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REVIEW ARTICLE **OPEN ACCESS**

A Review on the chemistry and pharmacological properties of Oxymatrine alkaloid compound obtained from the roots of *Sophora flavescens* Aiton.

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ABSTRACT
Oxymatrine- a quinolizidine alkaloid, which is extracted from the dried roots of a traditional Chinese herb named Sophora flavescens. There are several signalling pathways that have shown effects ofanti-cancer, anti-inflammatory, anti-pruritic and analgesic properties. Experiments have been conducted on mice showing significant results. The compound is extracted effectively by using the microwave assisted extraction (MAE) process as well as ultra-sound assisted extraction (UAE) method. The crude extract is used directly or has been isolated and purified to study the effects of various cytotoxic, neurological, dermatological ailments of the body as there has been enough evidence proving the traditional therapeutic applications of oxymatrine. The compound oxymatrine is also known as matrine N- oxide based upon the structure of the compound. Oxymatrine is a potential drug highly used in the traditional Chinese medicinal for healing multiple problems. The plant from which it is extracted belongs to sub-family fabaceae under the family leguminosae. A lot of studies have been done on the compound suggesting the use of the compound as a healing drug to cure inflammations, fibrosis, lower the diabetes, fungal infections, cardiovascular problems, as well as acts against tumor and related issues like angiogenesis, analgesic, mitosis, pruritis, asthma, and neuro-protective activities. This proves the compound holding a lot of potential to be used in the pharmaceutical industry in order to be useful for the treatment of several diseases.
Key words: *alkaloid, chemistry, extraction methods, oxymatrine, pharmacological properties, quinolizidine, Sophora flavescens.*

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INTRODUCTION

Oxymatrine is a quinolizidine alkaloid extracted from the roots of *Sophor aflavescens*.Oxymatrine is also known as matrine N-oxide[1]. Alkaloids are defined as cyclic organic compounds consisting nitrogen with negative oxidation state. The basic properties of alkaloids are linked to the heterocyclic tertiary nitrogen. The reason behind alkaloid being used in medicines is because they are soluble in water when in acidic conditions, while in neutral to basic conditions; alkaloids are soluble in lipids[2]. Natural sources or chemical nature are used to classify alkaloids. The distribution as per preliminary C-N skeleton structure is the most common way of classifying alkaloids. Alkaloids are divided into the large groups namely, pyrrolidine, pyridine, quinoline, isoquinoline, steroidal, diterpenoid, indole, quinazoline, and other alkaloids. NMR techniques and X-ray diffraction spectrometry help in identification of most of these compounds. Commercially near about 30 alkaloids are used as medicines, flavouring agents or as poisons [3]. Quinolizidine alkaloids are preliminary alkaloids observed in the family Leguminosae. They have over 170 structures that have been reported. These molecules have at least one quinolizidine ring which distinguishes it from the other alkaloids [4].*Sophora* genus is said to be traditional Chinese herb which has gained popularity in recent times. The plant is native to parts of China, Japan, Tibet, Magnolia, Taiwan, Korea, and recently been introduced to Great Britain. It is a perennial, non-climbing herb.The plant from which it is extracted belongs to the sub-family Fabaceae under the family Leguminosae. It has been studied that this genus is rich in phytochemicals like quinolizidinealkaloids, flavonoids which were used as markers in chemotaxonomy. The phytochemicals present shows alot of activities like anticancer, anti-asthmatic and anti-inflammatory. Oxymatrine has been extracted from the dried roots (known as kushen) of the plant *Sophora flavescens* [5]. In countries like China, the drug is used to treat viral hepatitis, cardiac arrhythmia and skin inflammations. Studies have proved the effectiveness of oxymatrine in inhibition of

tissue fibrosis along with the hepatic fibrosis, myocardial fibrosis, and pulmonary fibrosis [6]. Most of the literature explains the presence of alkaloids and flavonoids in*Sophora* plants which reveals several pharmacological activities. The root extracts contain the most amounts of bioactive compounds which include matrine and oxymatrine alkaloids, flavonoids like flavanones, flavonols, isoflavonols, prenylated flavonoids and isoflavonones. The bioactive ingredients could lead to drug discoveries and thus it becomes necessary to investigate all the pharmacological properties alongside its toxicity with clinical trial as well as *in vitro* and *in vivo* experiments [5].

PHYSICAL PROPERTIES OF OXYMATRINE

Molecular formula for Oxymatrine is $C_{15}H_{24}N_2O_2$. The molecular weight of the compound is 264.36 g/mol. Oxymatrine has white to off-whitish colour and a slight amber aroma. The melting point has been recorded as 208^{o}C. Oxymatrine is found to be soluble in water, methanol, and ethanol [7]. The molecule has two nitrogen atoms, one as amide, and other bound to an oxygen atom forming coordination bond (Figure 1). Due to the bond, the electron supply capacity become weak, so its alkalinity reduces, resulting in an increase in its hydrophilicity which makes it dissolved easily in water, chloroform, ethanol and poorly dissolved in petroleum ether and ethyl acetate[8].

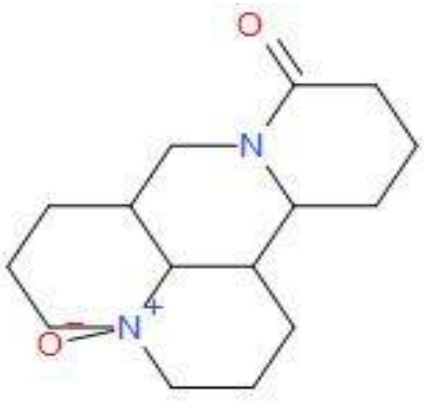

Fig 1: Chemical structure of Oxymatrine compound.

OXYMATRINE EXTRACTION METHODS

Oxymatrine has a large number of activities, and thus it becomes necessary to extract it carefully and effectively. The general process of extracting and separating any compound from the plant material is briefed in Table-1. There are many methods like high performance liquid chromatography (HPLC), high performance capillary electrophoresis (HPCE) and gas chromatography (GC) which have been used for the separation of alkaloids from roots [9]. But prior to the separation, extraction is done by maceration, decoction and refluxing or leakage method. Solvents like ethanol, water are used, instead of methanol or chloroform in order to avoid toxicity to humans and environment. The extraction temperature range is a factor alongside the solvents used, which determines the yield of the compound. Therefore, Microwave Assisted Extraction (MAE) method was proved to be much more effective in terms of yield in case of oxymatrine extraction.The optimal extraction conditions are determined as 60% ethanol 20:1 ratio of liquid to material for 10 minutes at 50ºC, with microwave radiation of 500W. The yield of oxymatrine under these conditions is observed as 14.37 mg/g of the sample. The crude extract is either processed further for isolation and purification of oxymatrine, or else used as a component for complex traditional medicines[10]. A few studies conducted using Ultra-Sound Assisted Extraction (UAE) has shown an even higher extraction rate of oxymatrine (52.3-53.4%). For Ultra-Sound Assisted Extraction (UAE), the parameters that provide optimal yield are 65% ethanol, liquid to material ratio of 30:1, for 10 minutes at 50ºC under ultrasonic radiation. Extraction of important active component from plant material can be done using MAE and UAE[11]. Table-2 summarizes the percentage yield of the compound obtained by using several extraction methods at varying temperature.

PHARMACOLOGICAL PROPERTIES OF OXYMATRINE COMPOUND

Oxymatrine compound is known to possess several vital properties (Figure-2) which are described below. The mechanism of action of the compound associated with each pharmacological property is briefed in Table-3.

Anti-inflammatory property:

Oxymatrine has shown great anti-inflammatory property in the earlier studies. The tissue is protected from inflammatory infiltration and its molecular mechanism relates with the inhibition of phosphorylation of nuclear factor and signal pathways of mitogen activated protein kinases. Oxymatrine enhances acute pancreatitis induced by L-arginine, by reducing plasma amylase, D-lactic acid, and pancreatic myeloperoxidase activity. Oxymatrine along with the sodium ferulate shows synergistic anti-inflammatory properties [12]. One of the most costly diseases in the dairy industry is considered to be bovine mastitis, which is an inflammatory reaction of the mammary glands. Many studies have already proved the use of oxymatrine in protecting reperfusion injury to the heart via anti-inflammation and anti-apoptosis. Oxymatrine does not have any toxic effects with intra-peritoneal injections, thus it was examined for the treatment of mastitis and the study suggested production of pro-inflammatory cytokines in the mammary glands that linked with the inhibition of signalling pathways showing promising therapeutic medicine for the treatment of mastitis[13].

Anti-fibrosis property:

Safe and effective treatment of hepatic fibrosis was seen using oxymatrine. Mesenchyme stem cell therapy is enhanced and serum levels of interleukin-4 (IL-4) and interleukin-10 (IL-10) are increasing. Serum alkaline phosphatase is reduced along with collagen deposits, which inhibits the gene expression related to fibrosis. There is a reduction in theproduction and deposition of collagen in liver tissue by modulating fibrogenic transduction of p39 MAPKs signalling and protection from the myocardial by transforming the growth factor signal pathway[12].The implications of the direct expressed proteins mediate oxymatrine effects on the fibroblasts. Proteomic analysis is used to confirm the changes in the expression of proteins along with western blotting and reverse transcription polymerase chain reaction (RT-PCR). The protein analysis explains the mechanism of protective effects of oxymatrine with the pulmonary fibrosis [6].

Protective effect on diabetes and hyperlipidemia:

Lipid and glucose metabolism is effectively protected by oxymatrine. Islet and liver is guarded by it and insulin secretion as well as sensitivity is improved via increase of mRNA and protein levels of peroxisome proliferator-activated receptor-α (PPA Rα), microsomal triglyceride transfer protein (MTTP), and carnitinepalymitoyltransferase 1A (CPT1A), decreasing the Srebf1, which contributes for the reduction of insulin resistance, hyperglycemia, and hyperlipidemia. Oxidative stress is defended by oxymatrine and contents of renal advanced glycation end products are also reduced [12]. Alkaloid molecules like oxymatrine target the insulin signalling pathway because they are able to lower the glucose and lipid levels as well as act as anti-inflammatory compounds [14].

Antifungal property:

The study suggests that oxymatrine has the strongest inhibitory effects on *Fusariumoxysporum* with the EC_{50} value of 26μg/ml, while the germinated spores treated with matrine in low concentration shows short germ tube formation in comparison to the control [15].

Cardiovascular protective effect:

Studies and researches show oxymatrine playing protective role in the cardiovascular system. It enhances the experimental remodelling of ventricular by reduction of serum content of asymmetric dimethylarginine (ADMA), increasing the expression of double methyl arginine hydrolase 2 antibodies (DDAH2), and inhibition of expression of ACE- angiotensin-converting enzyme and TGF-β1 and activating the extracellular signal- regulated kinase. Oxymatrine works against the cardiotoxicity induced by aldosterone by inhibiting apoptosis inducing factor signalling pathways, upregulation of sarcoplasmic reticulum Ca^{+2} ATPase helps to improve heart failure; arrhythmias are protected through sodium and calcium current inhibition, shortening action potential at the time of reducing L-type calcium current and enhancing transient outward potassium current, and inward rectifier potassium current is inhibited[12].

Anti-tumour property:

Matrine and oxymatrine, individually or in combination alongside chemotherapy are proved to exert anti-tumour activity while blocking the stages of cell-multiplication [16].

Anti-angiogenesis property:

The important process for the growth of tumour is angiogenesis. It plays essential role in the spread of cancer. Anti-angiogenesis is the wide accepted method for the therapy of cancer by suppressing growth of tumour by disrupting nutrient supply and oxygen to the tumour from the blood. Oxymatrine helps in decreasing the expression of associated factors like the vascular endothelial growth factor (VEGF). Oxymatrine along with angiogenesis inhibitor or chemotherapy drug in a low dose can inhibit the expression of VEGF and chemokine receptor-4, exerting synergistic effects on the growth of gastric cancer cells[12]. The inhibition in growth and survival of pancreatic cancer cells is done by oxymatrine while inhibiting capillary tube formation. The treatment with oxymatrine had significantly reduced the peak

intensity value of pancreatic cancer in mice which is used as quantification of tumor blood purification. Thus, matrine and oxymatrine prove to have great anti-angiogenic potentials [16].

Analgesic effect:

Cancer induces the pain and the relief from it is a preference. The high dose of oxymatrinecan inhibit the expression of morphine-induced P-glycoprotein and developing tolerance for morphine, leading to the effect of analgesic. Molecular mechanism helps regulating N-methyl-D-aspartate (NMDA) receptor 2b (NR2B)-containing the NMDA receptor-ERK/CAMP- response element binding protein (CREB) signalling. Along with sodium ferulate, prominent analgesic effect is seen with synergistic inhibition of receptor potential[12]. Administration of oxymatrine with morphine provides morphine tolerance. The analgesic activity of morphine depends upon the P-gp expression which is considered as a mechanism for the tolerance of morphine. The effect of oxymatrine on morphine induced P- gp expression is studied. In the morphine treated rats, the P- gp expression was decreased by the use of oxymatrine. Oxymatrine results in the partial reversal of multidrug resistance due to P- gp inhibitory action. Thus, oxymatrine has a potential analgesic activity related with other properties and components[17].

Apoptosis-promoting property:

Dose and time-dependent manner of oxymatrine proves to induce cell apoptosis. Anti-tumor action is exerted by stimulating caspase-triggering signal pathway. Down regulating anti-apoptotic Bcl-2 and IAP families (inhibitor of apoptosis protein), and up regulating pro-apoptotic Bax expression, releases cytochrome c and activates caspase in human cancer cell. The membranes of IAPs i.e. Livin and Survivin are important for inhibiting apoptosis by negative action on caspase. Overexpression of the membranes is associated with the prognosis in cultured tumors, since oxymatrine can down regulate the expression of livin and survivin, promoting the apoptosis effect[12]. The treatment of oxymatrine has pro-apoptotic effects on breast cancer MCF-7 cells which are correlated with the up regulation of Bax transcription and down regulation of Bcl-2 transcription as well as protein transcription, while being dependent on the time and dose[18].

Anti-mitotic property:

Methanolic extract of the plant *Sophora flavescens* was screened against stored grain insects, where it was observed to have contact toxicity and anti-feedant effects. Cytotoxic property has been evaluated via the allium test, where in *Allium cepa* was used, carefully removing the scales, which were later, cultivated on the test-tubes having varying concentrations of oxymatrine. After 72hr, the roots were measured, and later on fixed using glacial acetic acid, and stained. The root tips were squashed and smeared on to the slide and observed under the microscope to study the mitotic index (MI). Oxymatrine was seen to significantly reduce the mitotic index by 50%, which shows that oxymatrine has anti-mitotic activity which could stop the process of mitosis at any point in the cell cycle, inhibiting the cancerous cell growth[19].

Anti-pruritic activity:

Pruritus is an urge to itch caused by an uncomfortable sensation that might be due to some allergies. ACD-allergic contact dermatitis which is an inflammatory disease of the skin was mediated by hapten-specific T-cells. We lack at having an effecting therapy for pruritis, and studies show some evidence that the traditional Chinese herb- *Sophoraflavescens* can be used to relieve the itch with minimal side-effects, instead of using the current options like antihistamines and anticonvulsants. Oxymatrine combines with the cells in the nervous system (pruritus nerve condition dorsal root ganglion [DRG] cells) that depicts oxymatrine hold potential to act as the biochemically helpful agent for curing ACD [20].

Anti-asthma activity:

A chronic inflammatory disease of the airways is known as asthma. It has caused major distress, and if aggrevated, might lead to dead. Drugs like steroids, mast cell stabilizers are developed for the treatment of asthma. OVA-induced model is used to check if oxymatrine could be proved effective for the treatment of allergic asthma. Inflammatory cells like, eosinophils and the lymphocytes migrate inside the lungs contributing to the allergic inflammations, and an increase in the number of eosinophil cells results in asthma. Oxymatrine has shown to reduce the number of eosinophils in the lung, which gives prominent relief in asthma [21].

Neuro-protective property:

Ischemic stroke (IS) is a neurological disease with high rate of mortality. Oxymatrine has shown protecting activity against IS. *In-vivo* and *in-vitro* models are used to assess the effect of oxymatrine with association of the brain-blood barrier (BBB). It was evaluated via the neurological function scores and labelled staining. Oxymatrine has prominently improvised the functions of the brain, while reducing the BBB permeability. Also, oxymatrine improvised the cells that were in a deprivation of oxygen and glucose, which means it can be significantly effective against BBB injury after Ischemia-reperfusion[22].

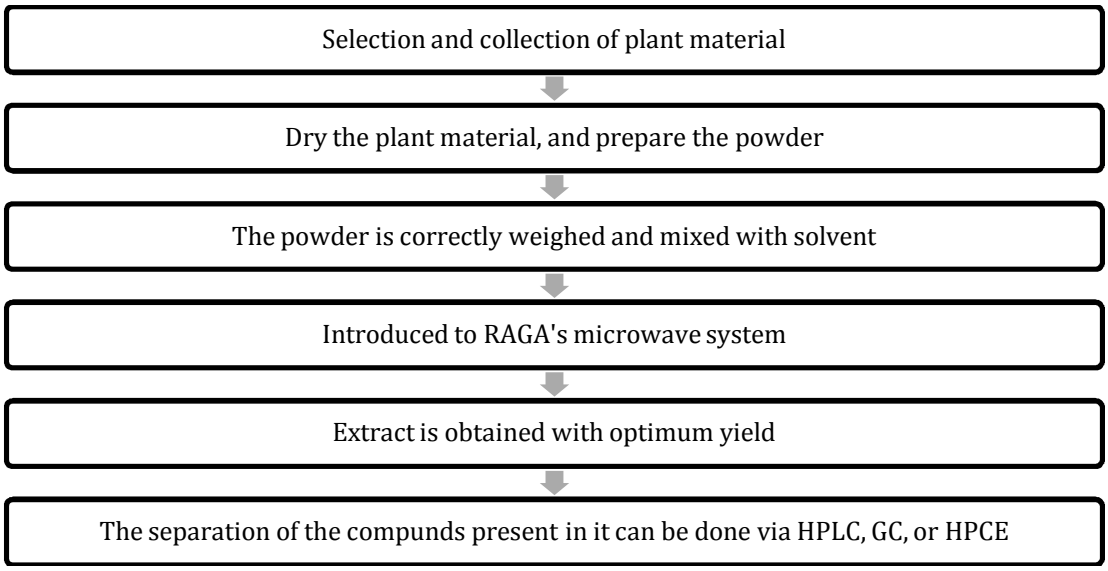

Table 1: showing the process of extraction and isolation of compounds in brief.

Sr no.	Method	Time	Temperature	% Yield
1.	Soxhlet Extraction	4hrs	90ºc	66.8%
2.	Conventional Solvent Extraction	24hr	25ºc	78.1%
3.	Microwave Assisted Extraction (MAE)	50min	50ºc	86.1%
4.	Ultra-Sound Assisted Extraction (UAE)	50min	50ºc	100%

Table 2: showing comparison for percentage of yield using different extraction methods

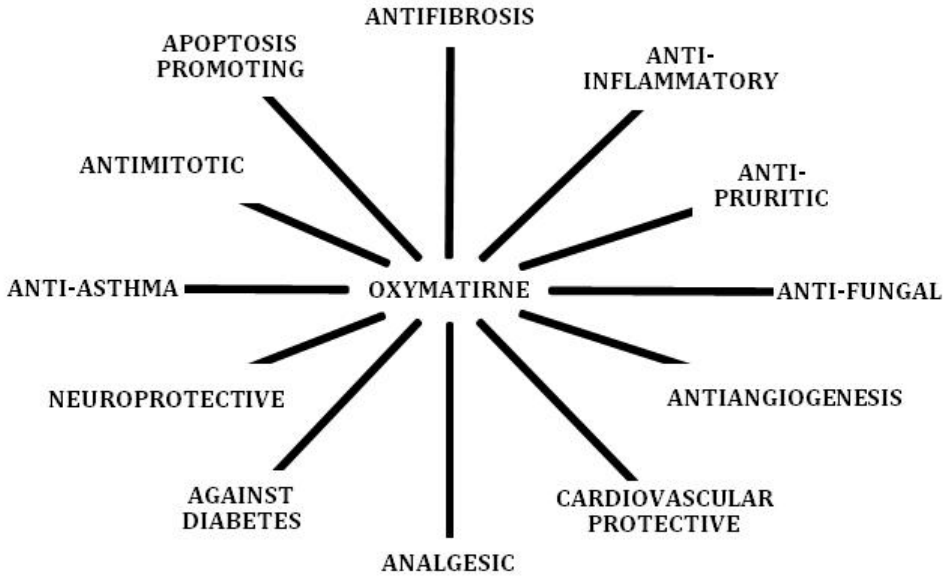

Fig 2: showing a summary of all the properties of oxymatrine compound.

Sr. no.	Property	Mechanism
1.	Anti-inflammatory	Inhibition of phosphorylation of nuclear factors, signal pathways of mitogen activated protein kinases[12. 13]
2.	Anti-fibrosis	Reduced production and deposition of collagen in liver tissue by modulating fibrogenic transduction of p39 MAPKs signalling [6, 12]
3.	Anti-angiogenesis	Decreasing the expression of associated factors like the vascular endothelial growth factor (VEGF) [12, 16]
4.	Anti-mitotic	Cytotoxic property has been evaluated via the allium test; Oxymatrine was seen to significantly reduce the mitotic index by 50%, inhibiting cancerous cell growth [19]
5.	Anti-pruritic	Oxymatrine combines with cells of nervous system (DRG cells) showing cure to ACD [20]
6.	Analgesic	High dose of oxymatrine can inhibit the expression of morphine-induced P-glycoprotein and developing the tolerance for morphine, leading to the effect of analgesic [12, 17]
7.	Diabetes	Increase in levels of PPA Rα, MTTP, CPT1A, decrease in Srebf1, resulting in insulin resistance [12, 14]
8	Anti-asthmatic	OVA-induced model, decreasing eosinophils, resulting in relief in asthma [21]
9.	Apoptosis promoting	Stimulated by caspase-triggering signal pathway [12, 18]

Table 3: showing the mechanism of action of oxymatrine compound for various pharmacological properties

CONCLUSION

This quinolizidine alkaloid, oxymatrine can be a wonderful natural treatment for a lot of ailments like skin allergies, inflammations, asthma, tumor, cancer and pruritis. The drug also shows apoptosis promoting mechanism, anti-angiogenesis, analgesic, neuro-protective, anti-mitotic, and anti-fibrosis properties. Matrine and oxymatrine are the alkaloids that can be useful for cardiovascular diseases and for diabetic control. The extraction, isolation and commercialization of the particular compound from the plant can be very impactful in curing and combating major life threatening diseases like cancer and asthma.The extraction of crude can be done using microwave assisted extraction (MAE) or ultrasound assisted extraction (UAE). Large scale utilisation of the drug will require commercializing the cultivation of the plant over being just a traditional Chinese herb.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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