Journal of Biosphere, 2(1): 22-27, 2013 ISSN 2278 – 3342

Therapeutic applications of ricin and some alkaloids

Rita Kaushal, Pardeep Singh, Vijayta Gupta, M.S. Parmar, Anu Gopal, Anu Gopalakrishnan and Vinay Kant

Abstract: Ricin is one of the most known toxic materials of plant origin. The 2007 Guinness World Records Book considers ricin the world's most potent plant toxin. It also possesses wide-ranging biological effects on higher organisms and it is a powerful inhibitor of cellular protein synthesis. Ricin is frequently therapeutically used in the construction of immunotoxins designed to kill specific target cells such as cancer cells. Some alkaloids such as nicotine, atropine and ergots have also shown various toxicities and side effects associated with their use. Instead of the various toxicities and side effects, these alkaloids have wide therapeutic potentials for various diseases. These have shown very good therapeutic potentials for the treating nicotine dependence, acetylcholinestrase poisoning, cardiac, respiratory and urinary affections, hyperhydrosis, pain, termination of labour, migraine, postural hypertension etc.

Key words: Atropine; ergot; nicotine; ricin; therapeutic potentials.

1. Introduction

The five most toxic materials of living origin known today includes (1) Tetanus toxin from *Clostridium tetani* (2) Botulinum toxin from *Clostridium botulinum* (3) Diphtheria toxin from *C. diphtheria* (4) Gramicidin from *Bacillus braves* and (5) Ricin, which is considered to be the most toxic substance of plant origin [1]. Ricin present in castor oil seed (*Ricinus communis*) is a protein of molecular size approximately 62 kDa and it comes under the group of proteins called ribosome inactivating proteins (RIPs), which is a family of functionally and structurally related toxins of plant and bacterial origin [2]. Out of the two subunits of Ricin protein, the 32 kDa catalytic subunit (RTA) is the major factor responsible for its toxicity and the 34 kDa galactose/N-acetylgalactosamine-binding subunit (RTB) is the one determining attachment, entry, and intracellular trafficking of ricin into host cells [3,4].

Rita Kaushal
Division of Veterinary Public Health,
GADVASU, Ludhiana, Punjab, India.

Pardeep Singh
SKUAST-J, R. S. Pura, Jammu 181102, J&K, India.

Vijayta Gupta
Department of Chemistry,
University of Jammu, Jammu - 180006, J&K, India.

M.S. Parmar
Division of Physiology and Climatology,
IVRI, Bareilly - 243122, UP, India.

Anu Gopal, Anu Gopalakrishnan and Vinay Kant(✉)
Division of Pharmacology and Toxicology,
IVRI, Bareilly - 243122, UP, India.
Email: drvinaykant2001@yahoo.co.in

2. Ricin

Average lethal dose of ricin in humans is approximately 2 mg and is twice as deadly as cobra venom. In an infamous political assassination happened in 1978 on the streets of London it is assumed that a minute drop of ricin on a needle tip of an umbrella was used as the weapon. Ricin also gained entry in the 2007 Guinness World Records Book as the world's most potent plant toxin. Since it is a powerful inhibitor of cellular protein synthesis ricin has wide-ranging biological effects on higher organism.

2.1 Therapeutic applications of ricin

The discovery of ricin and its lethal potential has captured the attention of scientists for a prolonged time. However, because of its non-specific toxicity, an early control trial of ricin as a therapeutic agent in leukemia patients was not found to be much successful. But when used with appropriate targeting modalities this highly potent toxin can specifically kill any unwanted cells in the body which is now being exploited for the development of immunotoxins in targeted cancer therapies [5]. Ricin A-chain and its modification are most frequently used to create immunotoxin by coupling with monoclonal antibody therapy. Here monoclonal antibodies (moAbs) raised against cancer cell markers[6] are coupled with ricin A chain molecule, forming Ricin immunotoxin (R-immunotoxin) The antibody recognizes the cancer cell, attaches it enabling ricin to enter and kill it.

Though normal cells too express the cancer markers, but the concentration of these markers is elevated during cancerous state that makes the cancer cell more prone to R-immunotoxin. Nevertheless, some normal cells in the host also get killed by such immunotoxins, that is an adverse outcome by non-specific toxicity. In addition, the reactivity of moAbs to normal tissue may create side effects and difficulty

in screening of Mobs for evaluation of drug targeting. Moreover, antibodies are generated against both moAbs and the toxin moiety of immunotoxin can decrease their efficacy.

Single moAb linked ricin immunotoxin seldom reacts with more than a small proportion of cancer cells within a tumor. Engert and Thorpe [7] prepared immunotoxins by linking ricin A-chain to several moAbs that recognize different markers on Hodgkin/Reed-Sternberg (H-RS) cells, tested their antitumor activity successfully in vitro and also in mice Hodgkin's tumor models. Therefore, they advocated its use for clinical trials in human patients with Hodgkin's disease. To establish the efficacy of such immunotoxins the most conventional method documented is by treating nude mice bearing engrafts of human tumors. The disadvantage of this system is that those tumors do not metastasize and hence only reduction in local tumor size can be measured. Moreover, the vasculature of the tumor engrafts in mice does not mimic that of humans. In addition, immunocompromised nude mouse cannot be compared with cancer patient. In a trial led by Le Moister and Rosen [8], 14 patients with cutaneous T-cell lymphoma were treated with an R-immunotoxin containing murine monoclonal antibody (H65) that reacted with CD5 and got partial responses in four patients.

R-immunotoxins still have a long way to go, before they can be used as successful therapeutics because of their high toxicity. There are several reports of using R-immunotoxins to treat cancers and many a times the clinical trials were discontinued due to their intolerable toxicity. In one trial, patients with metastatic carcinoma were treated with an antitransferrin receptor antibody coupled to recombinant ricin A-chain given intraperitoneally. One patient developed a fatal encephalopathy even though he had a normal brain CT scan at the start of the therapy therefore, the trials were discontinued. Several investigators have proved that R-immunotoxins are rapidly eliminated from the circulation, with preferential localization in liver causing liver damage.

It seems that the moAbs used for immunotoxins synthesis are as much responsible as is ricin which is relatively crude having non-specific toxicity. The drug designing and the clinical modifications will allow scientists to develop newer R-immunotoxins having minimum side effects. In the mean time, alterations in the clinical protocols are probably the main variable that will make the present immunotoxins valuable therapeutic tools.

3. Alkaloids

3.1 Nicotine

It is an alkaloid found in the nightshade family of plants (Solanaceae), predominantly in leaves of tobacco (0.3-5% by dry wt.), and in lower quantities in tomato, potato, eggplant (aborigine), and green pepper. Nicotine alkaloids are also found in the leaves of the coca plant. It is a potent neurotoxin with particular specificity to insects. It has been widely used as an insecticide in the past, and currently nicotine derivatives such as imidacloprid continue to be widely used. In lower concentrations (an average cigarette yields about 1mg of absorbed nicotine), the substance acts as a stimulant in mammals and is one of the main factors responsible for the dependence-forming properties of tobacco smoking. According to the American Heart Association, "Nicotine addiction has historically been one of the hardest addictions to break."

In 1550, Jean Nicot, a French ambassador sent tobacco and seeds from Portugal to Paris and promoted their medicinal use. Tobacco plant *Nicotiana tabacum*, has been named after Jean Nicot and Nicotine in turn is named after the tobacco plant *Nicotiana tabacum*. Nicotine is hygroscopic (moisture absorbing), oily liquid. It blocks the nicotinic acetylcholine receptors. In small concentrations it escalates the activity of these receptors and leads to an increased flow of adrenaline (epinephrine), a stimulating hormone. The release of adrenaline in turn causes an increase in heart rate, blood pressure and respiration, as well as higher glucose levels in the blood. In high doses, nicotine will cause a blocking of the nicotinic acetylcholine receptor, which is the reason for its toxicity and its effectiveness as an insecticide. The LD50 of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. 40–60 mg can be a lethal dosage for adult human beings [9].

Dopamine levels in the reward circuits of the brain were found to be increased in response to nicotine. Studies have shown that smoking tobacco inhibits monoamine oxidase (MAO), an enzyme responsible for breaking down monoaminergic neurotransmitters such as dopamine, in the brain.

3.1.1 Therapeutic uses

The primary therapeutic use of nicotine is in treating nicotine dependence in order to eliminate smoking with its risks to health. Controlled levels of nicotine are given to patients through gums, dermal patches, lozenges, or nasal sprays in an effort to wean them off their dependence. However, in a few situations, smoking has been observed to apparently be of therapeutic value to patients. These are often referred to as "Smoker's Paradoxes" [10]. Although in most cases the actual mechanism is understood only poorly or not at all, it is generally believed that the principal beneficial action is due to the nicotine administered, and that administration of nicotine without smoking may be as beneficial as smoking, without the high risk to health. For instance, recent studies suggest that smokers require less frequent repeated revascularization after percutaneous coronary intervention (PCI). Risk of ulcerative colitis has been

frequently shown to be reduced by smokers on a dose-dependent basis; the effect is eliminated if the individual stops smoking (Longmore, Oxford handbook of clinical medicine).

Smoking also appears to interfere with development of Kaposi's sarcoma, breast cancer among women carrying the very high risk BRCA gene, preeclampsia, and atopic disorders such as allergic asthma [11]. A plausible mechanism of action in these cases may be nicotine acting as an anti-inflammatory agent and thus interfering with the inflammation-related disease process, as nicotine has vasoconstrictive effects [12]. With regard to neurological diseases, a large body of evidences suggests that the risks of Parkinson's disease or Alzheimer's disease might be twice as high for non-smokers than for smokers [13]. A plausible mechanism of action in these cases may be the effect of nicotine, a cholinergic stimulant, in decreasing the levels of acetylcholine in the smoker's brain; Parkinson's disease occurs when the effect of dopamine is less than that of acetylcholine. Recent studies have indicated that nicotine can be used to help adults suffering from Autosomal dominant nocturnal frontal lobe epilepsy. The same areas that cause seizures in that form of epilepsy are also responsible for processing nicotine in the brain. Gary *et al*, demonstrated the ability of chronic nicotine treatment to enhance the cognitive performance of normal aged rats, suggesting that chronic nicotine or nicotinic agonist administration may be of considerable clinical benefit in the treatment of age-associated memory impairment [14].

The therapeutic use of nicotine as a means of appetite-control and to promote weight loss is anecdotally supported by many ex-smokers who claim to put on weight after quitting. However studies of nicotine in mice suggest that it may play a role in weight-loss that is independent of appetite. And studies involving the elderly suggest that nicotine not only affects weight loss, but also prevents some weight gain.

3.2 Atropine

It is a tropane alkaloid extracted from the deadly nightshade (*Atropa belladonna*) and other plants of the family Solanaceae. The most commonly found sources are *Atropa belladonna*, *Datura inoxia*, *D. metel*, and D. *stramonium*. Other sources include members of the *Brugmansia* and *Hyoscyamus* genera. The *Nicotiana* genus (including the tobacco plant, *N. tabacum*) is also found in the Solanaceae family, but these plants do not contain atropine or other tropane alkaloids.

Atropine is a racemic mixture of D-hyoscyamine and L-hyoscyamine, with most of its physiological effects due to L-hyoscyamine. The most common atropine compound used in medicine is atropine sulfate $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$. It exhibits its pharmacological effects by competitive binding to muscarinic acetylcholine receptors.

3.2.1 Therapeutic uses

3.2.1.1 Acetylcholinestrase poisoning

Atropine is widely used in treatment of organophosphate, nerve gases and carbamate insecticide poisoning and poisoning by certain mushrooms. Carbamate and organophosphorus insecticides inhibit acetylcholinestrases, therefore inhibiting the metabolism of acetylcholine, thus producing cholinergic toxicity. Atropine acts by blocking the action of acetylcholine at muscarinic receptors. Troops who are likely to be attacked with chemical weapons often carry autoinjectors with atropine and obidoxime which can be quickly injected into the thigh. It is often used in conjunction with Pralidoxime chloride. Atropine is given as an antidote to SLUDGE (Salivation, Lacrimation, Urination, Diaphoresis, Gastrointestinal distress, Emesis) symptoms caused by organophosphate poisoning [15].

3.2.1.2 Analgesic

The external uses of the drug mainly have pain relieving effect. The liniment or plaster of belladonna may relieve many forms of local pain. Atropine is more likely than iodine to relieve a pain of quite superficial origin. Totally to be reprobated is the use, in order to relieve pain, of belladonna or any other application which affects the skin, in cases where the surgeon may later be required to operate. In such cases, it is necessary to use such anodyne measures as will not interfere with the subsequent demands that may be made of the skin, *i.e.* that it be aseptic and in a condition so sound that it is able to undertake the process of healing itself after the operation has been performed.

3.2.1.3 Ophthalmology

Atropine is universally and constantly used topically as a cycloplegic, to temporarily paralyze the accommodation reflex, and as a mydriatic, to dilate the pupil for examination of the retina by the ophthalmoscope or in cases where the inflamed iris threatens to form adhesions to neighboring parts. Atropine degrades slowly, typically wearing off in 2 to 3 days, so tropicamide and phenylephrine Homatropine -an alkaloid prepared from atropine are generally preferred as mydriatics. The effects of atropine can last up to two weeks. In atropine-induced mydriasis, the mechanism of action involves blocking the contraction of the circular pupillary sphincter muscle which is normally stimulated by acetylcholine release, thereby allowing the radial pupillary dilator muscle to contract and dilate the pupil [16]. Atropine can also be given to patients who have direct globe

trauma. But, atropine is contraindicated in patients predisposed to narrow angle glaucoma.

3.2.1.4 Cardiac affections

Injections of atropine are used in order to treat bradycardia (an extremely low heart rate), asystole and pulseless electrical activity (PEA) in cardiac arrest. This works because the main action of the vagus nerve of the parasympathetic system on the heart is to slow it down. Atropine blocks that action and therefore may speed up the heart rate. The usual dose of atropine is 0.5-1 mg every three to five minutes, up to a maximum dose of 3mg.

Atropine is also useful in treating first degree heart block, second degree heart block Mobitz Type 1 (Wenckebach block), and also third degree heart block with a high Purkinje or AV-nodal escape rhythm. However it is usually not effective in second degree heart block Mobitz type 2, and in third degree heart block with a low Purkinje or ventricular escape rhythm. Atropine is contraindicated in ischaemia-induced conduction block because the drug enhances oxygen demand of the AV nodal tissue, thereby aggravating ischemia and the resulting heart block. One of the main actions of the parasympathetic nervous system is to stimulate the M_2 muscarinic receptor in the heart, but atropine blocks this action.

3.2.1.5 Secretions

The action of atropine on the parasympathetic nervous system inhibits salivary, sweat, and mucus glands secretions. Even though it has not been officially indicated for either of these purposes by the FDA, it has been used by physicians for these purposes.

3.2.1.6 Respiratory affections

Apart from number of minor applications of this drug, there are two therapeutic uses which are of unquestionable utility. In cases of whooping-cough or any other condition involving spasmodic action of the muscular fiber in the bronchia like asthma and cases of bronchitis - atropine is an almost invaluable drug. Not only does it relieve the spasm, but it reduces the amount of secretion - often dangerously excessive - which is often associated with it. In treating an actual and present attack of asthma, it is advisable to give the standardized tincture of belladonna - unless expense is no consideration, in which case atropine may itself be used - in doses of twenty minims every quarter of an hour as long as no evil effects appear. Relief is thereby constantly obtained. Smaller doses of the drug should be given three times a day between the attacks.

3.2.1.7 Urinary disturbances

The nocturnal enuresis or urinary incontinence of children and of adults is frequently relieved by this drug.

3.2.1.8 Hyperhydrosis

The anhydrite action of atropine is largely employed in controlling the night-sweats so characteristic of pulmonary tuberculosis, small doses of the solution of the sulphate being given at night.

3.2.2 Side effects and overdoses

Adverse reactions to atropine include ventricular fibrillation, supraventricular or ventricular tachycardia, dizziness, nausea, blurred vision, loss of balance, dilated pupils, photophobia, confusion, hallucinations, and excitation. These latter effects are due to the fact that atropine is able to cross the blood-brain barrier (BBB). Because of its hallucinogenic properties, some have used the drug for recreational purposes, though this is very dangerous and often unpleasant. Although atropine treats bradycardia (slow heart rate) in emergency settings, it can cause heart rate slowing when given at very low doses, presumably as a result of a weak partial agonist effect at the cardiac muscarinic receptors. A commonly used mnemonic used to describe the physiologic manifestions of atropine overdose is: "Hot as a Hare, Dry as a Bone, Red as a Beet, Mad as a Hatter, and Blind as a Bat." The antidote to atropine is physostigmine or pilocarpine.

3.3 Ergot alkaloids

Ergot is the dried sclerotium of the fungus *Claviceps purpurea*, a grain (rye, especially) fungus that can arise in the ovary of the rye *Secale cereale*. These sclerotia can substitute one or more of the kernels in a mature grain head with a hard, dark coloured, horn-like mass. Although ergot mostly attacks rye it can also infect barley, wheat, oats, and other grasses. Widespread infection of ergot, known as ergotism is exhibited by the signs of limb gangrene, disruption in functions of the CNS and even death. This fungus synthesizes many biologically active agents including:

(i) Many unique ergot alkaloids (up to 2%) - which effect; a) Alpha-adrenergic receptors b) Dopamine receptors and c) Serotonin receptors

(ii) Aromatic and heterocyclic amines - tyramine, histamine, agmatine

(iii) Free amino acids, ergosterin, choline, acetylcholine, betaine, ergothionine, uracil, guanidine

All ergot alkaloids may be regarded as derivatives of the tetracyclic compound 6-methylergoline. Naturally occurring ergot consists of two types of alkaloids:

(i) Clavine-type alkaloids, derivatives of 6, 8-dimethylergoline.

(ii) Lysergic acid derivatives (peptide alkaloids) - pharmacologically active alkaloids. Each active alkaloid occurs with an inactive isomer involving isolysergic acid.

The ergot alkaloids possess a high biological activity and have an extensive spectrum of pharmacological effects; hence they are of significant

importance to medicine. They exhibit adrenoblocking, antiserotonin and dopaminomimetic properties. Ergot alkaloids exert therapeutic effects on some forms of migraine, post-partum hemorrhages, mastopathy and also a sedative effect on the central nervous system. These compounds are now obtained both by methods of artificial parasitic cultivation on rye and by saprophytic growth techniques.

Ergot alkaloids

Group	Alkaloid	Formula
Ergometrine Group	Ergotmetrine	$C_{19}H_{22}O_2N_3$
	Ergotmetrinine	
	Ergotamine	$C_{33}H_{35}O_5N_5$
Ergotamine Group	Ergotaminine	
	Ergosine	$C_{30}H_{37}O_5N_5$
	Ergosinine	
	Ergocristine	$C_{35}H_{39}O_5N_5$
Ergotoxine Group	Ergocristinine	
	Ergocryptine	$C_{32}H_{41}O_5N_5$
	Ergocryptinine	
	Ergocornine	$C_{31}H_{39}O_5N_5$
	Ergocorninine	

http://www.pearson-college.uwc.ca/pearson/fungi/ergot.htm [17]

3.3.1 Therapeutic uses of ergot alkaloids

Ergot has its uses as medicine to reduce hemorrhage after childbirth by midwives as far back as 1582. The isolation of the ergot alkaloids from ergot not only revolutionized the treatment of attacks of migraine but also provided an alternative oxytocic [18]. Although ergot derivatives have been outdated in the treatment of migraine by drugs like sumatriptan. However other applications of the ergot alkaloids, for example their use to treat the symptoms of Parkinson's disease, may mean that they supply the active components of many mainstream drugs treating these ailments for years to come.

3.3.1.1 Termination of labour

All of the natural alkaloids of ergot radically amplify the motor activity of the uterus. After small doses contractions are augmented in force or frequency, or both, but are followed by a normal degree of relaxation. As the dose is further increased, contractions become more powerful and extended, resting tonus is markedly enhanced and sustained contracture may result. Although these characteristics preclude their use for induction or facilitation of labour, it is relatively compatible with their use postpartum or after abortion to control bleeding and maintain uterine contraction. The gravid uterus is very sensitive and small doses of ergot alkaloids can be given immediately postpartum to obtain a marked uterine response, usually without significant side effects [19].

Ergot was probably first used in medicine as an oxytocic drug. In the year 1582, Adam Loncier in Germany made the first note of ergot exciting uterine contractions of labour by administering three sclerotia. It was considered the most effective drug for this purpose at the time resulting in a rapid and sudden termination of labour, with a delivery time lasting less than three hours. But ergot was ultimately deemed unsuitable for this purpose as the dosage could not be given accurately due to large variations in the active ingredients. Violent nausea and vomition were severe adverse effects caused by Ergot. In 1822, Hosack from New York stated that many stillbirths were due to uterine rupture resulting in maternal death and by the end of the 19th century use of ergot as an oxytocic was virtually deserted. Although all natural ergot alkaloids have qualitatively the same effect on the uterus, ergonovine has proved to be most active and also less toxic than ergotamine. For these reasons in obstetrics, ergonovine and its semi-synthetic derivative methylergonovine have replaced other ergot preparations as uterine-stimulating agents. Methylergonovine differs little from ergonovine in its uterine actions. The dihydrogenated alkaloids do not have the uterine-stimulating properties of the parent alkaloids when tested in experimental animals; however they are capable of exerting a marked uterine-stimulating action on the pregnant human uterus at term. Ergonovine and methylergonovine are quickly and completely absorbed after oral administration and exert an uterotonic effect that can be seen within 10 minutes after per os administration of 0.2 mg of ergonovine to women postpartum.

3.3.1.2 Migraine

In the 1920's Ergot derivatives were first found to be useful as anti-migraine agents and they persist to be a main class of curative agents for the relief of moderate or severe migraine. Ergotamine shows best results when drug administered prior to the attack (prodromal phase) but its effectiveness reduces as the attack progresses. Ergotamine may also be used in conjuction with caffeine. Caffeine promotes ergot alkaloid absorption. Ergotamine can be given by oral, intravenous and intramuscular routes. Dihydroergotamine (IV administration mainly): may be suitable for intractable migraine (nasal or oral formulations of dihydroergotamine are being assessed). Migraine Prophylaxis: Ergonovine, Methysergide (Sansert) has been found to be useful in about 60% of patients with 40% frequency of toxicity. However it is not effective in treating an active migraine attack or even preventing an impending attack.

However, ergot alkaloids are non-selective pharmacological agents in that they interact with numerous neurotransmitter receptors, including 5-HT_1 and 5-HT_2 receptors, as well as adrenergic and dopaminergic receptors. Dihydroergotamine (DHE) can compete for radioligands for binding to a variety of receptor subpopulations. DHE is potent at all known 5-HT_1 receptors as well as at a number of other biogenic amine receptors, such as 5-HT_{2A}, 5-

HT_{2B}, D2 dopamine, and a_1- and a_2-adrenergic receptors. The multiple pharmacological effects of ergot alkaloids have intricated the determination of their precise mechanism of action in the acute treatment of migraine.

Another ergot derivative, lisuride, stimulates postsynaptic striatal D_2 receptors and is a mild D_1 receptor agonist. The antiparkinsonian efficacy of this drug is equivalent to that of bromocriptine and pergolide. Lisuride is highly water-soluble and can therefore be used for parenteral therapy. This compound has been shown to be very effective in controlling motor fluctuations in Parkinson's disease when administered by continuous infusion. However, long-term studies of lisuride have shown that its parenteral use is complicated by a high incidence of psychiatric adverse effects, possibly because of its serotonergic properties.

3.3.1.3 Postural hypertension

The natural amino acid alkaloids, particularly ergotamine, constrict both arteries and veins. While dihydroergotamine retains appreciable vasoconstrictor activity, it is far more effective on capacitance than on resistance vessels. This property is the basis for investigation of its usefulness in the treatment of postural hypotension.

References

1. Lee M.D. and Wang R.Y., Toxalbumins. In: Brent J, Wallace KL, Burkhart KH, Phillips SD, Donovan JW, editors. Critical care toxicology. Elsevier Mosby: Philadelphia. (2005) pp. 1345–1349.
2. Lord J.M., Roberts L.M. and Robertus J.D., Ricin- structure, mode of action, and some current applications, *FASEB J*., **8** (1994) 201–208.
3. May K.L., Yan Q. and Tumer N.E., Targeting ricin to the ribosome, Toxicon: In press (2013).
4. Yermakova A., Vance D.J. and Mantis N.J., Sub-domains of Ricin's B subunit as targets of toxin neutralizing and non-neutralizing monoclonal antibodies, *PLoS ONE*, **7** (2012) e44317.
5. de Virgilio M., Lombardi A., Caliandro R. and Fabbrini M.S., Ribosome inactivating proteins: from plant defense to tumor attack., *Toxins (Basel)*, **2** (2010) 2699–2737.
6. Becker N. and Benhar I., Antibody-Based Immunotoxins for the Treatment of Cancer, *Antibodies*, **1** (2012) 39–69.
7. Engert A. and Thorpe P., The development of ricin A-chain immunotoxins for clinical trials in patients with Hodgkin's disease. In: Gregoriadis G, Florence AT, Poste G, editors. Targeting of Drugs 3: The challenges of Peptides and Proteins. Pleum Press: New York. (1992) pp. 9–17.
8. Le Moistre C.F. and Rosen S., Phase I trial H65-RTA immunoconjugate in patients with cutaneous T cell lymphoma, *Blood*, **78** (1991) 1173–1182.
9. Okamoto M., Kita T., Okuda H., Tanaka T. and Nakashima T., Effects of aging on acute toxicity of nicotine in rats, *Pharmacol. Toxicol.*, **75** (1994) 1–6.
10. Cohen D.J., Doucet M., Cutlip DE., Ho K.K.L. and Popma J.J., et al., Impact of smoking on clinical and angiographic restenosis after percutaneous coronary intervention, *Circulation*, **104** (2001) 773–778.
11. Hjern A., Hedberg A., Haglund B. and Rosen M., Does tobacco smoke prevent atopic disorders? A study of two generations of Swedish residents, *Clin. Exp. Allergy*, **31** (2001) 908–914.
12. Lisa M., Body Blazes, *Scientific American*, (2006) p.24.
13. Fratiglioni L. and Wang H.X., Smoking and Parkinson's and Alzheimer's disease: review of the epidemiological studies, *Behav. Brain Res*., **113** (2000) 117–120.
14. Gary W., Arendash T., Paul R.S. and Gregory J.S., Nicotine Enhances the Learning and Memory of Aged Rats, *Pharmacol. Biochem. Behav*., **52** (1995) 517–523.
15. Balali Mood M. and Shariat M., Treatment of organophosphate poisoning. Experience of nerve agents and acute pesticide poisoning on the effects of oximes, *J. Physiol., (Paris)*, **92** (1998) 375–378.
16. Quinton R.M., The mydriatic response of mice to atropine, *J. Pharm. Pharmacol*., **15** (1963) 239–250.
17. http://www.pearson-college.uwc.ca/pearson/fungi/ergot.htm
18. Bigal M.E. and Tepper S.J., Ergotamine and dihydroergotamine: A review, *Curr. Pain Headache Rep*., **7** (2003) 55–62.
19. de Groot N.J.A., van Dongen P.W.J., Vree T.B., Hekster Y.A. and van Roosmalen J., Ergot alkaloids - current status and review of clinical pharmacology and therapeutic use compared with other oxytocics in obstetrics and gynaecology, *Drugs*, **56** (1998) 523–535.