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Effect of dehydrated *Salacia prinoides* on experimental mice and on NIDDM subjects

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Abstract
Studies conducted with dehydrated *Salacia prinoides* and soya flour individually show their beneficial effects on the physiological well being of animals and humans. Thus nutrient analysis of dehydrated *Salacia prinoides* and a supplementation study was conducted. Supplementation study included both animal and human subjects. It was observed that dehydrated *Salacia prinoides* is rich in protein and fibre. It is low in fat content. Copper and magnesium were present in negligible amounts. There was significant decrease in body weight, mean blood glucose levels, mean total serum cholesterol levels and an increase in mean serum total protein levels and serum urea level of the experimental albino mice. Histopathological findings revealed that there was a mild impact on liver, kidney and intestine, the representative segments showed mild changes in cell structure. Thus no significant toxicity was found in liver, kidney and intestine when the experimental albino mice were sacrificed. In the human study, purposive sampling technique was used to select NIDDM subjects. Five grams of *Salacia prinoides* was provided as a supplement for a period of 45 days. A reduction in body weight was observed among the experimental group subjects. There was significant reduction in fasting and post prandial blood sugar levels among the subjects in experimental group. A significant reduction in serum triglycerides and LDL cholesterol was also observed. Thus it can be concluded that dehydrated *Salacia prinoides* can be recommended as a food supplement with additional benefits of its active principle for its anti-diabetic activity

Keywords: *Salacia prinoides,* blood glucose levels, anti-diabetic activity.

Introduction
There is a worldwide interest and awareness towards naturopathy, since it is the only branch of medicine free from side effects. In South India wild shrubs from the family Celastraceae are shown to have hypoglycemic and hypolipidaemic effect. Salacia prinoides, commonly known as Ekanayakam (in Tamil-Koranthi) is one of the plant products used in ayurvedic medicine as a hypoglycemic agent (Kumar, 2000). It is a small erect or straggling tree or large woody climbing shrub found almost throughout India including Andaman and Nicobar Islands. Leaves are ovate to lanceolate; flowers 2 to 6 clustered together on axillary tubercles, yellowish; fruits small, globose, one to two centimeter in diameter, red when ripe, one-seeded; seeds surrounded by an edible pulp. Ripe fruits are eaten (Wealth of India, 1972). For thousands of years, it has been used in the treatment of diabetes mellitus, obesity, gonorrhea, rheumatism, itching, and asthma. As far as its effects in diabetes, several studies have shown that the extract targets multiple processes within cells that help with not only blood sugar management, but also has other beneficial effects. It has recently been consumed in Japan, USA, and other countries as a supplement for diabetes mellitus and obesity prevention. As far as its use in diabetes mellitus, several animal and some human studies have shown that this plant species causes lowering of blood sugar.

Two friedelane-type triterpenes, salasones D and E, a new norfriedelane-type triterpene, salaquinone B, and a new polyacylated eudesmane-type sesquiterpene, salasol B, were isolated from the stems of Salacia chinensis LINN. (S. prinoides DC, Hippocrateaceae) collected in Thailand (Kinobu *et al.,* 2003). Their stereo structures were elucidated on the basis of chemical and physicochemical evidence. Some norfriedelane-type triterpene, lignan, and catechin constituents were found to show radical scavenging activity. Chemical constituents of *Salacia prinoides* were isolated and purified with various column chromatography by Gao and co workers (2008). Sixteen compounds, including seven triterpenes, flavanoids, egallic acid were obtained which had antioxidant properties. The root bark contains two 1, 3- diketones (C30H48O3 and C30H46O3), fatty matter, rubber, dulcitol, magniferin, phlobatannin and glycodidal tannins. Roots also contain leucopelargonidin and its dimmer and tetramer (Dash & Bedi, 1967). *Salacia prinoides* is widely used in the treatment of different diseases in the ayurvedic system of medicine and is free from side effects (Kowsalya *et al.,* 1998). The methanolic extract from the roots and stems of Indian *Salacia prinoides* and its water-eluted fraction of Diaion HP-20 column were found to exhibit inhibitory activities against α-glucosidase (Yoshikawa *et al.,* 2008). According to Pillai and co-workers (1980), hypoglycemic effect was observed in the experimental animals on oral administration of 0.5 g per kg and 1 g per kg of decoction and infusion of root bark of Salacia prinoides.

The therapeutically effective food supplement, when given in excess of the recommended dosage leads

to toxic effects. Supplementation of a diet with specific active principle over a prolonged period of time and the monitoring of any physiological and clinical changes gives information about the impact of the active principle on the animals. Thus male albino mice which are used for experimental purposes as they are biologically stable and free from hormonal imbalance were chosen for the current study.

Materials and methods

Studies conducted with dehydrated *Salacia prinoides* and soya flour individually show their beneficial effects on the physiological well being of animals and humans (Nair *et al.,* 1986; Khalifa *et al.,* 1994). Thus nutrient analysis of dehydrated *Salacia prinoides* and a supplementation study was conducted. Supplementation study included both animal and human subjects.

Animal study

For the animal study experimental research design was followed. The research design selected for the animal study was "After only with control" design. The root bark of *Salacia prinoides* was collected and broken into pieces. The pieces were ground into a fine powder. The soya beans were collected from the local market and also ground into fine flour in a flour mill. The normal feed was given to the experimental albino mice. The supplementary feed was prepared using *Salacia prinoides* and soya flour in three different proportions as given in Table 1, in pellet form.

Depending on the proportion of combining dehydrated *Salacia prinoides* and soya flour the experimental albino mice were divided into three groups. Group I received pellets containing 50 percent of normal feed, 20 percent dehydrated *Salacia prinoides* and 30 percent soya flour. Group II received pellets containing 50 percent normal feed, 25 percent dehydrated *Salacia prinoides* and 25 percent soya flour. Group III received 50 percent of normal feed, 30 percent dehydrated *Salacia prinoides* and 20 percent soya flour. Group IV acted as control. Approximately 400 g of supplementary feed was fed to each experimental group (consisting of 16 experimental albino mice in each group) for a period of 30 days. Periodic clinical examination for deficiency disorders, illness, discharge from the eyes and mouth were conducted. The weights of the individual mice were monitored regularly. Biochemical analysis like blood glucose level, serum total protein levels, serum total cholesterol levels and serum urea levels were analyzed as per the advice of doctors in veterinary hospital. Histopathological investigations were carried out in the individual organs like liver, kidney and intestine of the experimental mice.

Human study

For the human study, the root bark of *Salacia prinoides* was collected and was broken into pieces in a sawing machine and pieces were ground to a fine powder. As per the advice of the clinicians of Government Siddha Hospital Chennai, a preliminary trial was conducted among 6 patients. They were administered with increasing concentration of dehydrated *Salacia prinoides* powder along with 250 ml of buttermilk at a time i.e., 1g, 1.5 g, 2.0g upto 5g respectively. This was conducted to assess the tolerance of dehydrated Salacia prinoides. Upto 5g concentration the patients did not experience any intolerance, but when the powder was increased further a few patients experienced mild nausea. Hence 5g of dehydrated Salacia powder was taken as the tolerance level for human subjects. As per the clinicians advice 5gm of dehydrated Salacia powder was administered as capsules in three divided doses per day. A single dose could not be administered because the largest capsule size could hold only 750mg of dehydrated Salacia powder. Thus six 750 mg capsules and one 500 mg capsule were given to subjects to be consumed as three divided doses. A purposive sampling method was adopted in selecting 12 NIDDM subjects of which six of the subjects were given dehydrated *Salacia prinoides* for 45 days and formed the experimental group, while the other six were not given any supplement and formed the control group. The criteria used for the selection of subjects were

- They should be on dietary restrictions with minimal dosage of oral hypoglycaemic drugs (1 tablet of dianil/day)
- They should be between the age ranges of 35 to 55 years.
- They should not have any other complications.
- They should be willing to co-operate for the entire period of supplementation.

Purposive sampling technique was used to select NIDDM subjects of which half of the subjects were given dehydrated *Salacia prinoides* (experimental group) while the remaining subjects served as control group. Anthropometric assessment (body weight and height) and biochemical parameters like fasting blood sugar, post prandial blood sugar, glycosylated hemoglobin, serum lipids and serum trace minerals like copper and magnesium were assessed before and after supplementation for both the groups.

Results and discussion

The results and discussion of the study is dealt under three main headings.

1. Nutrient analysis
2. Animal study
3. Human study

Nutrient analysis

The nutrient content of dehydrated *Salacia prinoides* is given in Table 2. Carbohydrates (Nitrogen free extract) content of dehydrated *Salacia prinoides* was found to be 44.93 percent comparable to that of fenugreek seeds (44.1 percent). This shows that dehydrated *Salacia prinoides* is an edible supplement in the human diet with its therapeutic values (Gopalan, 1996). Protein content of dehydrated Salacia prinoides, was found to be quite high (11.97 percent) comparable with that of wheat (11.8

Plate 1. Root bark of Salacia prinoides

Plate 5. Histopathological effects on experimental and contro group albino mice: Histopathological effects on liver

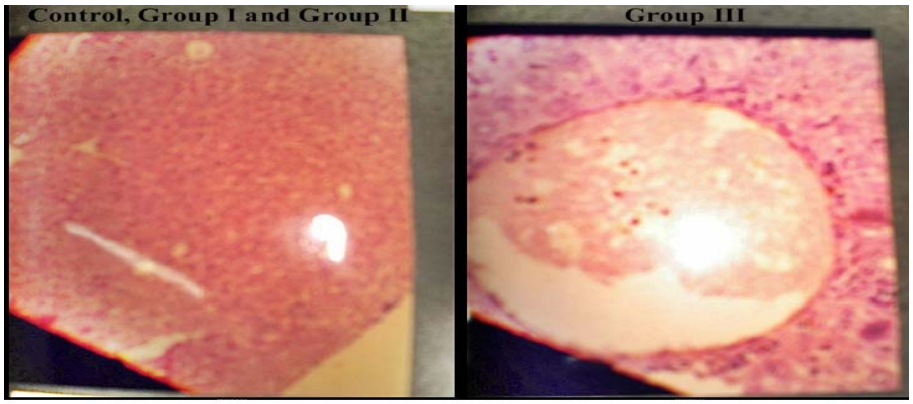


Plate 6. Histopathological effects on intestine

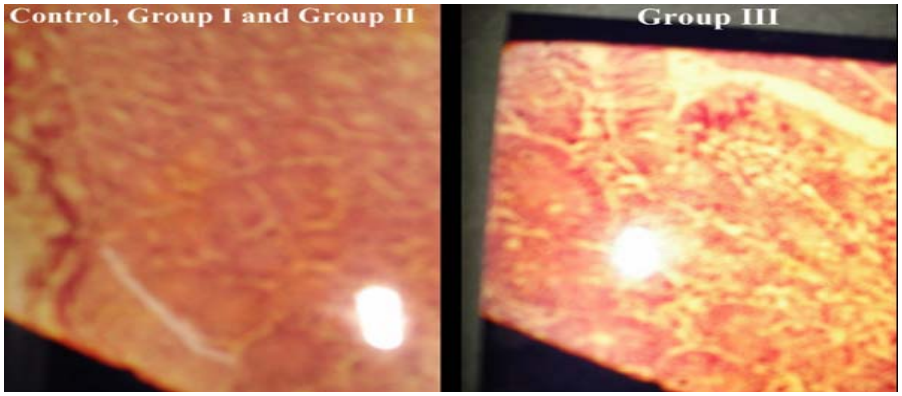
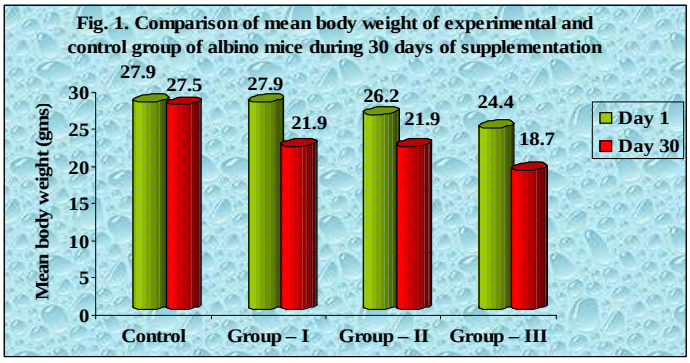


Plate 2. Soya flour and Salacia prinoides (powdered form)

Plate 4. Salacia prinoides - capsulated form for human study

Plate 3. Salacia and soy flour supplement as pellets for albino mice



percent) and higher than the protein content of rice (6.4 percent). Thus it is very clear that dehydrated *Salacia prinoides* is suitable as a good protein supplement in the human diet. Fat content of dehydrated *Salacia prinoides* was found to be quite negligible (5.95 percent) Comparable with that of Bengal gram (5.3 percent). Since, it is a root bark, it does not have significant amount of fat in it. The fibre content of dehydrated *Salacia prinoides* was found to be high (28.49 percent). It is comparable to the fibre content of green leafy vegetables (25-30 g) and to that of dry lotus stem (25.0 g/100g).The amount of total ash and insoluble ash present in dehydrated *Salacia prinoides* are 4.73 percent and 1.76 percent respectively. The copper content of dehydrated *Salacia prinoides* was found to be 22.41 μg per gm. The highest copper content is in barley (1.19 mg per 100 gm) The highest magnesium content is found in betal leaves as 447 mg per 100 gm, whereas dehydrated *Salacia prinoides* has very negligible amount of magnesium (6mg per gm).

Table 1. Different proportions of supplementary feed prepared (in percentage)

Ingredients	Control group	Group I	Group II	Group III
Normal feed	100	50	50	50
Dehydrated Salacia prinoides	-	20	25	30
Soya flour	-	30	25	20
Total	100	100	100	100

Table 2. Analysis of proximate principles in dehydrated Salacia prinoides (in percentage)

Proximate principles (in percentage)	Amount present
Moisture content	3.93
Carbohydrates (Nitrogen free extract).	44.93
Crude proteins	11.97
Fat (ether extract)	5.95
Crude fibre	28.49
Insoluble ash	1.76
Total ash	4.73
Copper (μg per gm)	22.41
Magnesium (mg per gm)	6

Animal study

Periodical clinical examination

Periodical clinical examination of the experimental albino mice was carried out before and after supplementation with dehydrated *Salacia prinoides* and soya flour in three different proportions. No significant difference was observed before and after supplementation when examining for external signs of deficiency disorders, illnesses, discharges from the eyes and mouth and behavioral changes.

Comparison of mean body weight of experimental & control group of albino mice during 30 days of supplementation

The mean body weight of experimental and control group of albino mice supplemented with dehydrated *Salacia prinoides* and Soya flour in three different proportions were compared and the results are presented in fig. 1. Results indicate that there is a significant difference at 1 percent level (weight reduction) in the animals belonging to all the experimental groups after supplementation. Between the experimental groups the albino mice belonging to group I and group III showed greater weight loss than those in group II. The reason for group III (50:30:20) experimental albino mice loosing weight rapidly could be associated with the active principles present in dehydrated *Salacia prinoides* especially fibre content.

Effect of dehydrated Salacia prinoides and soya flour on blood glucose levels of experimental group of albino mice during 30 days of supplementation

As the study design being "After only with Control", the biochemical parameters were tested only after supplementation and the values were compared with control group mice. The mean blood parameter levels of the control group and the experimental group mice are presented in Table 3. It is very clear from the table there is a significant difference among experimental and control group mice with respect to blood glucose, serum total protein and cholesterol levels. When comparing the mean values of blood glucose and total cholesterol levels, between the three experimental groups it was interesting to note that group III showed lower mean blood glucose and total cholesterol levels than groups I and II. This could be related to the fact that dehydrated *Salacia prinoides* is rich in fibre and a good hypocholesterolemic agent (Shobana, 1999). From table 3, we can infer that there is a significant increase in the serum total protein levels of Group III of experimental albino mice than that Group II and Group I. When comparing the mean serum total protein levels of the three experimental groups, it comes to light that group III (50:30:20) and group II (50:35:25), have the mean Serum total protein levels higher than the corresponding values of group I (50:20:30).

Table 3. Mean blood parameter levels of control and experimental group albino mice at the end of the supplementation period.

Blood parameters	Control	Group I	Group II	Group III	F value
Blood glucose (mg/ dl)	119.4±3.7	100.00±3.8	100.5±2.9	91.0±2.5	105.63**
Serum total protein (mg/dl)	7.3±0.35	8.3±0.37	9.1±0.24	9.3±0.33	60.88**
Serum total cholesterol (mg/dl)	120.5±2.2	104.0±4.1	98.1±3.3	93.2±4.1	92.64**
Serum Urea level (mg/dl)	38.0±1.8	47.5±2.5	38.7±2.4	47.2±1.9	46.35**

** Significant at 1 % level ($p<0.01$)

With respect to serum urea levels it is found that in groups I and III (50:20:30) and 50:30:20) respectively, there is significant increase in the serum urea levels where as in group II (50:25:25), the serum urea levels are comparable with the control group. Clear reasoning for

this is not available but it could be presumed that when either of the two powders that is dehydrated *Salacia prinoides* and soya flour are supplemented to the experimental albino mice in the proportion 30:20 or 20:30 along with the normal feed, the supplementation has an effect on raising the serum urea levels. Thus it can be concluded that the proportion of supplementation in group II (50:25:25) helps in stabilizing the mean serum urea levels. Tom and co workers (2006) demonstrated that chronic oral administration of the water extract from the root of Salacia to Zucker diabetic fatty (ZDF) rats, a genetic model of type 2 diabetes and obesity, lowered plasma triglyceride and total cholesterol (TC) levels, increased plasma high-density lipoprotein levels and reduced the liver contents of triglyceride, non-esterified fatty acids (NEFA) and the ratio of fatty droplets to total tissue. By contrast, the extract had no effect on plasma triglyceride and TC levels in fasted ZDF rats. After olive oil administration to ZDF the extract also inhibited the increase in plasma triglyceride levels. These results suggest that Salacia extract improves postprandial hyperlipidemia and hepatic steatosis in ZDF rats can be correlated to the fact that when a supplementation with active principles is given in excess it leads to toxic effects over and above its therapeutic values (Senden, 1981).

Table 4. Comparison of mean body weight of NIDDM human subjects of the experimental and control group on test days

Groups	Mean weight (kg) as on Day 1	Mean weight (kg) as on Day 45	't' value
Experimental	62.70 ± 11.99	62.55 ± 11.60	0.79^{NS}
Control	66.68 ± 14.39	67.13 ± 14.46	8.00^{**}

*NS - Not Significant** - Significant at 1% level (P<0.01)*

Table 5. Comparison of mean Body Mass Index (Kg/m^2) of NIDDM human subjects of the experimental and control group on test days

Groups	Day 1	Day 45	't' value
Experimental	22.85 ± 2.81	22.80 ± 2.67	0.69^{NS}
Control	27.41 ± 5.09	27.66 ± 5.10	11.13^{**}

*NS - Not Significant; ** - Significant at 1% level (P<0.01)*

3. Human study

Comparison of mean body weight of NIDDM human subjects of the experimental and control group on test days

The mean body weight of NIDDM subjects of both experimental and control groups are compared and the results are indicated in Table 4. Statistical analysis revealed that the mean body weight of the experimental subjects did not increase significantly at the end of the study period as compared to the mean body weight of the control group which showed a statistically significant increase in body weight. These results correlate with the fact that supplementation of dehydrated *Salacia prinoides* results in maintaining or lowering of body weight of the subjects belonging to the experimental group.

Comparison of mean Body Mass Index (BMI) of NIDDM human subjects of the experimental and control group on test days

Body Mass Index calculated using body weight and height of NIDDM human subjects on test days are given in Table 5. Statistical analysis revealed that the reduction in the mean Body Mass Index was not significant in the experimental group, where as in the control group the increase in mean Body Mass Index was statistically significant (P<0.01). This again brings to light, the beneficial effect of supplementing dehydrated *Salacia prinoides* to human subjects in experimental group.

Table 6. Comparison of lipid profile of NIDDM human subjects belonging to the experimental and control groups

Parameters	Experimental group		t' value	Control group		t' value
	Day 1	Day 45		Day 1	Day 45	
Triglyceride (mg/dl)	308.5	290.3	3.032**	275.5	292.3	3.12**
Total cholesterol (mg/dl)	221.8	214.5	1.052**	213.0	215.2	0.1875NS
HDL (mg/dl)	42.3	43.0	0.1617 NS	46.0	50.3	5.399**
LDL (mg/dl)	154.8	150.5	3.532**	150.7	151.3	0.2183 NS
VLDL (mg/dl)	24.7	24.5	0.0314 NS	17.0	15.5	0.322NS

*NS - Not Significant; ** - Significant at 1% level (P<0.01)*

The histopathological findings reveal the fact that the supplementation of dehydrated *Salacia prinoides* and soya flour does not have any significant impact on the experimental group of albino mice except for group III. The representative segments of liver showed congestion and dilatation of blood vessels and mild infiltration of cells. The representative segments of kidney showed mild degenerative and necrotic changes in the tubular epithelium. Mono Nuclear Cell (MNC) infiltration has been observed in the interstitial tissues. The intestinal structure revealed increased goblet cell activity and infiltration of MNC were seen in the lamina. The probable reason for these changes could be that the amount of Salacia and soya flour administered could be high. This

Comparison of mean fasting blood sugar level, post prandial blood sugar levels and glycosylated hemoglobin values of NIDDM subjects belonging to the experimental and control groups at Day 1 (initial) and Day 45 (final) of the supplementation period

There is a highly significant reduction in mean fasting and post prandial blood sugar levels at 1 percent level (P<0.01) among the experimental group subjects, whereas the mean glycosylated hemoglobin values did not show any significant reduction. From the above results, it is very clear that administration of dehydrated

Salacia prinoides even for a short duration (45 days) results in a marked lowering of blood sugar levels (fasting and post prandial). Thus endorsing the fact that it is a good oral hypoglycemic food supplement. The mean value of glycosylated hemoglobin was found to have been increased but the increase was not statistically significant. The reason for the increase in the mean value could be associated to the fact that the subjects were not adhering strictly to dietary control before the commencement of the study period since the glycosylated hemoglobin value shows the glucose level prevailing atleast three months before the study period.

Comparison of mean serum lipid profile of NIDDM subjects

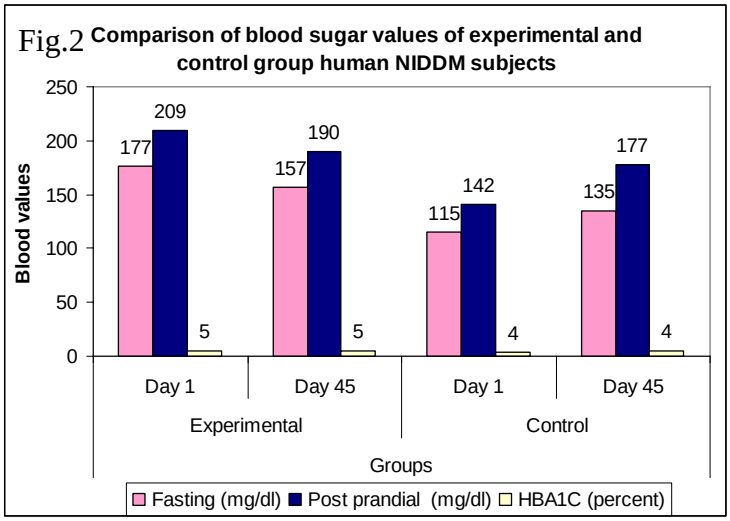


Fig.2 **Comparison of blood sugar values of experimental and control group human NIDDM subjects**

The above table represents that there is a significant decrease in the serum triglyceride, serum total cholesterol and serum LDL values of experimental group, while there was no significant difference in serum HDL and VLDL levels of the same group before and after the period of supplementation of dehydrated Salacia prinoides. Among the control group there was significant increase in the serum triglyceride and serum HDL levels while there was no significant difference among other blood lipid values, though the triglyceride, HDL and LDL levels were in the borderline range for both the groups. Thus it can be inferred that dehydrated *Salacia prinoides* does prove to be a good hypolesterolemia agent.

Summary and conclusion

Nutrient analysis of dehydrated Salacia prinoides

From the results obtained for the nutrient analysis, it has been found that dehydrated *Salacia prinoides* is rich in protein and fibre comparable with other edible food stuffs (like rice - 6.4 and wheat - 11.8). It is low in fat content (5.95 percent only). The minerals analysed were copper and magnesium. They were present in negligible amounts in dehydrated *Salacia prinoides* when compared to the commonly known food stuffs (barley - 1.19 mg per gm).

Animal study

Significant reduction in mean blood glucose and cholesterol levels of experimental group of albino mice belonging to the three groups (I, II and III) during the 30 day period of supplementation was observed (at one percent level). When compared to group I, group II and group III, group III shows maximum reduction in mean blood glucose and cholesterol levels after 30 days of supplementation while significant increase in mean serum total protein and serum urea levels of experimental group of albino mice belonging to the three groups (I, II and III) during the 30 day period of supplementation. When compared group III shows maximum increase in mean serum total protein levels after 30 days of supplementation. Group II of experimental albino mice did not show any marked changes in the mean serum urea levels. When comparing the mean serum urea levels of group I with group III it was observed that group I showed higher mean values. The histopathological findings of the experimental group of albino mice revealed that there is mild impact on liver, kidney and intestine. The representative segments of liver, kidney and intestine show mild changes in the cell structure.

Human study

A reduction in body weight was observed among the experimental group subjects. Statistical analysis revealed that the reduction in the mean Body Mass Index was not significant in the experimental group, whereas in the control group the increase in mean Body Mass Index was statistically significant ($P<0.01$). There was significant reduction in fasting and post prandial blood sugar among the experimental group subjects. A significant reduction in serum triglycerides and LDL cholesterol was also observed.

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