeutic efficacy of O. sanctum fixed oil against mastitis suggests that it has the potential to replace the steroid (if not both antibiotic and steroid) and offer a cheaper therapy for the disease47.

Conclusion

O. sanctum fixed oil possesses significant antiinflammatory, antipyretic, analgesic, antiarthritic, antiocoagulant, hypotensive, chemopreventive and antibacterial activities. The oil also possesses antiulcer activity due to dual inhibition of arachidonic acid metabolism. Most of the disorders e.g. inflammation or pyrexia, are caused by oxidative stress. α-Linolenic acid (an α-3 fatty acid), contained in the oil, could act as a reducing agent/antioxidant, and thereby could be responsible for the biological activities of the oil. However further studies are needed to comment more in this respect. Existence of antiinflammatory, antiulcer and antibacterial activities in single entity i.e. fixed oil also appears to be unique.

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