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Himalayan Medicinal Resources: Present and Future. A Case Study: Andidiabetic Activity of Shilajit

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ABSTRACT

A major portion of drugs used in Ayurvedic system of medicine which has been practiced since the early human civilization in Indian subcontinent were of plant origin. It should be noted that 70% of the population in this region depends on Ayurveda for their medical treatment and 60% of the drug resources are obtained from the Himalayan region. Therefore, Nepal becomes a potential source of plant drug resource since it occupies a major portion of the Himalaya. In the present paper, in general a current status of medicinal plant resources of Himalayan region especially Nepal will be discussed. In addition to this, a typical example of antidiabetic activity of Shilajit will be taken for the discussion.

Shilajit is one of the crucial elements in several formulations including those of Rasayana, a therapy in Ayurveda, which has been practiced in the prevention of ageing and mental disorder. Although, Shilajit is widely used for the treatment of diabetes, no satisfactory scientific reports are available up to now. The crude Shilajit in the market is a dark brown or black rock-like substance collected from the Himalayan region with a strong smell of cow's stale urine.

In our studies, Shilajit (collected in the central Himalayan region) prevented the diabetes in nonobese diabetic (NOD) mice model. Shilajit also prevented the diabetes in the rats against the action of multiple low-dose (10 mg/kg, i.v., 5 times) of streptozotocin. On the other hand, Shilajit did not show antioxidative activity. The preventive action of Shilajit on diabetes is mainly focused on the Th1 and Th2 cell activities, since Th2 cells activity was found to be significantly upregulated. Shilajit, however, showed a mild action in controlling the blood sugar level in young, old, and mild diabetic rats, but not in the severe diabetic rats. It also stimulated the nitric oxide production in macrophages. Based on these evidences, the antidiabetic activities of Shilajit appear to be immunomodulative probably by protecting or strengthening insulin-producing b-cells in the pancreas. Further systematic research on constituents of Shilajit and its quality evaluation is necessary to enable the use of natural medicines in the treatment of diabetes.

CURRENT SITUATION OF MEDICINAL PLANT RESOURCES IN NEPAL

Nepal is a small country situated on the laps of Himalayas. The unique topography of Himalaya has afforded a rich source of plant life. Some of these plants have been used as natural medicines since time immemorial. In Nepal, the information of such valuable

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medicinal plants has been compiled in the form of a book called Nighantu. It is estimated that more than 70% of the population in South Asian region depends on the plant resource i.e. herbal medicine based on Ayurveda for their medical treatment. More than 60% of the drugs used in Ayurveda, are derived from Himalayan region (Wealth of India, 1998).

Although small in size (147,181 sq. km.), Nepal possesses distinct ecological zones from almost sea level (100 m) to the highest point in the world (Mt. Everest, 8848 m). Such ecological diversity reflected in her rich and varied plant life. The estimated medicinal plants in different ecological zones are shown in Table 1 (Malla SB, 1984). In the current estimation, 7000 plant species has been recorded in Nepal. Among them 10% (700 plants) are supposed to be of medicinal use. Among the 700 plants only about 450 are used in Ayurveda and other are used by the traditional local healer. Among them 200 plants are of great demands. Among the 200 medicinal herbs of high demands, 20 of them in a state of endanger and the government has banned on their trade completely or partly.

TRADE ON MEDICINAL PLANTS IN NEPAL

Majority of the herbs is considered as the non-timber forest products in Nepal. Therefore, plants growing wild are major source of medicinal herbs. A collector needs permission from the Forest Department to collect and sell the medicinal plants. However, many of the remote area of high mountains are out of reach of Forest Department because of difficult topography therefore usual legal permission is ignored. In such context, there is no systematic methods of cultivation, harvesting and procession of medicinal plants especially in the high mountain area. Such herbs are randomly collected by the local people when the demand comes from the local dealer. Many often, they are collected in inappropriate time too. In many occasion, the mountain people when need money they pack the dried herbs or freshly cut herbs in a sac and come down to city to local healer or trader who can give them small sum of money. This is how small scale individual collection goes to bigger and bigger collectors and ultimately 90% of such crude drugs exported to India may be to third country later. In spite of all unorganized trade, the Himalayan medicinal plants contribute almost 4% to national GDP.

Some major herbs with potential demands are listed in Table 2. The demand of medicinal herbs is not regular. In some year, the demands is very high and the same demand will not be in the next year. This is because of ignorance in market study and scientific evaluation on the quality of the herbs. The local consumption is not very high because of very low population density in the high mountains.

Besides this some medicinal herbs are processed and partly purified. The major processed commercial herbs are shown in Table 3. Gaultheria fragrantissima Wall with its essential oils containing methyl salycilate is the most processed and produced medicinal plant (200

Table 1. Distribution of Plants of Medicinal Value in Ecological Zones of Nepal

Ecological zone	Altitude (m)	No of species available
Tropical zone	up to 1000	310
Subtropical zone	1000-2000	340
Temperate zone	2000-3000	225
The Subalpine zone	3000-4000	114
Alpine zone	4000-5500	45

Table 2. Estimated Quantity of Air Dried Herbs Available in Nepal

Herbs	Quantity (tons)
Zanthoxylum armatum	400
Lichens	300
Swertia chiraita	250
Nardostachys jatamansi	200
Dioscorea deltoidea	200
Sapindus mukorossi	200
Cinnamomum tamala	170
Cinnamomum glaucescens	100
Emblica officinalis	100
Rubia cordifolis	100
Terminalia bellirica	100
Terminalia chebula	100
Asparagus rasemosus	80
Picrorhiza kurroa	50
Lycopodium clavatum	40
Valeriana wallichii	30
Aconitum spicatum	20
Juniperus comuni	20

tons/year). Most of these processed herbs have the commercial value for essential oils.

MEDICINAL PLANT RELATED MAJOR ORGANIZATION

Government has three major organizations with the goal to conserve and develop the Himalayan medicinal resources.

1. Department of Plant Resources (Ministry of Forest)

2.Department of Drug Administration (Ministry of Health)

3.Herb Production and Processing Co. (Ministry of Industry and Commerce)

Department of Plant Resources has following functions:

- Research on medicinal plants
- Identification of herbs for medication and technology development for processing
- Botanical survey and herbarium for collecting and authenticating flora of country and maintaining herbarium
- Technology development for propagation of material and collection

Department of Drug Administration is responsible for licensing and regulating medicines including phytomedicines. Currently following regulation is adopted from the Department of Drug Administration.

For phytomedicines, the products should be registered with Department of Drug Administration, Ministry of Health, His Majesty' s Government,

Table 3. Approximate Average Annual Air-dried Weight of six Major Products Processed in Nepal by the Public and Private Sector

Herbs trade name	Botanical name	Quantity (tons)
Winter Green	Gaultheria fragrantissima	200
Timur	Zanthoxylum armatum	100
Jatamansi	Nardostachys jatamansi	80
Sughandha kokila	Cinnamomum glaucescens	70
Jhau	Parmelia sp., Usnea sp.	60
Sunpati	Rhododendron anthopogon	15

Kathmandu, Nepal.

Regulatory requirement:

- Up to date manufacturing licenses issued by concerned drug control authority
- Price approval and valid price list
- Letter of warrenty-manufacture' s responsibility for safety, efficacy and quality of their products
- Authorization for import/export/distribution of product, mode of distribution and promotion

Pharmaceutical requirement:

- Quantitative formula including all excipients
- Stability, self life, bioavailability data wherever applicable
- Description of product (physical identity) container/packaging labeling and photograph of each product

Herb Production and Processing Co. prepares the medicinal products or ingredients for medicines. It maintains herbal farm, collect, stores and sells herbs.

In addition to the governmental organization, several nongovernmental organizations are also involved in the field of herbal medicines. Tribhuvan University, Pokhara University, Kathmandu University offers academic courses related medicinal plants. Singha Darbar Vaidya Khana is government undertaking organization for production of Ayurvedic medicines. Private production unit such as Gurkha Ayurveda, Cosmos Herbal, Dabur Nepal, Kunsen Co. are also emerging.

CONCLUSION

Medicinal plants, its products and traditional practices are facing difficult time because of natural deforestation as well as encroachment of forest for agriculture land and urbanization. On the other hand, common people are more attracted towards the natural herbal use day by day. The short sighted unscrupulous collection has added more problem. New generation of traditional healers are opting for other profession. A right solution is difficult to come by but some plausible solutions may include respect to nature, strict forest law, public awareness, generous investment in teaching and research on traditional knowledge of medicine and sound resource management. In essence, man' s ability to live in harmony with nature is key to ensure sustainable use of medicinal plant resources.

A CASE STUDY: ANTIDIABETIC ACTIVITY OF SHILAJIT

Diabetes mellitus (DM) is regarded as the one of the oldest known diseases in human societies. The

symptoms of diabetes such as polyurea, polyphagea, excessive thrust, weakness, losing weight etc., had already been well illustrated in Chinese or Indian ancient literature dated back 3500 years (Hengesh and Holcomb, 1981). The treatment for DM such as exercise, controlled food habit and some herbal remedies had already been practiced. In spite of all this, DM is often wrongly referred as the western disease. However, DM is the disease related to metabolic disorder, therefore, we cannot ignore the food habit and living style as the main cause of DM. In an attempt of evidence-based medicine, an epoch-making discovery in 1921, that a pancreatic extract (insulin) would lessen the symptoms of diabetes, was a landmark in the area of antidiabetic drugs from natural sources. However, the treatment of insulin was life saving and prolonged the life of those people who have to die early because of ketoacidosis or diabetic complications. On the other hand, the daily pain of using insulin and its difficulty of handling for DM patient encouraged the scientists to discover some other oral hypoglycemic agents. In the mean time, during World War II when insulin was not available in many of the countries, search was made for an insulin substitute from plant sources. Soon after the World War II, oral hypoglycemic agents were made available for the clinical use that exceeds more than 88 types up to now (Soejarto and Farnsworth 1989).

In spite of all efforts, even in developed countries such as Japan, USA, etc. where sophisticated therapy is widely available, more deaths are attributed to diabetes than to cancers or motor vehicle accidents (World Health Organization, 1985). It has been considered that DM is the third leading cause of human death in the world. The report of WHO shows that the population of diabetics was 60 millions in 1980, 118 millions in 1995 and there will be 220 millions in 2020 (Amos et al, 1997). Every year diabetic population is increasing in spite of all research and education in this area. More than 10% Japanese population of over 40 years is suffering from type II diabetes (NIDDM: Non Insulin Dependent Diabetes Mellitus, i.e. insulin is insufficiently produced or hyperinsulinema or insulin does not functions properly however external insulin is not required). Although, the number of Type I diabetes (IDDM: Insulin Dependent Diabetes Mellitus, i.e. insulin can not be produced within the body, therefore external insulin is required) population is very low in Japan comparing to western countries, more than 6000 people are suffering from such incurable diseases in Japan. Therefore, it is very important to focus on the research aim to find cure for such disease which is not only killing the lives, but deteriorating health and also a huge social economic burden to the nation. Only in USA, direct medical costs due to diabetes are estimated to have been 9.6 billion US$, and indirect costs for short term morbidity, long-term disability, and mortality are estimated to have been 10 billion US$ (Center for Economic Studies in Medicine 1988). The enormous costs of modern treatment indicate that alternative strategies for the prevention and treatment of diabetes must be developed. Since almost 90% of the people in rural areas of developing countries still rely on traditional medicines for their primary health care. A synthesis of traditional and modern knowledge may lead to effective and cost-efficient alternatives.

Whether it is Type I or II, the main cause of diabetes is the loss of b-cells either due to toxic chemicals from external environment, viral infection or diet. In addition to this, immunological factors such as autoimmune disorders have been considered as the focus for the cause of Type I to genetically vulnerable individuals (Unger and Foster, 1985). Type II diabetes is generally found in older population and often associates with obesity. More than 15% of people over 65 years of age have Type II (Ilarde and Tuck, 1994). In Type II, either insulin is excessively produced with its improper function or production of insulin is not sufficient. In both conditions, the patients suffer with an elevated

blood glucose level causing the metabolic disorder.

Before the modern therapy, herbal treatment, diet and exercise were the way of treatment for the DM patients. Such treatment still exist as a part of treatment but this treatment is not beneficial to the Type I diabetes, although it is often beneficial for the several cases of Type II diabetes. The focus on pancreas has been started since 1889 after von Mering and Minkowski who found that pancreas has a great role to control glycemia. The successful experiment was carried out by Banting and Best in 1921 for the use of pancreatic extract, what we call insulin today, to reduce the elevated blood glucose level, which has been considered as the foundation of modern treatment for the diabetic people. Insulin controls the absorption of glucose in the intestine, reverses the neoglucogenesis process, activates the hepatic enzymes, activates and generates the glucose transporter in the fibroblasts, suppresses the function of glucagon and other hormones, activates the glycogenesis and fat synthesis. All these actions of insulin help to lower the elevated glucose level to normal. Therefore, the major concern for the research on diabetes remained mainly to be the action mechanism of insulin after its discovery since 1921. Insulin plays a vital role to control the glycemia, however, it could not be the total solution to overcome the diabetes. Moreover, insulin being a polypeptide (51 aminoacids) the oral route of administration is not effective, since it is digested in the intestine. In addition to this, it has shorter life span in the human body, therefore, its effect is not long lasting.

Several attempts were made to discover the effect of the natural medicine, especially the herbal remedy, to potentiate the insulin producing b-cells and to produce more insulin. In this context, Shilajit has been considered traditionally as a beneficial drug for diabetes, however there are no reports available so far for its control over glycemia. Shilajit is considered as one of the controversial drugs in the Ayurvedic medicine regarding its source, nature and processing. In general, Shilajit is blackish brown rock exudates with the smell of stale cow urine (Koirala, 1993). It is especially collected from the Himalayan mountain at an altitude between 2500-5000 m in Nepal (Kong et al., 1987) and used in the Ayurvedic system of medicine to increase sexual potency besides to treat DM and urinary disorder. Shilajit has been used to treat the DM since long back but there is no systematic study to explain its antidiabetic activity (Chopra, 1976). There are a few reports showing its constituent related to the plants growing on such area. However, the quality of Shilajit remains to be questionable. In our experiment, a systematic antidiabetic activity of Shilajit was studied. Therefore, we mostly studied the preventive action of Shilajit on diabetes using nonobese diabetes (NOD) mice model as well as the glucose tolerance in normal and streptozotocin (STZ)-induced diabetic rats.

RESULTS AND DISCUSSION

In the present experiment, Shilajit was collected from Central Himalayan part of Nepal. The Shilajit extract refers the water extract obtained by the usual procedure.

1. Effect of Shilajit in NOD Mice

The NOD mouse, genetically defective animal, develops spontaneous and severe diabetes at the age of 16 to 20 weeks (Faust et al., 1994). This mouse model has been considered to be very close to Type 1 diabetes in human (Solimena and Camilli, 1996). The female NOD mice are more prone to diabetes than male one. At the age of 30 weeks more than 80% of female NOD mice die because of severe diabetes developed with the of destruction of b-cells by autoimmune disorder (Faust et al., 1994). In our experiment, the Shilajit rock was ground to fine powder and given as the diet supplement with an approximate dose of 200 mg/kg/day to the female NOD mice at the age of 5 weeks. Blood glucose

level was measured once in two weeks and food consumption calculated twice in a week. The mice with blood glucose level more than 200 mg/dl in two consecutive measurements were considered to be as diabetic. In the control group, the diabetic symptoms appeared at the age of 16 weeks while in Shilajit treated group, the first diabetic symptoms appeared at the age of 24 weeks. At the age of 30 weeks, 60% of the mice in the control group developed diabetes and all diabetic mice died because of severe ketoacidosis. In contrast, in the Shilajit-treated group only 20% animals showed diabetes and 10% of them died. This result clearly shows that Shilajit as diet supplement, at least delays or prevents the diabetes in NOD mice.

2. Effect of Shilajit Extract on Multiple Low-Doses of Streptozotocin in Rats

In this experiment, the effects of Shilajit extract on multiple low-doses streptozotocin in rats were studies (Takamura et al., 1999). Male SD rats, at the age of 8 weeks, were treated seven times with the Shilajit extract at a dose of 100 mg/kg, p.o. Then five times low-dose (10 mg/kg, i.v.,) STZ in ice-cold saline was administered. During STZ-injection period, animals were fasted over night and drugs were given before STZ-injection and food was given after 30 min of STZ injection. After four days of final dose of STZ-injection, the blood glucose level were measured and compared with that of the control group. Nicotinamide was used as the positive control. Both in Shilajit and nicotinamide treated groups, no diabetic symptoms were observed while in the control group, average blood glucose level was 346 mg/dl. This result showed that the Shilajit extract prevented the diabetes induced by the multiple low-doses of STZ.

3. Antioxidative Activity of Shilajit Extract

The antioxidative activity was studied by DPPH assay (Basnet P. et al., 1997). The Shilajit extract did not show antioxidative activity at the concentration of 1, 10 or 50 mg/ml. For the comparison, vitamin C was taken as the positive control showing a concentration dependent antioxidative activity. More than 90% of the free radicals of DPPH were scavenged by 10 or 50 mg/ml of vitamin C and 25% of the free radicals were scavenged at the concentration of 1 mg/ml of vitamin C. This result shows that Shilajit extract does not have any antioxidative activity.

4. Effect of Shilajit Extract on Th1 and Th2 cells in mice

The Th1 and Th2 cells activities were measured by ELISA method (Mosmann and Coffiman, 1989) in pancreas and spleen before and after STZ treatment (150 mg/kg, i.p.) in mice. By the treatment of STZ the Th1 cell activity was increased by six folds while Th2 cells activity is just doubled. By treating STZ together with the Shilajit, Th1 cell activity is not changed while Th2 cells activity is increased two folds comparing to control. This result clearly suggested that Shilajit modulates the immunoregulatory Th1 and Th2 cells. It is well known that Th1 and Th2 cells are cytotoxic and cytoprotective, respectively. Shilajit is very important drug for preventing diabetes by strengthening the cytoprotective cell activities and reducing the cytotoxic cell activities.

5. Effect of Shilajit Extract on Glucose Tolerance in Rats

The effect of Shilajit extract was studied on glucose tolerance in rats. Glucose tolerance test was performed on young rats, old rats, mild diabetic rats and severe diabetic rats. Young rats were 6 weeks old, male, SD rats. Old rats were more than one year old, male, SD rats. Mild diabetic rats were prepared by administering considerably low-dose (30 mg/kg, i. v.) and severe diabetic rats by high-dose (55 mg/kg, i.v.) of STZ,

respectively. The glucose tolerance test on normal, mild diabetic and severe diabetic rats were done by the usual method. Before the glucose tolerance test, animals were fasted about 18 h (food is removed on 18:00 of previous night and glucose loaded on 12:00 next day). Drugs (Shilajit extract: 100 or 200mg/kg; tolbutamide as a positive control: 100 mg/kg) are given two times p. o. at 18 h and 2 h before the glucose loading. For the control group same volume of saline water (vehicle) was given. Glucose (20% solution, p. o., 2 g/kg) was given to rats. Blood glucose level was measured just before called as 0 h, and 30 min, 60 min and 120 min after glucose loading. Food was not given during the tolerance test. The glucose tolerance test showed that Shilajit water extract has a mild but significant improvement of glucose tolerance in normal and mild diabetes with the dose of 200 mg/kg but not in severe diabetic rats. The glucose tolerance effect of Shilajit (200 mg/kg) was found to be weaker than tolbutamide (100 mg/kg).

9. Effect of Shilajit Extract on Nitric Oxide Production in Murine Macrophages

The effect of Shilajit extract was studied on J774.1 murine macrophage (Sakaguchi et al., 1995). The cells were cultured by usual procedure. The cells were treated 100, 10 and 1 mg/ml of Shilajit with (10 mg/ml) or without LPS. The nitric oxide production was expressed by measuring the nitrite (NO2-) concentration produced in the medium by Griess reagent. In both, presence or absence of LPS the production of NO induced as the concentration dependent way by the Shilajit comparing to the respective control. This result clearly suggests that Shilajit enhances the production of NO.

CONCLUSION

Shilajit is widely used for the treatment of diabetes in Nepal or South Asian region, however, no scientific reports available so far. In our experiment, we found that Shilajit at least prevent or delay the diabetes in NOD mice and multiple low-doses STZ-treated rats. Regarding its glucose tolerance test, it improved glycemia only with very high dose comparing to tolbutamide, therefore, its beneficial antidiabetic effect is due to the prevention more than controlling blood glucose level. Based on its action of mechanism point, Shilajit did not show any antioxidative activity, therefore its preventive action must be immunoregulatory. Shilajit upregulated the Th2 cell significantly together with the down regulation of Th1 cell activity, therefore, its cytoprotective action against STZ-toxicity or prevention from autoimmune action were due to the up regulation of Th2 activity.

On the other hand, it induced NO production. NO has dual role, since its controlled induction shall led to cytoprotective activity. Vascular complications are major causes of morbidity and mortality in patient with DM. NO has an important role in the regulation of vascular tone and impaired NO activity could be implicated in the development of diabetic vasculopathy (Chan et al., 2000). Experimental evidence suggests that decreased bioavailability of NO is plausible in Type I diabetes. For example, it has been shown that the vasodilating effect of insulin in skeletal muscle is mediated through an increase in NO release (Steinberg et al., 1994). Hence in the presence of insulin deficiency in Type I diabetes there could be reduced NO release. Even if NO release is normal, its bioavailability could be reduced because advanced glycation end products, as a result of chronic hypoglycemia, quench NO in vitro (Bucala et al., 1991). In our experiment, Shilajit induced the production of NO in vitro, therefore, at least in part, the microvascular complication might reduced in NOD mice and hence the mortality rate was reduced in Shilajit treated group.

Many traditional antidiabetic plants probably act at

least in part through their fiber, vitamin, mineral contents. Mineral deficiencies are common in diabetes and can exacerbate insulin resistance. Several of these minerals are co-factors for signaling intermediaries of insulin action and key enzymes of glucose metabolism. Mineral supplement can benefit patient with mineral deficiencies, as demonstrated with magnesium and zinc. Plants rich in minerals have also shown to benefit in diabetic patients (Day, 1990). Shilajit is being a rock substance containing minerals. It is not clear whether the mineral or organic constituents of Shilajit is beneficial for preventing diabetes; however, the beneficial effect of mineral cannot be excluded.

Probably Shilajit enhances the microvascular activity and prevents diabetes by strengthening the cytoprotective cell activities and reducing the cytotoxic cell activities. In addition to this, Shilajit also helps to control glycemia in rats, however the effect is weaker than that of tolbutamide. In conclusion, Shilajit is beneficial for the prevention of diabetes, however, systematic research on constituents of Shilajit and its quality evaluation is necessary to enable the use of natural Shilajit for the treatment of diabetes.

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