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Review & Future Perspectives of Using Vasicine, and Related Compounds

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Abstract— Vasicine molecule was first isolated in 1924 and most of the work on this molecule was done between 1960s-1980s. The plant *Adhatoda vasica* (Acanthaceae) is the main source of this molecule. This plant has been used in India for 2000 years for the treatment of respiratory ailments and for its abortifacient activities. It is surprising to note that, even if, it has been used effectively and extensively in the above mentioned areas since ancient times till date, and a huge amount of work has already been done on its derivative "Bromhexine", very few recent reports are available for its molecular mechanism of action. The present article is an attempt to compile the literature available for this molecule and to further analyze its probable potential use in the above areas, including few more other areas as well.

Keywords : Vasicine, Peganine, *Adhatoda vasica*(Zeylanica), Asthama, Bisolvon, Bromhexine.

INTRODUCTION

Vasicine/Peganine is a quinazoline type alkaloid mainly obtained from the plant *Adhatoda vasica (zeylanica)/Justicia adhatoda* (Acanthaceae) (Figure 1). Few of the main chemical constituents of this plant are vasicine (derived from leaves), 2'-hydroxy-4-glucosyloxychalcone, vasicol (from leaves), vasicinone (from leaves, stem and roots), vasicinol (contained in stem and roots), and deoxyvasicinone (from leaves) etc. as shown in figure 2.

It was first isolated from by Sen and Ghose in 1924 [1]. Different morphotypes of this species of the plant are found in nature. These species are classified in different groups and can be characterized with the help of fingerprint profile and vasicine content [2]. *Adhatoda vasica* (AV) is found in tropical regions of Southeast Asia, including India [3]. Pharmacological properties of this plant are known since very old times. It is well established now that, vasicine is the major, as well as, the most important active principle of this medicinal plant. It is reported to be responsible for most of its activities including: antioxidant, anti-inflammatory and bronchodilatory activity. It is an optically active molecule in its natural condition but, gets racemized when extracted. Although, its importance has been recognized since years and it is being used extensively in many herbal formulations for respiratory diseases and disease related to female reproductive system, limited data is available for its molecular mechanism of action.

Fig 1*: Adhatoda vasica* plant (Courtesy, Patanjali Yog Peeth, Haridwar, India)

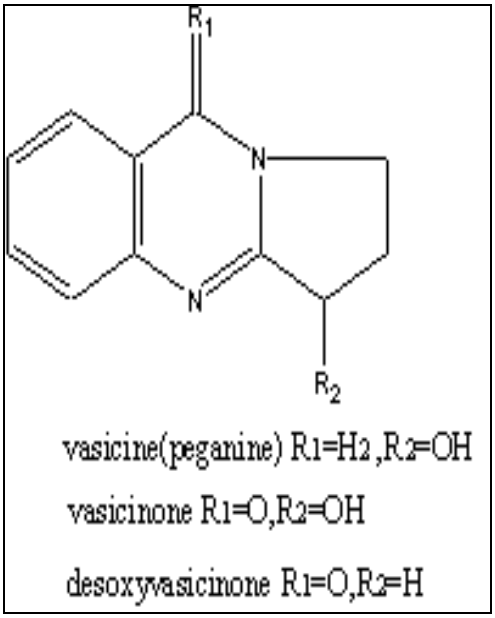

Fig.2: Structures of vasicine, vasicinone, deoxyvasicinone

Realizing its medicinal importance, Shivanna (2009) [4] has worked upon its pollination biology. There are studies describing various methods for extraction of vasicine from the plant AV [5]. HPLC determination of vasicine with photo diode (PD) detector is not well established [6]. C_{18} column (pH 3.9) with acetonitrile - phosphate – acetic acid (15:85:1, v/v/v) buffer can be used to separate vasicinone and vasicine. Soni *et al.* (2008) [7] compared different methods of extraction of vasicine including the classical method in which, the crushed leaves are subjected to wet heat followed by squeezing the extract as well as modernization of the same by exposing the leaves to steam at 15 lb. The total alkaloid content varied from 0.3 mg/ml to 5.93 mg/ml and that of vasicine from 0.2 mg/ml to 5.64 mg/ml for different methods. International Application Published under the Patent Cooperation Treaty (PCT) has published method for the production of vasicine in 2003 [8].

OTHER SOURCES OF VASICINE

Although AV is the main source of obtaining vasicine, other plants were also worked upon to isolate this molecule. In 1975, vasicine was isolated from a different plant *Sida cordifolia* [9] by Ghoshal *et al*. (1975). Susag *et al*. (2003) [10] isolated vasicine from two species of Afrogalega: *Galega battiscombei* (Bak.f) Gillett and *Galega.lindblomi* (Harms) Gillett. Bagchi *et al.* (2003) [11] reported its isolation from *A. beddomei* and the content of vasicine was compared to *Adhatoda zeylanica/vasica*. They reported that *Adhatoda zeylanica/vasica* showed wide seasonal variation throughout the year. The variation of vasicine was found from 1.22 % to 2.57 % twice a year. Peganine/vasicine hydrochloride was isolated from *Peganum harmala* seeds in dihydrated form by Khaliq *et al.* [12].

PHARMACOLOGICAL PROFILE

Antioxidant and anti-inflammatory activity

Methanolic extract of *Adhatoda* has been reported to have antiinflammatory activity. At a dose of 50 µg/pellet alkaloid fraction, Chakraborty *et al.* (2001) had shown its potent activity in modified hen's egg chorioallantoic membrane test. Srinivasrao *et al.* (2006) [13] have worked upon the antioxidant and anti-inflammatory activity of vasicine against ovalbumin and aluminum hydroxide induced lung damage in rats. They had shown that, vasicine treatment has effect on superoxide dismutase, catalase, glutathione peroxidase and "reduced glutathione".

The extracts of AV, along with few other plants were evaluated for their effect on reduced glutathione and lipid peroxidation in liver (antioxidant) and in DPPH▪ and ABTS assays (radical-scavenging activities). All plant extracts showed antioxidant activity (*A. vasica* – 337 µg/ml). Besides this, the antioxidant, antidiabetic and antibacterial activity of various extracts of AV has also been investigated by Ilango *et al.* (2009) [15]. Among hexane, chloroform and methanolic extracts, the methanolic extract had shown to have maximum antioxidant activity and blood cholesterol lowering activity. Hexane extract (400 µg/ml) showed to have maximum antibacterial activity.

Genoprotective role

Oxygen species are reported to be harmful for the important cellular constituents like lipids, proteins and nucleic acids. Antioxidants are used to protect this kind of damage caused due to an imbalance in the oxygen radical content in the cells. Both *Adhatoda* as well as its pure component vasicine are shown to have a very strong antioxidant activity, as described above. Research has been conducted by Jahangir *et al.* (2006) [16] to investigate the radioprotective/genoprotective activity of this plant. They found that, the oxidative stress caused by cadmium chloride (and so the genotoxicity) can be reverted by AV. Vasicine and vasicinone as the major alkaloids in the extract of this plant were suggested to play a key role in this case.

Kumar *et al.* (2005) [17] investigated the hematological changes in the blood of Swiss albino mice after the treatment with ethanolic extract of AV (800 mg/kg body weight, 6-30 d post irradiation intervals). Mice exposed to radiation (8.0 Gy) without AV leaf extract pre-treatment, exhibited signs of radiation sickness like anorexia, lethargy, ruffled hairs and diarrhoea and such animals died within 25 days of post-irradiation. Conversely, animals pre-treated with AV leaf extract showed 81.25% survival till 30th day. A significant decrease in blood "reduced glutathione" (GSH) content and increase in lipid peroxidation (LPO) level was observed in control animals (radiation alone). However, AV leaf extract pretreated irradiated animals exhibited a significant increase in GSH content and decrease in LPO level. A significant increase in the serum alkaline phosphatase activity and decrease in acid phosphatase activity was observed in AV leaf extract pretreated irradiated animals during the entire period of study.

Further, Kumar *et al*. (2007) [18] investigated the genoprotective role of extracts of AV on radiation induced damage at cellular, biochemical and chromosomal level. The ethanolic extract have shown to have effect on histology of testis, glutathione (GSH) activity, lipid peroxidation (LPO) level, acid and alkaline phosphatases activity and chromosomal alterations, in Swiss albino mice.

They have shown that, if 800 mg/kg ethanolic extract is given to mice prior to the exposure to radiation, 70% improvement is observed in terms of mortality. The level of GSH is found to be increased and LPO to be decreased. The tissue damage in testis was also shown to be reduced. Their investigation indicates that, the antioxidant mechanism of radioprotection and free-radical scavenging appears to be likely mechanisms of radiation protection by the plant extract.

Sharma *et al.* (2009) [19] also reported the radiation protective ability of 50% methanolic extract of AV by chromosomal aberration assay in human peripheral cell culture. Patients undergoing radiotherapy were pretreated with low and high (50 and 100 mg/kg body wt) doses. Both the doses were able to increase the number of normal metaphases (in total 200 metaphases studied in control and experimental sets), increasing to 179 and 184 (for low and high doses respectively) in comparison to 118, in control.

Hepatoprotective activity

AV was reported to be hepatoprotective and, it is believed to be based on its antioxidant property. Pandit *et al.* (2004) [20] investigated the antioxidant property of *Adhatoda* on carbon tetrachloride induced hepatotoxicity in rats. They had shown that AV (100 mg/kg and 200 mg/kg) increased the activity of protective enzymes in the liver. Doses from 50 to100 mg/kg, per os, of AV leaf have also been shown to have significant hepatoprotective effect on liver damage induced by d-galactosamine in rats [21].

Antitussive and bronchodilatory activity

AV is well known for its use in respiratory ailments. Taking a lead from the nature, scientists are trying to synthesize molecules similar to, or derivatives of vasicine. Both pure vasicine and its derivatives are worked upon to investigate their bronchodilatory and antitussive effects. One of those derivatives is Bisolvon/bromhexine (N-cyclo-N-methyl-(2-amino-3,5-dibromo-benzyl)amine hydrochloride). It has been reported to possess mucus liquefying/expectorant activity by Amin *et al*. (1959) [22] and Sharafkhaneh *et al.* (2007) [23].

A clinical trial was conducted with the derivative: bromhexine (bisolvon) with 30 patients (20 d, 8 mg, thrice a day) suffering from variety of respiratory complaints [24]. It was found that, there was a major change in the viscosity and acid mucopolysaccharide (AMPS) structure in the mucus of infected and uninfected patients. Similar study was conducted by Gent *et al.* also in 1969 [25]. Bruce and Kumar further observed AMPS fiber system to be disintegrated and disorganized with a concomitant fall in viscosity. A total of 100 human patients were given this drug for trial and were reported to respond well for the ease for expectoration of less viscous sputum. On the contrary, Langlands (1970) [26] did not observe any significant change after this treatment. In this study, Bromhexine was compared with a placebo in a double-blind clinical trial in patients with exacerbations of chronic bronchitis who had mucoid sputum. Treatment with either Bromhexine 8 mg, three times a day or with identical placebo tablets was continued for 14 days. There was no significant effect on the characteristics of the sputum, improvement in ventilatory capacity, or clinical advantage in patients on Bromhexine. Similarly, a report with usage of a higher dose of Bromhexine was published in "*British Journal of Disease Chest*" in 1973 [27]. It states that 48 mg Bromhexine dosage daily for 2-3 weeks brought about an indistinguishable effect with the placebo tablets with respect to stickiness of sputum, difficultly of expectoration or time taken to clear the chest in the morning.

Similarly in 1974, Thomson [28] investigated the effect of Bromhexine on 9 patients for its (16 mg, thrice a day) mucociliary clearance rate of removal of previously inhaled particles tagged with a radioisotope (99 mTc). Serial whole lung gamma counts showed on average a small but, statistically significant faster clearance after the drug administration than in identical control runs ($P < 0.05$). The effect of Bromhexine after 72 h of treatment in 23 patients with chest infection was also tested [29]. Bromhexine was

appeared to cause a small decrease in the capacity of the bronchial secretion to become viscid. There was no evidence that, Bromhexine at a dose of 24 mg daily, altered the capillary permeability of the bronchial epithelium.

Racle *et al.* (1976) [30] investigated the effect on 40 patients in a randomization, half of whom received Bisolvon intravenously. Observations were made for the following parameters: fewer bronchial aspirations, less fluid secretions, a decrease in alveolar cells, an increase in bronchial cells, a reduced increase in total mucus. These results evidenced an original action ascribable to Bisolvon on the bronchial cells.

Antitussive effect of AV extract was also investigated in mechanical or chemical induced coughing in guinea-pigs [31]. Oral administration of AV extract (ED_{50}: 75.6-200 mg/kg) inhibited chemically induced coughing, in dose dependent manner (up to 75%) and was comparable to codeine. An i.v administration of the extract (5-20mg/kg) shown to have almost complete inhibition (at 20mg/kg) of the coughing induced by mechanical methods. Electrically induced coughing was also found to be inhibited by i.v. administration of AV extract (ED50: 15.5 mg/kg) but was not comparable to codeine. Another derivative of vasicine: 2,4-diethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-12-one, exhibited marked bronchodilator activity as evaluated on contracted trachea or constricted tracheo-bronchial tree. Cumulative dose response study with acetylcholine and histamine indicated its non specific direct effect on smooth muscle [32].

Ambroxol, a widely used secretolytic agent developed from vasicine, is found to inhibit IgE-dependent mediator secretion from human mast cells and basophils, which are the main effector cells of allergic inflammation. This compound was found to be more potent than vasicine in attenuating basophil IL-4 and IL-13 secretions respectively. It also reduced IgE-dependent p38 MAPK phosphorylation in basophils [33].

Richardson and Phipps [34] reviewed the drugs including Bromhexine and briefed the studies conducted around the world. Very few studies from India were included in the same. It is interesting to note that, the results obtained in those studies were not consistent and were contradictory. The evidences suggest that Bromhexine did not cause spectacular improvements in bronchitis patients. Still Bromhexine is the main constituent of many expectorants available in the market, for example: Tuspil from “Indoco remedies” is being used for treatment.

It is clear from the above discussion that, contradictory results were obtained in history about the antitussive effect of vasicine and its derivatives. No recent report is available for this kind of analysis. The summary of these studies is given in table 1.

Table 1 Summary of clinical trials performed with the bromhexine

Sr.no.	Compound used	Dose	Scientist	Outcome
1	Bisolvon/ bromhexine	30 patients (20 days, 8 mg, thrice a day	Bruce and Kumar (1968)	major change in the viscosity and acid mucopolysaccharide
2	Bisolvon	100 human patients, 8 mg, thrice a day	Bruce and Kumar (1969)	AMPS fiber system to be disintegrated and disorganized
3	Bisolvon	-	Gent *et al* (1969)	Symptomatic improvement
4	Bisolvon	8 mg three times a day for 14 days	Langlands (1970)	No any significant change
5	Bisolvon	48 mg daily for 2, 3-weeks	British Journal of Disease Chest (1973)	No significant change
6	Bisolvon	42 mg/per day, 2-3 weeks) to 42 patients	Stark (1973)	Uncertain
7	Bisolvon	9 patients, 16 mg, thrice a day	Thomson (1974)	Statistically significant
8	Bisolvon	23 patients, 72 hrs, 24 mg daily	Brogan (1974)	Statistically significant
9	Bisolvon	40 patients, intravenously	Racle *et al* (1976)	Significant improvement
10	Pure vasicine	-	Dhuley (1999)	Marked bronchodilator activity evaluated on contracted trachea or constricted tracheo-bronchial tree.
11	2,4-diethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-12-one	-	Zabeer *et al* (2006)	Marked activity

Muscle Stimulant activity

Madappa *et al.* (1989) [35] studied the effect of vasicine (1 and 10 µg/ml) on uterus, mammary gland, guinea pig ileum and guinea pig tracheal muscle. They found that, vasicine has stimulatory effects on rat/guinea pig uterus and tracheal muscle as well as, on other tissues. They compared its effect with (+) INPEA (nifenolol). The effect of (+) INPEA showed selectivity for uterine tissue. Vasicine potentiated the action of oxytocin in isolated rat mammary strip preparation. It also showed smooth muscle stimulant activity and is thus used for bronchodilation, abortion.

Abortifacient activity

As mentioned above vasicine from AV has been reported to be abortifacient too. Gupta *et al.* (1977) [37] investigated the uterotonic activity on uterus of different species of animals in different hormonal states both *in vitro* and *in vivo.* It was found that, in a comparative study with methergen and pitocin, the uterotonic effect of vasicine was similar to that of these two known oxytocics. As, the changes in the responsiveness of uterus to vasicine varied according to its hormonal status, similar changes in the responsiveness of uterus were observed with methergen and pitocin also. They suggested that, vasicine might be acting through the release of prostaglandins. Also it was suggested that, vasicine being a respiratory stimulant can antagonize the respiratory effects of narcotic analgesics when used in labour and it is also useful to control post partum hemorrhage.

Chandokhe *et al.* (1978) [36] has reported this compound to mediate the effect via prostaglandins. Further Gupta *et al.* (1978) [38] have also reported vasicine to enhance the uterotonic effect in rats and guinea pigs depending upon the stage of pregnancy and prior priming with oestradiol and supported the idea of prostaglandins being the mediators again. But, in rats they reported that, it does not (5-15 mg/kg intraperitoneal on 18^{th} and 16^{th} day of pregnancy) have abortifacient effect. Vasicine primed with oestradiol (50 µg/ml, 40 h before vasicine administration, 10^{th} day of pregnancy vasicine administration) had shown 100% resorption. In case of guinea pigs, when vasicine (30 mg/kg) was given on late stage of pregnancy, 50% abortion was observed. When vasicine is primed with oestradiol (10 mg/kg vasicine, 50 µg oestradiol), 3 out of 8 at early stage, 50% at middle stage and 10 out of 12 aborted.

Vasicine, at a dose of 1 mg itself has been shown to have no effect on contractions [38]. It has stimulatory effect on $F_2\alpha$ and PGE_1 evoked contractions in isolated rat uterus. Vasicine increased the contractions when it was given before PGE_1. Further Gautam *et al.* (1982) [40] studied the effect of vasicine and (+) sotalol and deoxysotalol on oxytocin induced contractions. They found that, (+) sotalol (10 µg/ml), deoxysotalol (10 µg/ml), and vasicine (1 µg/ml) produced marked potentiation in the contractile responses of oxytocin, while (+) sotalol and deoxysotalol did not potentiate the responses of oxytocin on mammary strip (selective for uterus tissue), vasicine HCL potentiate these responses. No explanation was available for this "non selectivity" of vasicine for this action at that time.

Further aqueous or 90% ethanolic extracts of leaf of *Adhatoda vasica* has been reported to be 100 % abortive on rats after 10^{th} day of insemination, at a doses equivalent to 175 mg/kg of the starting dry material [41].

As in case of bronchodilation, synthetic derivative of vasicine: bromhexine, was also worked upon for its abortifacient activity in rats. Bromhexine modifies the onset, appearance and regulation of cascade of glycol/sialoproteins and this may interfere with the events leading to implantation of the trophoblast. Kinetics of bromhexine-mediated down regulation of focal adhesive molecules (glycol/sialoproteins) of uterus and trophectoderm affecting conception in the rat was studied by Singh and Malaviya (2006) [42]. 10 mg/kg bromhexine was given to the 'day 1' pregnant rats twice daily for 3 days. Bromhexine inhibited the incorporation of sialic acid, galactose, glucose and glucosamine into proteins of pregnant rats, making protein more susceptible to proteolytic degradation. Bromhexine had shown interference in blastocyst attachment, conception, reduction in number of implantation sites and dwarfing of fetuses and hence, it was suggested to be a potential candidate for anti-implantation.

As an additional advantage of using vasicine during induction of child birth or during abortion is that, it helps to control post-partum hemorrhage. Atal *et al.* (1982) [43] experimented with animals and reported that repeated oral and intramuscular administration of vasicine hydrochloride resulted in an increase in platelet count in normal rats, mice, rabbits and dogs. There was no effect seen on Hb amount, RBC and WBC number. The increase in platelet number was found to be associated with hyperplasia of megakaryocytes. They suggested that, vasicine can be used for controlling the capillary hemorrhages and correction of drug induced bone marrow depression.

Anti-diabetic activity

Bromhexine, as it found to have effect on mucus glycoproteins, was tried on diabetic patients and it was reported by Clamp *et al.* (1979) [44] that, it can restore the balance in glucose level in the urine of diabetic patients but, has no effect on normal patient. They suggested that, this change may be due to reduction in the amount of glycoprotein and related material in the body, or from a change in the catabolism of these materials.

A different study by Gao *et al.* (2008) [45] highlights the role of vasicine in sucrose metabolism. Epidemiological studies and clinical trials conducted by them strongly support that, control of hyperglycemia is critical in treatment of not only, diabetic patients but also, individuals with impaired glucose tolerance. This recent report explains that, vasicine can act as irreversible sucrase (α-glucosidase)

inhibitor which further helps in reducing the glucose level by inhibiting the conversion of sucrose to glucose from the intestinal track. Vasicine and vasicinol, the main alkaloids from AV have an IC_{50} value of 125 μM and 250 μM. They both inhibited sucrose-hydrolyzing activity of rat intestinal α-glucosidase competitively with K_i values of 82 μM and 183 μM, respectively. Ilango *et al.* (2009) [41] have already evaluated the antidiabetic, antioxidant and antibacterial activity of various extracts of AV. Chloroform and methanolic extracts (200 mg/kg body wt) were found to reduce the blood and urine/glucose level. Among hexane, chloroform and methanolic extract, methanolic extract had shown to have maximum antioxidant activity and cholesterol lowering activity.

FEW UNRELATED ACTIVITIES

Anticestodal activity

The plant AV has been used indigenously by Naga tribes for curing intestinal worm infections. The study has been conducted by Yadav *et al.* (2008) [46] with the methanolic plant extract using *Hymenolepis diminuta* model for rat. 800 mg/kg double dose was found to be profoundly efficacious and the egg number/gm of the feces was reduced to 79.6%. The percentage recovery from the eggs was found to be 16.6% with comparison to the control. Although, it does not specifically indicate that only vasicine is responsible for its activity, the fact that the methanolic extract of the leaves contains mainly vasicine and vasicinone and glycosides and might indicate the reason for its possible anticestodal activity.

Antileishmanial activity

Peganine hydrochloride isolated from *Peganum harmala* seeds in dihydrated form is shown to have exhibited *in-vitro* activity against both extracellular promastigotes as well as, intracellular amastigotes residing within murine macrophages in *Leishmania donovani* by Khaliq *et al.* (2009). Furthermore, it also exhibited *in-vivo* anti-leishmanial activity, 79.6 (±8.07) % in hamsters at a dose of 100 mg/kg body weight.

Anti-helminthic activity

Al-Shaibani *et al.* (2008) [47] studied the ovicidal and larvicidal properties of AV extracts against gastrointestinal nematodes of sheep *in vitro*. The aqueous and ethanolic extracts of the plant at 25-50 mg/ml concentration were studied and shown to be ovicidal and larvicidal. The effect was dose dependent and ethanolic extract was more effective. The highest ED_{50} values of AV extracts were recorded against the eggs of *Chaberita ovina* (18.2 mg/ml for both the extracts). The lowest values were recorded against the eggs of *O. circumcinta* as 12.59 and 11.48 mg/ml for ethanolic and aqueous extracts, respectively. Similarly, the ED_{50} values of AV extracts against larvae, the highest ED_{50} values for *O. Columbianum* was 19.5 and 18.62 mg/ml and lowest against the *H. contotus* larvae : 15, 14 and 12.88 mg/ml for aqueous and ethanolic extracts respectively.

Anti-bacterial activity

The semi-synthetic derivatives of vasicine: benzylamines, bromhexine and ambroxol, widely used as mucolytics, have also been shown to have pH-dependent growth-inhibitory effect on *Mycobacterium tuberculosis* (MT). These compounds are reported to be accumulating in the macrophages and they also help in removing mucus, containing the bacteria. It is also reported that, they increase the secretion of lysozymes and rifampicin (if given along) in the lung tissue which might make it potentially useful adjunctive in the therapy of tuberculosis [48].

Water, ethanolic and petroleum ether extracts of *Adhatoda* leaves were tested by Karthikeyan *et al.* (2009) [49] for their antibacterial activity against *S. epidermidis, S. aureus B. subtilis, E. faecalis, E. coli, P aeroginosa, P. vulgaris, K. pneumoniae and C. albicans*.

Ethanolic extracts and petroleum ether were found to be antibacterial to many of them. These extracts contain alkaloids, phenols, tannins and reducing sugars *etc.* including its main active principle vasicine. The antibacterial activity of these extracts can be a result of individual or combination effect.

Anti ulcer activity

It is also reported to be an antiulcer agent [50] against ulcer caused by ethanol and pylorus with aspirin. 80% recovery was observed in case of ethanol-induced ulcer in rats in comparison to the control rats and 41% in case of pylorus+aspirin induced peptic ulcer.

A detailed study by Gupta *et al* (1977)

While reviewing the literature for vasicine, it is important to mention here that Gupta *et al* (1977) [51] attempted to do a detailed pharmacological study for vasicine and vasicinone from AV. They showed that, vasicine has cardio-depressant effect at a dose of 5.0, 7.5 and 10 mg/kg i.v. and was able to decrease blood pressure to 45, 50 and 55 mm Hg of the initial blood pressure in Mongrel dogs. These effects lasted for 70, 95 and 130 min, respectively. While tried on isolated heart, at a dose of 300 µg and above, it caused appreciable negative inotropic and chronotropic effects and decrease in coronary outflow, (20 – 30% decrease at 1 mg dose). Vasicinone did not show any effect at similar doses. In case of blood vessel perfusion, isolated rat hind quarter perfusion experiments showed that, vasicine has direct vasodilatory effect on the blood vessels. Perfusion outflow was increased on the average by 0.6, 1.3 and 1.8 ml/min with 50, 100 and 200 µg doses from the initial average out flow of 3.2 ml/min.

Respiratory stimulating effects of vasicine were observed on anaesthetized dogs. Respiratory rate ranged from 10 – 30/min (on an average of 18/min) with 2.5, 5, 7.5 mg/kg i.v. doses. Stimulation of respiration occurred in both rate and amplitude (2 - 4 times in 2 - 3 min which lasted for 40 - 60 min) and was dose dependent. On rabbits, vasicine had shown marked respiratory stimulant effects, both after i.v. (10 mg/kg) and i.m. (30 mg/kg) administration. Rabbits whose respiration was markedly depressed with 5 mg/kg s.c. morphine (116 to 35/min) and remained near normal after 70^{th} min in comparison to "micoren" which stimulates the depressed rate to 140 at 10^{th} min but goes down again to 34/min at 90^{th} min.

Vasicine at a dose of 1 mg/ml was shown to decrease the travel time of a poppy seed to 40 - 50% when applied internally in the oesophagus. It was reported that, it had an inhibitory effect on bronchial secretion (15 - 20%). A dose upto 10 mg/kg was not shown to have inhibitory effects on the cough reflex caused by the polythene tube in the mucosal surface of trachea. Vasicine and vasicinone at a dose of 2.5 to 10 mg/kg i.v. had initial vasoconstriction effect in histamine induced anaesthetized guinea pigs. In case of vasicine it was followed by dilation. At a dose of 5 mg/kg vasicine, appreciable responses were observed against 2 mg/kg i.v. histamine. It lasted for 15 - 20 min and the effect was found to be similar to theophylline. Effect of vasicine was also observed on histamine aerosol induced bronchospasm in guinea pigs. Vasicine at a dose of 20 mg/kg i.p. was shown to appreciably increase the pre-convulsive time and reduced mortality rate. Vasicinone on the other hand had a reverse effect. Vasicine and vasicinone in combination were more effective (20 mg/kg, i.p.). Vasicine was also reported to inhibit 5-HT, histamine and carbachol induced bronchoconstriction in anaesthetized dogs by measuring the intratracheal pressure changes by 5-10 mg/kg i.v. vasicine. In case of isolated guinea pig ileum, vasicine had shown antagonism against both acetylcholine and histamine. In contrast the contractions were potentiated by vasicine for rectus abdominus muscle, caused by 1 µg/ml acetylcholine. No effect was found on pentabarbitone sleeping time in rats with 20 mg/kg i.p dose. LD_{50} of vasicine in mice was 78.5 mg/kg by i.p. route (24 h).

In brief, they suggested that, the blood pressure fall was probably due to direct vasodilation. It could partly be due to negative inotropic and chronotropic effects of vasicine on heart. Respiratory stimulant effect of vasicine seems to be mediated mainly by its action on the respiratory center and partly peripherally through the chemosensory fibers.

The detailed investigation done by them, confirmed bronchodilatory activity of vasicine; *in vivo* bronchoconstriction and *in vitro* tracheal relaxation by vasicinone. Cambridge and coworkers (1962) [52] and Lahiri and Pradhan (1964) [53] have made similar observation while Amin and associates (1959) have reported that vasicine caused bronchoconstriction and vasicinone bronchodilation. They suggested that, due to similarity of their structure, vasicine and vasicinone were acting through the same receptors and also being metabolized by the same enzyme. Enhancement of bronchodilatory effects of vasicine *in vivo* may be due to not only, the occupation of the receptor sites by vasicine molecules and letting the bio-transformed vasicinone molecules remain unattached (which cause broncho-constriction) but also, perhaps to the fact that bio-transformed vasicinone served as a substrate for the metabolizing enzyme and afforded protection of vasicine against its metabolic breakdown thereby, enhancing its activity. In their study, vasicine did not show antitussive activity or increase of tracheobronchial secretions.

PHARMACOKINETIC STUDIES

The pharmacokinetic studies have also been conducted for vasicine. Atal *et al.* (1980) [54] reported that, 20 mg/kg body wt. of vasicine given intramuscularly gets well absorbed thereby reaching a maximum concentration of about 50 µg/ml in blood in both pregnant and non pregnant rats. Vasicine given intravenously in rat and mice showed high concentrations in uterus within 5 min and the peak was achieved after 10 min. In this case the half life was 5 - 7 min, 1.5 and 2 h after intravenous, intramuscular and subcutaneous administration respectively. Similar results were obtained by Zutshi in 1980 [55] in rats. Very low concentration of vasicine was observed after oral administration. Vasicine was metabolized in the liver to vasicinone and other metabolites which contributes to the first pass effect and was found to be an important way of elimination of vasicine [56]. Amla *et al.* (1987) [57] had conducted a human trial in healthy volunteers to study the pharmacokinetics of vasicine and reported that, 1.5mg/kg bolus i.v reached the peak in 15 min (65 ± 5µmol/L) and just after 4 hrs 1/3 of the peak was detected.

Ram *et al.* (2007) [58] determined the site of absorption of vasicine in the intestine. They used everted sac method to asses the absorption. Duodenum was reported to have the maximum capacity to absorb isolated vasicine from the methanolic and ethanolic extracts of vasaka (82.3±5.3%).

VASICINE DERIVATIVES

Using vasicine as a template, many scientists are isolating and developing new derivatives of the same for various biological activities.

Desoxypeganine hydrochloride isolated from *Peganum harmala* L. caused a pronounced depression of cholinesterase activity in animals. By anticholinesterase activity, desoxypeganine was ten times superior to peganine hydrochloride and 2 times to galanthamine hydrochloride. In the experiments on anesthetized cats, desoxypeganine hydrochloride eliminated blockade of neuromuscular conductivity induced by diplacine and on the contrary enhanced blockade induced by ditilin. Desoxypeganine hydrochloride was used for treatment of patients with lesions of the peripheral nervous system by Tuliaganov (1986) [59].

6, 7, 8, 9, 10, 12-hexahydro-azepino-[2, 1-b] quinazolin-12-one-(RLX) was synthesized by Johri *et al.* (2000) [60] as well as by Mahajan *et al.* (2010) [61]. It was found to be a potent bronchodilator. The effect of RLX (p.o.) was observed on: (a) mast cell degranulation, (b) release of histamine and prostaglandin E (PGE), (c) ^{45}Ca uptake and (d) activities of cAMP phosphodiesterase (PDEase) and lipoxygenase enzymes in mesenteries/peritoneal mast cells/lung tissue homogenates in rats under systemic anaphylaxis. RLX (10 and 20 mg/kg) inhibited antigen-induced mast cell degranulation and released of histamine from target tissues. Lung PDEase and lipoxygenase activities were decreased. These results suggested that, RLX could be acting like disodium cromoglycate and

aminophylline with additional attributes to its oral efficacy and long duration of action. Most recent study reports the formation and investigation of the bronchodilatory activity of various stereoisomeric forms of RLX. They assumed these forms to be more efficient bronchodilators (antihistaminic activity) than RLX on isolated guinea pig tracheal chain preparations. Loss of activity of these compounds was reported probably due to antagonistic effect of the stereoisomers.

3-lithiodeoxyvasicine and biaryl derivatives of (+/-)-vasicine were developed by Shevyakov *et al.* (2006) [62]. 2,4-diethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-12-one was synthesized along with other compounds by Zabeer *et al.* (2006). It was found to have bronchodilator activity on contracted trachea or constricted tracheobronchial tree. It showed relaxant effect on intestinal smooth muscle too, which was more potent than theophylline but, less to that of salbutamol on dose basis.

7-hydroxy vasicine was isolated from *Linaria vulgaris along with many other chemicals* by Hua *et al.* (2002) [63]. Its biological activities are not analyzed.

SAFETY & TOXICOLOGICAL STUDIES

Wakhloo *et al.* (1980) [64] investigated the safety of use of vasicine in 24 human volunteers using 0.5-16 mg dose of vasicine injected i.v. in 500 ml saline in 3 h with the objective of determining any acute human toxicity, tolerance, pharmacological action, any untoward effect and safe dosage range. Vasicine tried upto 16 mg dose on the hospital in-patients on 2^{nd} to 8^{th} day of normal puerperium was well tolerated and showed no undesirable effect in clinical observations, haematological and biochemical investigations and kidney and liver function tests carried out before, during and after vasicine treatment. However, uterus became firm and contracted after vasicine treatment which indicated its effectiveness as an oxytocic as discussed above and having abortifacient activity. Pahwa *et al.* (1987) [65] have conducted the chronic toxicity study of *Adhatoda vasica* in rats (2.5 mg/kg, 5 mg/kg and 10 mg/kg, low dose, 2x ED_{50}, med dose, 4x ED_{50} and 8x ED_{50} respectively) and monkeys (5, 10 and 20 mg/kg as above criteria) for 6 months. They reported that, there is no change in mortality rate and body weight. Autopsy and histological examination of major organs did not reveal any abnormality.

MOLECULAR MECHANISM

Very few specific molecular mechanisms for the activity of vasicine molecule are reported so far. Antioxidant nature of the herb AV and its components is suggested to be the its main characteristic, responsible for their physiological effects. In 1977, Gupta et al. had suggested that, the bronchodilatory activity of vasicine works through respiratory sensors and peripheral receptors as well. In case of cardio-depressant effect, it is suggested that, it dilates the blood vessel locally to reduce the blood pressure. For anti-diabetic properties, two main mechanisms have been suggested: one that it inhibits glucosidase activity and the other its effect on glycoprotein metabolism and its secretion in blood plasma and urine, to regulate the sugar level in the blood.

CONCLUSION & FUTURE PROSPECTS

The plant *Adhatoda vasica* and its component: Vasicine and its derivatives are extensively being used for bronchodilatory/mucolytic preparations since history till date. The antioxidant properties of this herb and its components including vasicine are reported to be responsible for anticancer, anti-aging, antidiabetic, hepatoprotective, genoprotective, cardiotonic properties. It is clear from the above literature survey that, vasicine might have multiple targets which are responsible for its beneficial effect for the treatment of respiratory ailments for example: mucolytic property, anti microbial activity, antioxidant activity etc. which, can improve the

pathological conditions synergistically. It is also possible that, vasicine is helpful in regulating the inflammatory reaction as well and, other than that, its smooth muscle stimulating activities might also be useful to remove the phlegm from the respiratory tract. It has become necessary now to explore molecular mechanism of this potent herb and its active principles. This plant might also have a specific application in case of induction of childbirth in diabetic women due to its anti-diabetic potential for optimized doses, and might also helps to control post partum haemorrhage. Being antibacterial it can further help to prevent infection during the above mentioned cases. In short, Adhatoda vasica and its phytoconstituents including: vasicine and its derivatives have great potentials to develop safe medications for respiratory and reproductive medicine. Our lab is currently in process of finding out the molecular targets of vaccine. We are hoping to identify few molecular targets so that, further strategy for utilization of this molecule can be directed.

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