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Review Article

CHEMICAL AND BIOLOGICAL REVIEW OF *OROXYLUM INDICUM* (KURTZ.) WITH SPECIAL ACCENT TO HEPATOPROTECTIVE ACTIVITY

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Abstract:

Oroxylum indicum (Kurtz.), commonly known as *Shyonaka*, described in Ayurveda under the broad group of drug *Dashamula*, and is widely available throughout India. The plant is reputed for its anti-inflammatory effect. Several advanced chemical study with this plant has already been conducted and about 28 varieties of chemical compounds are obtained. Most important compounds are baircalein and Ellagic acid (EA). Both these two compounds exhibit important biological activities like anti-inflammatory on neuro-inflammation, anti-cancer and hepatoprotective effect. Potent hepatoprotective activity was observed in CCl₄ induced hepatotoxicity and ethanol induced isolated goat liver. Probable mechanism of action of *O. indicum* may be due to free radical scavenging activities. The anti-inflammatory activity is due to its property of suppression the pro-inflammatory mediators like TNF- α , IL-6, NF- κ B-p65 and iNOS.

Keyword: *Oroxylum indicum*, Ellagic acid, baircalein, hepato-protective activity, anti-inflammatory effect.

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INTRODUCTION:

Medicinal plants are essential source to treat or prevent different types of diseases. Drugs of different kind may be developed from medicinal plants either in crude form or in the form of isolated compounds or synthetic drugs. Many developed or undeveloped countries use herbal or traditional medicine as a source of primary treatment for wide ranges of diseases [1]. Medicinal plants play an important role in maintenance of health [2] and emphasis has been drawn on this natural product for research to explore the medicinal value in global scale [3]. It has been observed that medicinal plants are found mainly in Asian and south American countries [4]. It is also reported that near about 75,000 to 1,000,000 medicated plant species are present in the world but 5, 00,000 plants are identified by their name. According to WHO, it is reported that, 20,000 numbers of plants are used for treatment traditionally. In recent years credence on research is also given on isolated compounds or active principles of medicinal plants. Plant products are generally used by patients who have chronic medical conditions such as - cancers, liver diseases, HIV, asthma, rheumatologic disorders, etc. [5]. In Asian country, especially in Bangladesh, there are more than 400 herbal drug manufacturing companies but about 90% of plants as raw ingredients are imported from other countries, such as India, Nepal and Pakistan etc [6]. In India, description regarding herbal drugs are available in traditional systems like Ayurveda, Unani, Siddha, etc. which are used for treating various diseases. Near about 80% peoples in India depend on traditional medicine for health care. In India, the history of healthcare systems is around 3500 years B.C. which is mentioned in traditional Ayurvedic texts like Charak Samhita, Sushruta Samhita, etc [7].

Sonyaka (Oroxylum indicum) is an important plant described in Ayurveda used for classical preparations like *Brahma Rasayana*, *Dashmularishta*, *Dhanawantara Ghrita*, *Amritarishta*, *Narayana Taila*, *Dantadyarista*, *Chyavanprasha* etc. It is reported that in Chinese medicine, the seeds of *Sonyaka* is treated for ulcers, boils, liver, and stomach problems. *Sonyaka* bark is used for

treatment of rheumatism and dysentery in Myanmar, the Philippines and Vietnam [8]. *O. indicum* is a most common plant mentioned in various texts of Ayurveda. Various parts of this plant like root, leaves, fruit, etc. are used traditionally as anti-inflammatory, antiulcer, hepatoprotective, anticancer, antioxidant, photocytotoxic, antiproliferative, antiarthritic, antimicrobial, anti-mutagenic, immuno stimulant, anti-diabetic, hypolipidemic and nephroprotective properties [9]. The plant contains various chemical compounds like baicalein-7-O-diglucoside (Oroxylum B), baicalein-7-O-glucoside, chrysin, apegenin, prunetin, sitosterol, oroxindin, biochanin-A, ellagic acid, baicalein and its 6- and 7-glucuronides, scutellarein, tetuin, anthraquinone and aloe-emodin [9]. Besides, stem bark is specifically contains the chemical components like baicalein, oroxylum and pinostrobin along with one sterol, Stigmast-7-en-3-ol [10]. However, Ellagic acid is reported as important chemical constituents of this plant present in various parts [11] but no report is available for Ellagic acid contents in seed. The seed of the plant is used traditionally in Ayurveda for treatment of cancer, scabies and other skin diseases. The plant is one of the important ingredients for the popular Ayurvedic medicine Chyawanprash awaleha [9].

Chemical components of *O indicum*

Several studies of phytochemical constituents of *O. indicum* were conducted and a group of constituents like flavonoids and phenolic compounds were obtained [12]. A review on this plant reveals that it contains about 28 varieties of chemical constituents, of which important members are Baicalein (fig. 1), Biochanin A, Chrysin (fig. 1), Ellagic acid (fig. 1), Oroxylum A (fig. 1), Oroxindin (fig. 1), β -Sitosterol, Scutellarein, Ursolic acid, Pinocembrin, Pinobanksin, Lupeol, Echinulin, Adenosine, Dimethyl Sulfone, etc. [13-14]. Studies reveal that the phenolic and flavonoids group of chemical compound like Ellagic acid is one of the major chemical constituents of *O. indicum*, particularly found in stem part [8]. In general, Ellagic acid belongs to the class of polyphenol extractives (tannins) widely spread among dicotyledons [15].

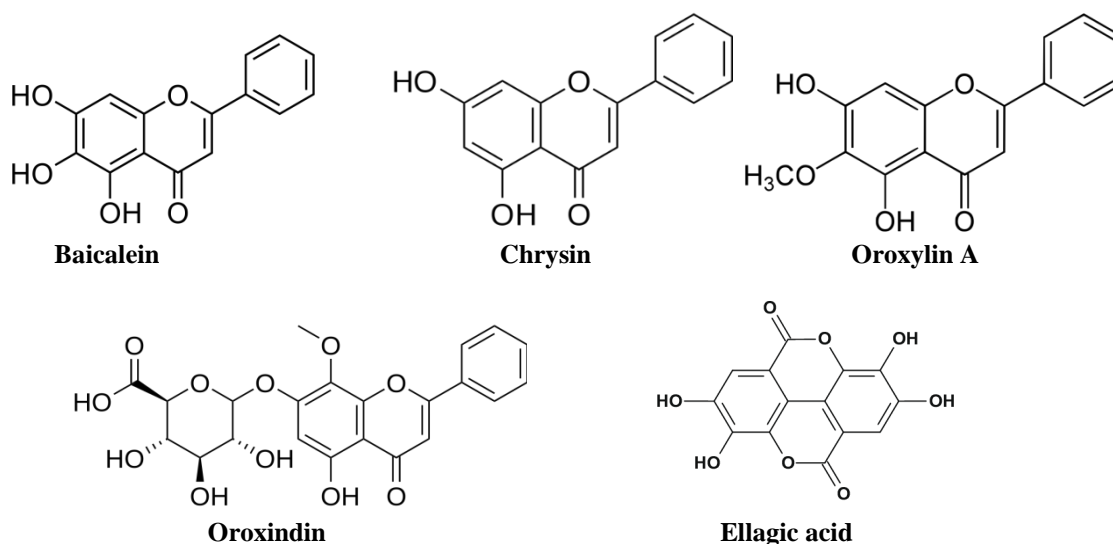


Fig. 1 Different chemical constituents of *Oroxylum indicum* (Kurtz.)

A detailed pharmacognostic study of the seed of the *O. indicum* was conducted by the authors to screen moisture contents, total ash and pH value. Moisture or water contents of the seed was done by conventional method and an amount of 7.94 g of test sample was placed in hot air oven for 20 minutes. This process was repeated for consecutive five times. After five times repetition of the process, weight of test sample with petridish was taken and the reducing weight was calculated. The moisture content of *O. indicum* sample was expressed in per cent (%). Total ash value was measured by converting the mineral constituents to the respective oxides at 600°C. An amount of 3.594 g test sample was used for experimental purpose in the muffle furnace for 2 hours. After the experiment was completed and cooled down, it was observed that the test sample was totally carbene free and looking white in colour. Then, final weight of test sample was taken and the per cent (%) of total ash present in *O. indicum* sample was calculated. pH value of the extract was measured with pH instrument. Result of the study is depicted in table 1.

Chemical fingerprinting of the seed of the plant was also performed by the authors using HPTLC, UV-visible spectroscopy and FT-IR spectroscopy methods. Initial chemical fingerprinting was done by HPTLC.

Table 1. Different pharmacognostic values of *O. indicum* seed

Pharmacognosy tests	Amount of sample (g/ mg/ ml)	Results
Moisture content	7.94 g	10.91% w/w
Total ash value	3.594 g	5.57% w/w
pH value	20 mg / ml	5.40

The HPTLC was done by taking 20.0 µl each samples of hydroalcoholic extract of *Oroxylum indicum* (HAOi), Gallic acid, Quercetine and Ellagic acid and were injected one after another in HPTLC instrument (CAMAG Linomat5_210131" S/N 210131). The size of 6.0 x 10.0 cm of HPTLC plate was prepared with HPTLC plates silica gel 60 F 254 for experimental purpose. HPTLC chromatograms were detected at two different wavelengths (254 and 366 nm) using AMD detector. The plate was spotted automatically and prepared chemical solvent system with ratio of Benzene: Chloroform: Methanol = 6:3:2 in TLC chamber. Plate was placed in TLC chamber and plate slides were running under TLC chamber. Plate was placed in TLC scanner instrument to identify chemical compounds and calculate R_f value automatically (table 2, fig. 2).

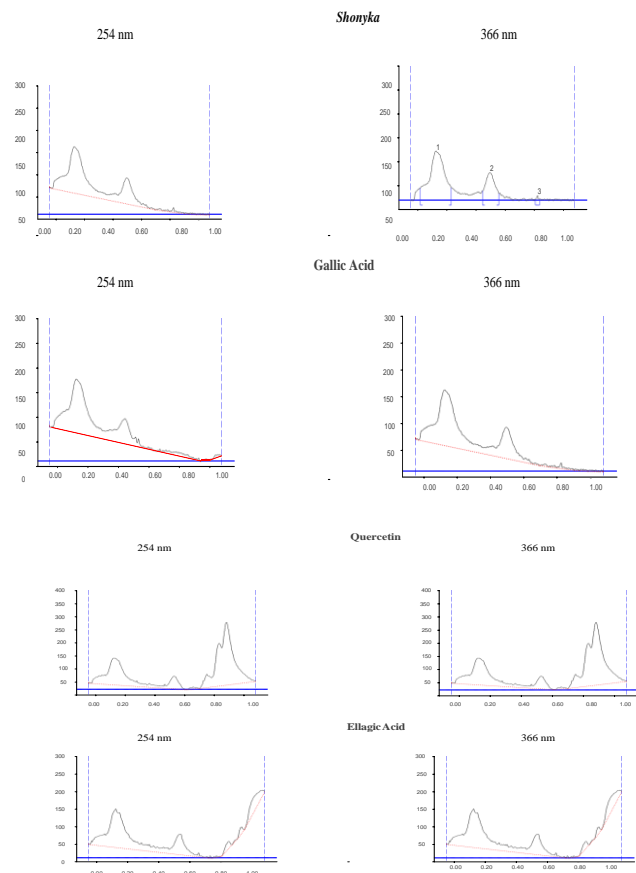


Fig. 2 HPTLC peaks of Shyonaka, Gallic acid, Quercetin & Ellagic acid at 254 and 366 nm
Table 2. HPTLC Rf values of *O. indicum* at 254 and 366 nm

Samples	Peak	Rf Values	
		254 nm	366 nm
Shonyaka (<i>O. indicum</i>)	1	0.30	0.23
	2	0.39	0.50
	3	0.51	0.55
	4	0.57	-
Gallic acid	1	0.32	0.23
	2	0.41	0.56
	3	0.57	0.84
	4	0.84	-
Quercetin	1	0.27	0.07
	2	0.43	0.22
	3	0.63	0.59
	4	0.73	0.78
	5	0.77	0.85
	6	0.86	1.03
	7	0.99	-
Ellagic Acid	1	0.26	0.06
	2	0.31	0.20
	3	0.35	0.61
	4	0.42	0.87
	5	0.62	0.95
	6	0.69	1.08
	7	0.74	-
	8	0.88	-
	9	0.95	-

The UV-visible spectroscopy was also done with HAQi and for this purpose an amount of 0.5 and 1 mg/ml extract solution were prepared for UV-spectroscopy study which were placed 1 cm of two cuvettes for experiment. An amount of 1 ml extract solution and 1 ml distilled water were taken in two different cuvettes for dose of 0.5mg/ ml with the help of wave length range 190.00 to 900.00 nm. Two graphs of sample (*O. indicum*) were automatically prepared and calculated (fig.3). Finally, the chemical fingerprinting of the crude powder of the seed of *O. indicum* was done by FT-IR method. Software of the instrument was started and a few amount of raw sample (*O. indicum*) was placed in instrument sensor machine. Process was started and automatically graph (fig.4) was represented in an instrument and calculates automatically at wave number 1500 cm^{-1} .

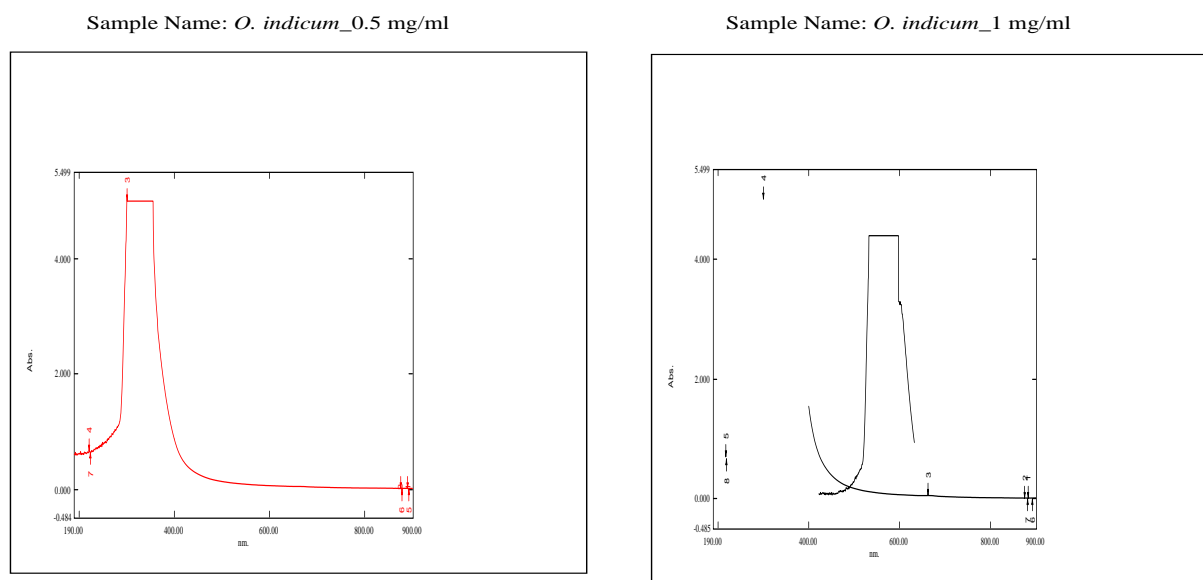


Fig. 3 UV-Visible Spectroscopy of *O. indicum*

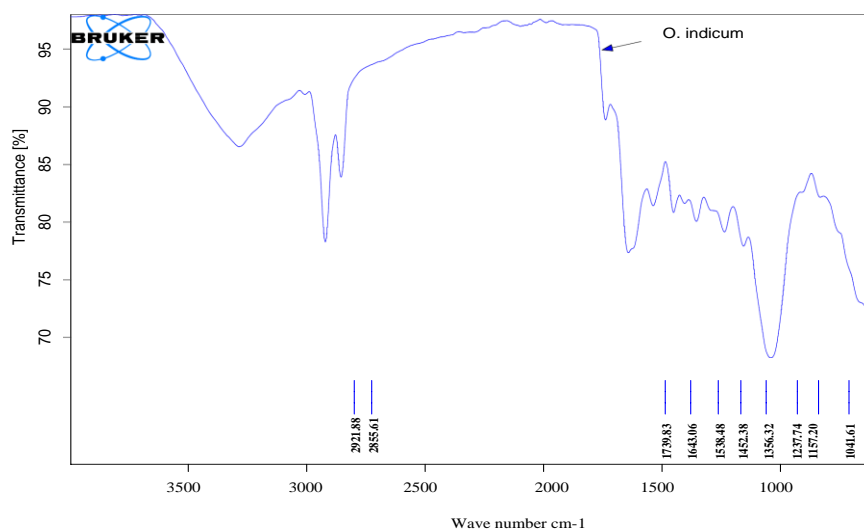


Fig 5. Result of FT-IR in *O. indicum* at wave number 1500 cm^{-1}

Biological properties of different chemical compounds of *O. indicum*

The phenolic compounds like baicalein, oroxylin A and chrysin present in this species have shown therapeutic potential in some areas such as anticancer, anti-inflammatory antiviral etc. [16]. The biological activities and therapeutic potential of baicalein originated from the *O. indicum* plant has potent anti-cancer, antibacterial, anti-hyperglycemia, neurogenesis, cardioprotective, anti-adipogenesis, anti-inflammatory and wound healing effects. Baicalein (5,6,7-trihydroxyflavone) is a flavones, originally isolated from *Oroxylum indicum* or Indian trumpet flower which inhibit the cell proliferation in a concentration-dependent manner in BFTC905 cells, which is responsible for bladder cancer. Higher concentrations of baicalein (60 and 80 $\mu\text{mol/L}$) almost completely blocked the cell proliferation ability. Baicalein (60–80 $\mu\text{mol/L}$ for 24 h) significantly decreased the G0–G1 phases, but increased the G2–M phases in BFTC905 cells. The administration of baicalein is also reported that it is associated with common side effects, such as fatigue and shortness of breath [17].

The multiple biological activities like antioxidant, cytotoxic, antimicrobial, etc. of *Oroxylum indicum* were detected. Different parts of this plant are used for medicinal purpose. Antioxidants assays like ABTS, DPPH, free radical scavenging methods etc. were done. It is reported that, the crude methanolic extract of *O. indicum* revealed free radical scavenging activity, phenolics, etc for detection biological property present in this plant [12]. The plant has preventive role as anti-inflammatory, anthelmintic, antiarthritis, antidiabetic, anticancer, activities etc. Therapeutically it is used for rheumatic pain, spleen problems, ulcers, cough, bronchitis, etc. In tribal communities of India bark and seeds of *O. indicum* are used for treatment of fever, pneumonia, stomach problems and respiratory troubles. The chemical compound Ellagic acid is particularly reported for its hepatoprotective activity. A study was conducted in CCl₄ induced hepatotoxicity in normal rat followed by treatment with oral feeding with ellagic acid (50 mg/kg) provided a significant protection against the biochemical alterations in serum and liver. In vitro experiments showed that liver microsomes from animals treated with ellagic acid and CCl₄, decreased lipid peroxidation compared to microsome prepared from rats exposed to CCl₄ alone [18].

Hepatoprotective effect of *O. indicum*

The liver is a largest internal organ of human body which performs various kinds of function like

metabolism of lipid, fat, protein, foreign compounds etc. Different parts of *O. indicum* like stem bark, fruit, leave, seed etc. are reported that has protective role of liver [19]. A study was carried out with aqueous extract of root bark of the plant against CCl₄ induced hepato-toxicity where significant ($p < 0.05$) reduction of bilirubin, SGPT, SGOT and ALP were found in comparison to control group [20]. A pilot study was also carried out by authors groups for evaluation of the hepatoprotective activity of the ethanolic extract of seed of *O. indicum* in isolated goat liver hepatocytes by the perfusion technique and evaluated on the basis of the hepatoprotective markers like AST, ALT, LDH and MTT. In spite promising results were obtained promising but the study needs more extensive procedures. Besides, anti-oxidant activity of the seed extract was also done on the basis of the assay of ABTS and DPPH, which resemble with the results of activities with other parts of the plant as reported by different scientists. There is a close relationship between the anti-oxidant and hepatoprotective activity and simultaneous study of these two activities were performed in most of the cases with *Oroxylum indicum*. It is observed that the leaf extract of *Oroxylum indicum* have present good antioxidant and hepatoprotective activity [21].

It is reported that acetaminophen (APAP) is a common antipyretic drug and used for analgesic and antipyretic drug purpose when it is over dosed liver can be damaged. In an experiment it was studied that, biological parameters like serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid peroxidation (LPO) etc. were reduced significantly against APAP. It is, therefore, confirmed the hepatoprotective activities of APAP induced liver damage in rat [22].

Modus operandi through phytochemistry

The *O. indicum* plant parts are used in several traditional medicines for prevention of various diseases. The plant has anti-microbial, anti-inflammatory, anti-cancerous, anti-ulcer and hepatoprotective activities and the bioactive compounds like aloe-emodin, chrysin, baicalein, oroxylin A, oroxin A-D, hispiludin and ursolic acid have been present in this plant [23].

There are some chemical constituents obtained from different parts of this plant i.e. baicalein-7-O-diglucoside (Oroxylum B), baicalein-7-O-glucoside, chrysin, apegenin, prunetin, sitosterol, oroxindin, biochanin-A, ellagic acid, baicalein and its 6- and 7-glucuronides, scutellarein, tetuin, antraquinone, aloe-emodin etc. Various parts of the plant are used in Ayurveda and folk medicine for the treatment such as

cancer, diarrhea, fever, ulcer, jaundice etc. It is reported that, *in vivo* and *in vitro* studies of this plant have present anti-inflammatory, antiulcer, hepatoprotective, anticancer, antioxidant, photo-cytotoxic, anti-proliferative, anti-arthritis, antimicrobial, anti-mutagenic, immuno-stimulant as well as hepatoprotective properties. The plant *O. indicum* scientifically proved for different biological activities utilizing traditional and folkloric sources [24]. In spite, different pharmacological activities of the plant were tested with crude extract as well as with some isolated chemical compounds but the pharmacokinetics and pharmacodynamics are yet to be explored in most of the conditions. However, some of the study directly dealt with establishment of mechanism of action with specific chemical constituents of the plant.

A study was carried out with Ellagic acid (EA) for establishment of hepatoprotective activity and it was observed that EA possesses antioxidant, antihepatotoxic, antisteatotic, anticholestatic, antifibrogenic, antihepatocarcinogenic and antiviral properties which improves the hepatic architectural and functions against toxic and pathological conditions. The molecular mechanisms exposed that EA activates scavenging of free radicals, regulation of phase I and II enzymes, modulation of proinflammatory and profibrotic cytokines synthesis in liver. EA also inhibits hepatic stellate cells and mast cells activation in liver resulting hepatoprotection [25].

Baicalein, another important chemical component of the plant, exhibits potent anti-inflammatory effect. Study proves that this activity of the compound is mediated by suppressing the pro-inflammatory mediators like TNF- α , IL-6, NF- κ B-p65 and iNOS [26]. This anti-inflammatory activity of baicalein is mainly oriented to neuro-inflammation and study was conducted with the compound isolated from a Chinese plant *Scutellaria baicalensis* but the activity of compound, is probably will be same for other plant like *O. indicum*. The baicalein is also reported for its anti-cancer activity, specifically in lung cancer. Its mechanism of action for anticancer activity is claimed to be due to regulation of cell proliferation, metastasis, apoptosis and autophagy [27].

CONCLUSION:

Oroxylum indicum (Kurtz.) is an Indian medicinal plant, which is used in Ayurveda and other traditional system of medicine for several therapeutic effects. The plant in Ayurvedic text is categories as one of the ten components in the group of drug named 'Dashamula'. Pharmacologically the plant is reputed

for its biological properties like anti-cancer, antibacterial, anti-hyperglycemia, neurogenesis, cardioprotective, anti-adipogenesis, anti-inflammatory, hepatoprotective and wound healing effects. Chemical analysis with various procedures envisages presence of multiple compounds, specifically phenolics and flavonoids in nature. Most important chemical compounds of the plant are baicalein and Ellagic acids. The major therapeutic activity of the plant as mentioned in different classical Ayurvedic texts is anti-inflammatory, particularly in rheumatism. Evidence of hepatoprotective activity of the plant is not mentioned in Ayurvedic texts but scientific study reveals such activity in different pharmacological models. Hepatoprotective activity of the aqueous extract of seed and stem bark of *O. indicum* was observed both in *in-vivo* and *in-vitro* models by inducing hepatotoxicity with CCl₄ and isolated goat liver models treated with ethanol respectively. Promising results were obtained with the plant extract both in these two models. The hepatoprotective activity may be due to its potent antioxidant activities as observed through estimation of biochemical markers like ABTS and DPPH. Ellagic acid of the plant could have a potent hepatoprotective activity due to its property of scavenging of free radicals, regulation of phase I and II enzymes, modulation of proinflammatory and profibrotic cytokines synthesis in liver. The other chemical compound baicalein is claimed to be a potent anti-inflammatory agent particularly against neuro-inflammation by suppressing the pro-inflammatory mediators like TNF- α , IL-6, NF- κ B-p65 and iNOS. Therefore, it could be recommended that *O. indicum* is a potent medicinal plant of Ayurvedic origin which can be used as potent hepatoprotective and anti-inflammatory therapeutic agent.

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