

REVIEW ON ETHNOBOTANY, PHYTOCHEMICAL AND PHARMACOLOGICAL PROFILE OF *ALSTONIA SCHOLARIS*Meena A. K.¹, Garg Nitika², Nain Jaspreet^{2*} Meena R. P.³ and Rao M.M.¹¹National Institute of Ayurvedic Pharmaceutical Research, Patiala, Punjab, India²M. M. College of Pharmacy, M. M University, Mullana, Ambala, India³Regional Research Institute of Unani Medicine, Chennai, India

*Mrs. Jaspreet Nain, Assistant Prof. M. M. College of Pharmacy, M. M University, Mullana, Ambala, India Email: ajaysheera@gmail.com

Article Received on: 01/01/11 Revised on: 09/01/11 Approved for publication: 18/01/11

ABSTRACT

Alstonia scholaris Linn., which is popularly known as the “Saptaparni” or the Devil tree, which are used as a well known remedy for the treatment of various types of disorders in the Ayurvedic, Homoeopathic and Folklore system of medicine in India. *Alstonia scholaris* is mainly used for the treatment of diarrhoea and malaria, as a tonic, febrifuge, emmenagogue, anticholeric and vulnerary. Over the past two decades, many reports have appeared in mainstream scientific journals describing its nutritional and medicinal properties. *Alstonia scholaris* mainly contain ditamine, echitamine and echitenine. As with many reports of the nutritional or medicinal value of a natural product, there are an alarming number of purveyors of “healthful” food who are now promoting *Alstonia scholaris*. This paper explains the evidence-based information regarding the pharmacological activities of this plant. It has many ethnobotanical uses and is medicinally used in the traditional Ayurvedic system.

KEYWORDS: Ayurvedic, *Alstonia scholaris*, Pharmacological activity, Folklore**INTRODUCTION**

Alstonia scholaris Linn., (Family, Apocynaceae) which is popularly known as the “Saptaparni” or ‘Devil’s tree’. It is widely distributed in dried forests of India, Western Himalayas, Western Ghats and in the Southern region. It is a well known remedy for the treatment of various types of disorders in the Ayurvedic, Homoeopathic and Folklore system of medicine in India^{1,2}

Common names of *Alstonia scholaris* Linn are Bengali (satiani, chattin, chatium); English (white cheese wood, birrba, milkwood pine, milk wood, milky pine, black board tree, devil's tree, dita bark); Gujarati (satuparni); Hindi (chatian, satni, satwin, saitan-ki-jhad); Sanskrit (saptaparna); Tamil (elalaipalai, palegaruda, pala); Trade name (pulai, shaitan wood, chatiyan wood, white cheese wood); Urdu (chatiana).³

Alstonia scholaris Linn. is a medium to large tree, to about 40 m high with a somewhat tessellated corky grey to grey-white bark. The outer blaze is cream to yellowish in colour with abundant, milky latex that flows rapidly when cut. Leaves in whorls of 4-8 in the upper axils; leaf stalks 1-1.5 cm long, the lamina obovate to elliptical or elliptical-lanceolate, glabrous or sparsely hairy, tapering towards the base, 11.5-23 x 4-7.5 cm. Upper surface is dark green, the lower green-white with 25-40 pairs of lateral veins on each side of the midrib and 2-6 mm apart. The tip of the leaf is rounded or shortly pointed, tapering towards the base. Flowers 7-10 mm long white, cream or green; the tube hairy; lobes sparsely or densely pubescent, 1.5-4 mm long, the left margins overlapping; strongly perfumed. Fruit a pendulous, two-lobed, dehiscent follicle, brown or green, dry or woody, spindle-shaped, 15-32 cm long, 4-6 mm in diameter, containing numerous flat, oblong, brown seeds, 4-5 x 0.9-1.2 mm, with a tuft of hairs 7-13 mm long at each end. The seed does not taper to a point at either end.⁴

ETHNOBOTANICAL USES

Decoction of the bark was used to treat diarrhoea and malaria, as a tonic, febrifuge, emmenagogue, anticholeric and vulnerary. Decoctions of the leaves were used for beriberi. Ayurveda recommends *Alstonia scholaris* Linn. for bowel complaints. In Sri Lanka its light wood is used for coffins. In Borneo the wood close to the root is very light and of white colour, and is used for net floats, household utensils, trenchers, corks, etc.⁵

Alstonia scholaris has following health benefit-It is used to treat chronic skin ulcers, the herb is given to lactating mothers to increase lactation, helps post delivery weakness and digestion, the herb improves the digestive system, and acts as an antipyretic, it is also used for various liver disorders, The herb also benefits in expulsion of worms in stomach, It is used to cure asthma and to cure chronic cough. *Alstonia scholaris* is used in various Ayurvedic Preparations like Saptaparnasatvadi vati, Saptachadadi vati, Saptachhadadi vati, Saptacchadadi taila, Saptacchadadi kvatha and Saptaparna ghanasara.⁶ Folk or uses of *Alstonia scholaris* mainly in Whooping cough Malaria, Jaundice, Gastric complaint, Headache, Asthma, Stomachache, Wound, Fever.⁷

Indole alkaloids 19,20-(E)-vallesamine, angustilobine B N4-oxide, 20(S)-tubotaiwine, and 6,7-seco-angustilobine B, and in particular the structure identification of the new indole alkaloids, manilamine, a new seco-uleine alkaloid derivative and N4-methyl angustilobine B.(indole alkaloids)isolation of two novel alkaloids, scholarisine I and (±)-scholarisine II. *Alstonia scholaris* Linn. seeds are rich in hallucinogenic indole-alkaloids (alstovenine, venenatine, chlorogenine, reserpine, ditamine, echitamine) and chlorogenic acid. Chlorogenic acid is a mild bladder and urethra irritant, resulting in increased sensitivity of the genital region. The only alkaloids present in the bark and latex are ditamine, echitamine and echitenine.^{8,9,10}

PHARMACOLOGICAL ACTIVITY

Antioxidant activity

Ethanol extract of *Alstonia scholaris* Linn using various in vitro tests including 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging, and ferric thiocyanate reducing ability. Dichloromethane and ethyl acetate fractions were found to have significant ($p < 0.01$) free radical scavenging and metal ion chelating properties, whereas the petroleum ether and n-butanol fractions lack the in vitro antioxidant property. These various antioxidant activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA) and l-ascorbic acid. These results indicate that dichloromethane and ethyl acetate fractions of *Alstonia scholaris* Linn. possess antioxidant property. The results observed were comparable to antioxidant properties of BHA and l-ascorbic acid.¹¹

Antibacterial activity

In-vitro antibacterial activity of methanolic, aqueous and total alkaloid extracts from the trunk bark against two gram-positive bacteria including bacillus subtilis and streptococcus pyrogens and four gram negative bacteria, Escherichia coli, pneumoniae, Pseudomonas aeruginosa and proteus mirabilis using disk diffusion method. All extracts showed varying degrees of inhibitory activity against all bacteria. Aqueous extract was found very active against both gram-positive and gram-negative bacteria in comparison to other extracts. Total alkaloid extract was found only active against gram-negative bacteria.¹² The methanol leaves extract exhibited broad-spectrum antibacterial activity against tested organisms. Maximum activity was exhibited against *Bacillus subtilis* followed by *Escherichia coli* and *Staphylococcus aureus*. Chloroform and acetone leaf extracts exhibited lesser activity, while petroleum ether extract showed no inhibition.¹³

Broncho-vasodilatory

The ethanol extract of *Alstonia scholaris* Linn. leaves induced pronounced bronchodilatory activity in anaesthetized rats with the probable involvement of prostaglandins. The vasodilatory activity of the extract was independent of adrenergic or muscarinic receptors or prostaglandins but was mainly via endothelial-derived relaxing factor, nitric oxide. The extract inhibited the spontaneous movements of rabbit jejunum and contractile effects of acetylcholine and histamine on guinea-pig ileum. Additionally,

the extract caused marked reduction of barium chloride-, potassium chloride- and calcium chloride-induced contraction on guinea-pig ileum and pulmonary artery, implying a direct interference of plant extract with the influx of calcium ions into cells. However, the extract has no detectable effect on mobilization of intracellular calcium. These results coupled with the *in vivo* effects of ethanol extract reveal that the *Alstonia scholaris* Linn. leaves possess broncho-vasodilatory activity mediated presumably by prostaglandins, calcium antagonism and endothelium-derived relaxing factor.¹⁴

Hepatoprotective activity

The hepatoprotective effect of *Alstonia scholaris* Linn. on liver injuries induced by carbon tetrachloride (CCl₄), β-D-galactosamine, acetaminophen and ethanol were investigated by means of serum-biochemical and histopathological examinations. Post treatment of *Alstonia scholaris* Linn. reduced dose-dependently the elevation of serum transaminases level and histopathological changes such as cell necrosis, inflammatory cell infiltration, which were caused by the single administration of 32 μl/kg CCl₄ or 600 mg/kg acetaminophen in mice. *Alstonia scholaris* Linn. significantly lowered 288 mg/kg β-D-galactosamine induced serum transaminases elevation in the serum-biochemical analysis in rats. A tendency was also shown to inhibit cell necrosis and inflammatory cell infiltration caused by β-D-galactosamine in histopathological examination. All serological and histopathological effects of *Alstonia scholaris* Linn. has been reported previously as a treatment criteria of hepatitis.¹⁵

Anticancer activity

The anticancer effect of various doses of an alkaloid fraction of *Alstonia scholaris* Linn. (ASERS), was studied *in vitro* in cultured human neoplastic cell lines (HeLa, HepG₂, HL60, KB and MCF-7) and in Ehrlich ascites carcinoma bearing mice. Treatment of HeLa cells with 25 μg/mL ASERS resulted in a time dependent increase in the antineoplastic activity and the greatest activity was observed when the cells were exposed to ASERS for 24 h. However, exposure of cells to ASERS for 4 h resulted in 25% viable cells and hence this time interval was considered to be the optimum time for treatment and further studies were carried out using this time. Treatment of various cells with ASERS resulted in a concentration dependent decline in the viable cells and a nadir was reached at 200 μg/mL in all the cell lines studied. The IC₅₀ was found to be 5.53, 25, 11.16, 10 and 29.76 μg/mL for HeLa, HePG₂, HL60, KB and MCF-7 cells, respectively. Similarly, administration of ASERS, once daily for 9 consecutive days to the tumor bearing mice caused a dose dependent remission of the tumor up to 240 mg/kg body weight, where the greatest antitumor effect was observed. Since 240 mg/kg ASERS showed toxic manifestations, the next lower dose of 210 mg/kg was considered as the best effective dose, in which 20% of the animals survived up to 120 days post-tumor-cell inoculation as against no survivors in the saline treated control group. The effect of ASERS was better than cyclophosphamide, which was used as a positive control, where all the animals succumbed to death by 40 days and the MST and AST were 19.5 and 18.3 days, respectively. The effective dose of 210 mg of ASERS was 3/10 of the LD₅₀ dose, which increased the MST and AST up to 54 and 49.5 days, respectively.¹⁶

The chemomodulatory activity of *Alstonia scholaris* Linn. extract (ASE) was studied in combination with berberine hydrochloride (BCL), a topoisomerase inhibitor, in Ehrlich ascites carcinoma-bearing mice. The tumor-bearing animals were injected with various doses of ASE, and 8 mg/kg of BCL (one-fifth of the 50% lethal dose) was combined with different doses of ASE (60-240 mg/kg). The combination of 180 mg/kg of ASE with 8 mg/kg of BCL showed the greatest antitumor effect; the number of tumor-free survivors was more, and the median survival time and the average survival time increased up to 47 and 40.5 days, respectively, when compared with either treatment alone. Similarly, when 180 mg/kg of ASE was combined with different doses of BCL (2-12 mg/kg), a dose-dependent increase in the anticancer activity was observed up to 8 mg/kg of BCL. However, a further increase in the BCL dose to 10 and 12 mg/kg resulted in toxic side effects. The best effect was observed when 180 mg/kg of ASE was combined with 6 or 8 mg/kg of BCL, where an increase in the antineoplastic activity was reported. The efficacy of the combination of 180 mg/kg of ASE was also tested with 6 mg/kg body weight of BCL in various stages of tumorigenesis, and it was effective when given in the early stages, although the efficiency decreased with an increase in the tumor developmental stages.¹⁷

The possible chemopreventive and anti-oxidative properties of this medicinal plant on two-stage process of skin carcinogenesis induced by a single application of 7, 12-dimethylbenz(a) anthracene (100 µg/100 µl acetone), and two weeks later, promoted by repeated application of croton oil (1% in acetone/thrice a week) till the end of the experiment (16 weeks) in Swiss albino mice. The tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were found to be higher in the carcinogen treated control (without ASE treatment) as compared to experimental animals (ASE treated). Furthermore, a significant increase in reduced glutathione, superoxide dismutase and catalase but decrease in lipid peroxidation was measured in ASE administered experimental groups than the carcinogen treated control.¹⁸

Anti diabetic activity

Potent α-glucosidase inhibitory activity was found in aqueous methanol extract of dried Devil tree (*Alstonia scholaris* Linn.) leaves. Active principles against α-glucosidase, prepared from rat small intestine acetone powder, were isolated and identified. The structures of these isolated compounds were found to be quercetin 3-O-β-D-xylopyranosyl (1000/200)-β-D-galactopyranoside and () -lyoniresinol 3-O-β-D-glucopyranoside on the basis of chemical and spectral evidence. The latter exhibited an inhibitory activity against both sucrase and maltase with IC₅₀ values of 1.95 and 1.43 mM, respectively, whereas the former inhibited only maltase with IC₅₀ values of 1.96 mM. This preliminary observation will provide the basis for further examination of the suitability of *Alstonia scholaris* Linn. as a medicinal supplement that contributes toward the treatment and prevention of diabetes.¹⁹

EEAS and glibenclamide were found to significantly ($p < 0.001$) reduce the blood glucose level, glycosylated hemoglobin and lipid peroxidation, whereas they increased body weight, liver and muscle glycogen and antioxidant status. The antidiabetic effect was sustained from 1 week onwards till the end of the study. The histopathology of pancreas revealed that the pancreatic β-cell damage with streptozotocin did not reverse in any of the treatment groups.²⁰

Antistress activity

Effect of stress and its modulation by methanolic extract of bark of *Alstonia scholaris* Linn. was studied using acute restraint stress model in mice. Acute restraint stress resulted in significant increase of plasma corticosterone, glucose, protein, cholesterol and triglyceride levels in stress group of animals. Methanolic extracts pretreatment at 100, 250 and 500 mg/kg for 7 days displayed promising anti-stress effect by normalizing these stress-induced biochemical perturbations in plasma of mice. Effect on cognitive functions was evaluated using passive avoidance model and elevated plus maze model. Diazepam (2 mg/kg, i.p) and piracetam (200 mg/kg, p.o) were used as standard drugs for antistress activity.²¹

Antidiarrhoeal and antispasmodic activity

The medicinal use of *Alstonia scholaris* Linn. as an antidiarrhoeal and antispasmodic by using *in vivo* and *in vitro* techniques. In the *in vivo* study the crude extract of *Alstonia scholaris* Linn. (As. Cr), which tested positive for the presence of alkaloids, provided 31–84% protection against castor oil-induced diarrhoea in mice at 100–1000 mg/kg doses, similar to loperamide. In isolated rabbit jejunum preparation, the As, Cr caused inhibition of spontaneous and high K⁺ (80 mM)-induced contractions, with respective EC₅₀ values of 1.04 (0.73–1.48) and 1.02 mg/mL (0.56–1.84; 95% CI), thus showing spasmolytic activity mediated possibly through calcium channel blockade (CCB). The CCB activity was further confirmed when pretreatment of the tissue with the As.Cr (0.3–1 mg/mL) caused a rightward shift in the Ca⁺⁺ concentration-response curves similar to verapamil, a standard calcium channel blocker. Loperamide also inhibited spontaneous and high K⁺ preconstruction as well as shifted the Ca⁺⁺ CRCs to the right. These results indicate that the crude extract of *Alstonia scholaris* Linn. possesses antidiarrhoeal and spasmolytic effects, mediated possibly through the presence of CCB-like constituent(s) and the study provides a mechanistic base for its medicinal use in diarrhoea and colic.²²

Immune stimulating effect

The immune stimulating effect of “Pule” (*Alstonia scholaris* Linn.) bark extracts was studied in BALB/c mouse. The extracts were administered orally, once a day for 7 consecutive days. The results showed that at the same doses (50, 100 and 200 mg/kg b.w.) the aqueous extract had higher phagocytic index (1.39–1.79) than the ethanolic extracts (0.81–0.93) in normal mice. The aqueous extract at 50 mg/kg b.w. also

enhanced phagocytic activity of immune suppressed mice significantly ($P < 0.01$). At 50 and 100 mg/kg b.w. the extract prevents the decrease of immune system induced by prednisone. The aqueous extract at 100 mg/kg b.w. increased lytic activity of peritoneal exudate cells against *Escherichia coli* significantly ($P < 0.05$). At the doses of 50 and 100 mg/kg b.w. the aqueous extract had no effect on primary antibody level. The aqueous extract at 50 mg/kg b.w. induced the cellular immune response while at 100 mg/kg b.w. inhibited the delayed type of hypersensitivity reaction.²³

Analgesic and anti-inflammatory activity

The alkaloids fraction of *Alstonia scholaris* Linn. leaf, three main alkaloids, picrinine, vallesamine and scholaricine, may produce the anti-inflammatory and analgesic effect peripherally based on several in vivo assays. In in vitro tests, alkaloids exhibited inhibition of inflammatory mediators (COX-1, COX-2 and 5-LOX), which is accordant with results on animal models.²⁴

Antitussive and anti-asthmatic activities

The alkaloids fraction significantly inhibited mice's frequency of cough induced by ammonia, increased mice's latent period of cough induced by sulfur dioxide, and increased guinea pigs' latent period of cough and inhibited frequency of cough. Besides, the alkaloids fraction increased delitescence of convulsion, and tumbles of guinea pigs in anti-asthmatic test, and enhanced tracheal phenol red output in expectorant evaluation. Moreover, the main alkaloid, picrinine exhibited anti-tussive and anti-asthmatic activities in vivo.²⁵

Molluscicidal and anti-cholinesterase activity

Alstonia scholaris Linn is a common medicinal plant of India. The aqueous as well as partially purified extracts of stem bark and leaf of *alstonia scholaris* has molluscicidal as well as in vivo and invitro anti-cholinesterase (AChE) activity against the snail *Lymnaea acuminata*. The aqueous stem-bark extract shows strong molluscicidal activity in comparison to leaf at all exposure periods in time as well as dose dependent manner. The anti-AChE activity of *Alstonia scholaris* was also time as well as dose dependent. At LC50 (24h) of aqueous and partially purified stem bark and leaf extracts of this plant parts did not cause any mortality among fish in a mix population of snails and fish, which support the view that plant product are safer in use as molluscicides for non-target organism.²⁶

CONCLUSION

The above collected information regarding the use of *Alstonia scholaris* Linn in world is matched with available literature. Recent years, ethno-botanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. It is best classical approach in the search of new molecules for management of various diseases. Thorough screening of literature available on *Alstonia scholaris* depicted the fact that it is a popular remedy among the various ethnic groups, Ayurvedic and traditional practitioners for treatment of ailments. Researchers are exploring the therapeutic potential of this plant as it has more therapeutic properties which are not known.

REFERENCES

1. Nadkarni AK. Indian Materia medica, Popular Prakashan, Bombay, 1976.
2. Joshi SG. Medicinal plants, Oxford and IBH publishing Co. Pvt.Ltd., New Delhi, 2000.
3. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi. 1956.
4. http://www.worldagroforestry.org/treedb2/AFTPDFS/Alstonia_scholaris.pdf.
5. http://en.wikipedia.org/wiki/Alstonia_scholaris.
6. <http://ayurveda.ygoy.com/2010/08/19/medicinal-uses-of-alstonia-scholaris>
7. http://fincomindia.nic.in/writereaddata/html_en_files/assam/book2.pdf.
8. Allan PG, Karsten K, Dietmar G, Roger WR, Joseph JB, Geoffrey AC, Scott GF, Alicia MA. Indole alkaloids from the leaves of Philippine *Alstonia scholaris*. *Phytochemistry* 66 2005; 1158–1162.
9. Cai XH, Shang JH, Feng T, Luo XD. Novel Alkaloids from *Alstonia scholaris* 2010; 65: 1164 – 1168.

10. The Ayurvedic Pharmacopoeia of India, Part I, Vol. I. Government of India Ministry of health and Family Welfare. Department of Ayush; 1989. p. 97.
11. Arulmozhi S, Mazumder PM, L Narayanan S, Prasad AT. In vitro antioxidant and free radical scavenging activity of fractions from *Alstonia scholaris* Linn. International Journal of Pharm Tech Research 2010; 2: 18-25.
12. Hussain A, Zaman MK, Ramteke AM. Antibacterial activity of trunk bark of *Alstonia scholaris*. Asian Journal of Pharmaceutical and Clinical Research 2010; 3: 46-47.
13. Khyade, MS, Vaikos N. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. African Journal of Biotechnology 2009; 8(22): 6434-6436.
14. Channa S, Dar A, Ahmed S, Rahman AU. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. Journal of ethnopharmacology 2005; 97: 469 - 476.
15. Linn SC, Linn CC, Linn YH, Supriyatna S, Pan SL. The protective effect of *Alstonia scholaris* R. Br. on hepatotoxin-induced acute liver damage. The American Journal of Chinese Medicine 1996; 24(2): 153-64.
16. Jagetia GC, Baliga MS. Evaluation of anticancer activity of the alkaloid fraction of *Alstonia scholaris* (Sapthaparna) *in vitro* and *in vivo*. Phytotherapy Research 2006; 20: 103–109.
17. Jagetia GC, Baliga MS. Effect of *Alstonia scholaris* in enhancing the anticancer activity of berberine in the Ehrlich ascites carcinoma –bearing mice. Journal of Medicinal Food 2004; 235-244.
18. Jahan S, Chaudhary R, Goyal P. Anticancer Activity of an Indian Medicinal Plant, *Alstonia scholaris*, on Skin Carcinogenesis in Mice Integrative Cancer Therapies 2009; 8(3): 273-279.
19. Anurakkun NJ, Bhandari MR, Kawabata J. α -Glucosidase inhibitors from Devil tree *Alstonia scholaris*. Food Chemistry 2007; 103: 1319–1323.
20. Arulmozhi S, Mazumder PM, Lohidasan S, Thakurdesai P. Antidiabetic and antihyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R.Br. European Journal of Integrative Medicine 2010; 2: 23-32.
21. Kulkarni MP, Juvekar AR. Effect of *Alstonia scholaris* (Linn.) R.Br. on stress and cognition in mice. Indian Journal of Experimental Biology 2008; 47: 47-52.
22. Shah AJ, Gowani SA, Zuberi AJ, Ghayur MN, Gill AH. Antidiarrhoeal and Spasmolytic Activities of the methanolic Crude Extract of *Alstonia scholaris* L. are mediated through Calcium Channel Blockade. Phytotherapy Research 2010; 24: 28–32.
23. Immaculata M, Soemardji AA, Retnoningrun DS, Sukrasno UM. Immunostimulating effect of Pule *Alstonia scholaris* L. R.Br., (Apocynaceae) bark extracts. Clinical Hemorheology and Microcirculation 2000; 23: 177-183.
24. Shang JH, Cai XH, Feng T, Zhao YL, Wang JK, Zhang LY, Yan M and Luo XD. Pharmacological evaluation of *Alstonia scholaris* :Anti-inflammatory and analgesic effects. Journal of Ethnopharmacology 2010; 129(2):174-81.
25. Shang JH, Cai XH, Zhao YL, Feng T, Luo XD. Pharmacological evaluation of *Alstonia scholaris*: Anti-tussive, Anti-asthmatic and Expectorant activities. Journal of ethnopharmacology 2010; 129 (3): 293-298.
26. Singh SK, Singh A. Molluscicidal and Anti-cholinesterase Activity of *Alstonia scholaris* plant against Freshwater Snail *Lymnaea acuminata*. Pakistan Journal of Biological Sciences 2003; 6(16): 1442-1446.