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Senna Leaves Treatment to Diabetics Improves Defence of Red Blood Cells and Other Diabetic Complications

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Abstract

Aims: Diabetes has been reported with constipation leading to the development of other diabetic were to treat diabetics with a new method of preparation of *Senna* leaves extract to control constipation and to observe the defence of red blood cells, liver dysfunction and other diabetic complications.

Methods: *Senna* leaves extract was prepared in hot water and orally administered to diabetics with constipation in 5 males and 4 females over the age of 50. This treatment was performed thrice a week in the 1st month, twice a week in the corresponding 2nd and 3rd months and once a week from 4th to 6th months. The defensive enzyme, the glutathione peroxidase was measured in the red blood cells along with the levels of reduced glutathione. The liver function test was performed by measuring AlaAT and AsAT levels. The other diabetic complications were also observed throughout the treatment.

Results: The diabetic persons were found to be vulnerable to skin infection particularly in the pelvic regions, backside, legs, etc. which were reversed to normal after six months of *Senna* leaves treatments. The neurological disorder like sustaining pain in legs was also recovered with the treatment. The stools quality also changed to normal. The physiological disorder like swelling of legs also improved by the treatment. The fasting glucose and glucose after meal remained to be in between 80-170 mg/dl of blood. In four of the diabetic persons who lost 15-20% of body weight, gained body weight by 7-10%. The percentage of glycosylated haemoglobin improved also and was found to be in normal range showing a long-term management of diabetes. The glutathione peroxidase decreased in red blood cells of diabetics by 20-30%, normalised after six months of treatment with *Senna*. The reduced glutathione found to be decreased in diabetics by 15-20%, normalised also. An increase in AlaAT in diabetic RBCs also got normalised after *Senna* treatment.

Conclusion: *Senna* leaves treatment to diabetics not only corrects the defence of red blood cells but also corrects skin infection, liver disorder, neurological disorder (pain in legs), physiological disorder (swelling in legs) and found to be a better alternative for a long-term management of diabetes even after treatment with antidiabetic drugs.

Keywords: Px - Glutathione Peroxidase; GSH - Reduced Glutathione; GHb - Glycosylated Haemoglobin; AlaAT - Alanine Aminotransferase; AsAT - Aspartate Aminotransferase

Introduction

Diabetes characterised by hyperglycaemia causes constipation which is one of the most devastating diabetic complications that occur in humans [1]. The excess glucose makes dysfunction of cells of Canal which are pacemakers of the intestine helping in defecation [2]. The defecation is delayed in constipation by holding the stool in the intestine for a longer time causing many metabolic disorders [3].

The metabolic disorders may be like gases formation, indigestion, less appetite, liver dysfunction, laziness, less immune system, etc [4]. In diabetes also, the defence of tissues decreases due to decreases in many defensive enzymes and metabolites [5]. Whether constipation in diabetes adds these decreases in enzymes and metabolites or not, have not been reported so far. It has been observed that the other symptoms of constipated related diabetic complications may be a skin infection, neuropathy (less movement

of legs due to swelling and pain), puffiness of the face, etc [6,7].

Many laxatives have been used for constipation but the *Senna* leaves components - sennosides A and B have been used as laxatives by most of the people [8]. The ayurvedic preparation of *Senna* leaves in powders and tablets have been used in Indian context [9]. The *Senna* leaves have mucilaginous properties and may have the degradation property by unknown factors [10]. Therefore, the powder and tablets of *Senna* may not be beneficial for human health as some toxicity has been defined [8].

Therefore, the present paper aims to find out a new methodology for the preparation of *Senna* leaves and to apply on diabetic constipated persons so that their defence of red blood cells and liver function tests may confirm a well-being of the diabetic person with the control on diabetic complications in the long-term management of diabetes.

Methodology

Design

Nine diabetic persons including 5 males and 4 females above the age of 50 years were chosen for the study. It was also observed that they all had constipation in spite of taking regular antidiabetic drugs. They also felt weaknesses, tired very soon and fewer movements of legs with a swelling and pain. They also had gas formation, bowel enlargement, indigestion, etc. with other diabetic complications as presented in Table 1. A design for experimentation was done to correct constipation by the specific preparation of *Senna* leaves extract for a longer time and had to observe the defence of red blood cells and liver function so that the body does not find weaknesses and also observed the other diabetic complications as mentioned in Table 1. The hot water treatment to *Senna* leaves may kill bacteria and fungi which infected the leaves and also separate laxatives in the extract and not taking the extra contents present in the *Senna* leaves. The treatment was designed carefully by giving thrice a dose of *Senna* leaves extract for the 1stmonth, twice a week for the 2nd and 3rd month and once in a week from 4th to 6th months. The observations on diabetic complications as mentioned in Table I were performed throughout the treatments. The defensive enzyme, the glutathione peroxidase, along with the defensive metabolite, the reduced glutathione, was measured in the red blood cells. The liver function tests were done by assaying AlaAT and AsAT enzymes. The long-term management of diabetes was performed by measuring the percentage of glycosylated haemoglobin in the blood.

Data Collection Method

All the data collection was done in the laboratory of Department of Biotechnology, Jamia Millia Islamia, New Delhi, India following all the norms by collecting the blood samples of diabetics with the consultation of a physician.

Procedure

Preparation of *Senna* leaves extract

4-5 gms of *Senna* leaves (*Cassia angustifolia*) were added to 150ml of boiling water and kept boiling for one minute. It was then cooled down, filtered and the extract was taken orally before one hour of dinner. In the morning the diabetic persons get relieved from constipation by visiting 4-6 times to toilets for defecation. The dose of *Senna* leaves extract was adjusted by the diabetics themselves so that the stomach pain should not occur while passing out the stools from the body.

The Blood Collection and Preparation of Hemolysate

The collected blood from constipated diabetics was passed through a column of a-cellulose and microcrystalline cellulose (1:1) as described by Butler, et al (1976) [11]. The eluate was centrifuged at 3000 rpm in a Serval RC 5C refrigerated centrifuge for the separation (97-98%) of RBCs. These cells were washed three times with cold phosphate buffered saline and then a hemolysate was made by forceful lysis with phosphate buffer (5 mm, pH 8.0). The hemolysates were kept at - 70°C for 4 h and then thawed at room temperature so that the haemoglobin may settle at the bottom of the test tube [11]. The upper layer of hemolysate was used for the assay of enzymes and metabolites.

Enzymes measurements

All the enzymes measurements were carried out in a spectrophotometer.

- **GSH-Px peroxidase** EC (1.11.1.9) was measured as described by Butler (1988) [12]. The following ingredients were taken in 1 ml of the cuvette with a final concentration of potassium phosphate buffer, 0.1 M, pH 7.0; GSH 1 mm; EDTA 4 mm; GSSG-R 3U per assay, sodium azide 0.4 mm; NADPH 0.2 mm; and 0.ml of diluted hemolysate (1: 40). The reaction was started by adding 0.5 ml of tert-butyl hydroperoxide 0.1 mm and the change in absorbance was measured at 340 nm.
- **Aspartate Aminotransferase** (AsAT) (EC 2.6.1.1) and Alanine Aminotransferase (AlaAT) (EC 2.6.1.2.) were measured by the method of Bergmeyer and Bernt (1974) [13]. For AsAT assay the reaction mixture in 1.5 ml in a final concentration contained potassium phosphate buffer 80 mm, pH 7.4; L-aspartate 66 mm, pH 7.4; malate dehydrogenase (dialysed) one unit; NADH 0.18 mm; and 10 ml of 1: 40 diluted hemolysate. The reaction was started by adding 2-oxoglutarate 12 mm, pH 7.4 and the change in absorbance was measured at 340 nm.

AlaAT was measured by taking the following in final concentration of assay mixture: potassium phosphate buffer 80 mm, pH 7.4; L-alanine 200 mm, pH 7.4, Lactate Dehydrogenase (LDH) (dialysed) 2 units; NADH 0.18 mm; and 50 ml of 1:40 diluted hemolysate. The reaction was started by adding 2- oxoglutarate 0.18

mm, pH 7.0 and the change in absorbance was measured at 340 nm.

Enzyme units were defined as the oxidation/reduction of 1 µmol of NADH/NADPH per ml hemolysate/min and were converted to mmol/g of haemoglobin in the case of all enzymes using NADH/NADPH.

GSH Measurement

Reduced glutathione (GSH) was measured by the method of Griffith (1980) [14]. The reaction mixture contained the following in a final concentration of: 0.20 mm NADPH, 0.6 mm 5,5¢-dithiobis-(2-nitrobenzoic acid, 0.5 U glutathione reductase in 125 mm sodium phosphate buffer (pH 7.5), 6.3 mm EDTA and appropriate sample volume. The rate of reduction of 5,5-dithiobis-(2-nitrobenzoic acid) was measured at 412 nm.

The Other Determinations

The glucose oxidase kits were used to measure the levels of blood glucose taking 10 ml of plasma. The percentage of glycosylated haemoglobin was measured by Raheja et al (1981) [15] using carbon tetrachloride and thiobarbituric acid. The optical density was measured at 443 nm. GHb percent was calculated on assumption that 1% corresponds to an absorbance of 0.029 at 443 nm.

Ethical Consideration

All the diabetic persons were ready to get treatments of *Senna* leaves voluntarily in the care of a physician. As *Senna* leaves have been reported to be used in constipation in our ayurvedic literature, therefore, voluntarily contribution in experimentation was ethically verified.

Analysis

All the data collected were statically tested by measuring mean, standard deviation and probability of significance by using standard formulae of Biostatistics. The significance of differences between the data pairs was evaluated by the Analysis of Variance (ANOVA) followed by Mann-Whitney U test.

Results

Most of the diabetic persons over the age of 50 years had skin infection particularly in the pelvic regions, backside, legs, etc. The improvement in these infections was observed after *Senna* treatment. The more or less swollen cheek, swollen legs with numbness reversed to normal after six months of treatment of *Senna* leaves. The results have been presented in Table 1. The difficulty in walking particularly swelling and pain in the left leg was more pronounced in comparison to the right leg, vanished after six months of treatment with *Senna*. The stools which were hard like pellets also became soft and smooth, though, passing out of stools used to occur after 3-4 days in diabetics. The appetite to drink more water also increased.

Parameters	Diabetes	Diabetes + *Senna* Treatment
Skin Infection	Pelvic regions, legs, backside	Reversed to normal
Puffiness of face	More or less swollen cheek	Reversed to normal
Swelling of legs and numbness	Continuous swelling and numbness	Reversed to normal
Legs pain and movement	Sustainable legs pain and difficulty in walking	Better legs movement and improvement in pain
Appetite	Less	Increased
Stools quality	Hard and pellet-like	Soft and semisolid

Table 1: Diabetic complications and *Senna* leave treatment after 6 months.

The fasting glucose and after meal glucose remained to be in between 80-170mg/dl of blood but before the treatment, the fasting glucose used to be 130-150mg/dl of blood and after a meal it was more than 200mg/dl of blood. In four of the diabetic persons who lost 15-20% of body weight, gained body weight by 7-10% after the treatment. The percentage of glycosylated haemoglobin improved also and was found to be in normal range showing a long-term management of diabetes. The results have been presented in Table 2.

Parameters	0 day of treatment	One month of treatment	3 months of treatment	6 months of treatment
Blood glucose (mg/dl of blood)				
Fasting	140±12[a]	130±9[b]	130±6[b]	120±8
PPP	210±14[a]	202±12[a]	160±15[a]	160±11[a]
	Control 88±120			

Glycosylated Haemoglobin (in%)	>8%	>7%	>7%	>6.5
Body weight	≥15%-20% loss	0% Weight Gain	5% Wight Gain	10% Weight Gain
Values are Mean±SEM of nine experiments. Fisher's p values are shown as $^{a}p < 0.001$ $^{b}p < 0.05$				

Table 2: Levels of blood glucose, glycosylated haemoglobin and the body weight of diabetics and diabetics treated with *Senna* leave extract after one month, 3 months and 6 months.

The first line of the body's defensive enzyme, the glutathione peroxidase, decreased in diabetics by 20-30%, normalised after six months of treatment with *Senna*. The defensive metabolite, the reduced glutathione, which decreased in diabetics by 15-20%, normalised also. The liver function tests performed by the assay of AlaAT and AsAT, were found only a slight increase in AAT in diabetic RBCs which also got normalised after *Senna* treatment. The results have been presented in Table 3.

Parameters	0 day of treatment	One month of treatment	3 months of treatment	6 months of treatment
GPx (U/g Hb)	28.34±2.6^{a}	32.41±3.2^{a}	37.6±3.8^{a}	44.25±2.3^{a}
	Control - 48.16±5.3			
GSH (m mol/L)	1.2±0.05^{a}	1.6±0.07^{a}	1.8±0.06^{a}	2.2±0.08^{b}
	Control - 2.8±0.43			
AsAT (U/g Hb)	1.12±0.04	1.21±0.07	1.18±0.05	1.17±0.07
	Control - 1.09±0.02			
AlaAT (U/g Hb)	1.81±0.04^{b}	1.62±0.06^{b}	1.61±0.04^{b}	1.52±0.07
	Control - 1.41±0.07			
The enzyme activity is expressed as µmol/min/g Hb. Values are Mean±SEM of nine experiments. Fisher's p values are shown as $^{a}p < 0.001$ $^{b}p< 0.05$				

Table 3: Levels of GPx, GSH, AlaAT and AsAT in diabetics and diabetics treated with *Senna* leaves extract after one month, 3 months and 6 months.

Discussion

Senna leaves have been thought to be effective as a laxative for treating constipation. The side effects of this leaves have been defined as acute hepatoxicity, renal impairment and skin breakdown, etc. [16-18]. But as the observations carried out in the treatments, when *Senna* leaves receive moisture and air, the leaves are broken down into pieces with the mucilaginous property. This may be due to rich contents of carbohydrates and proteins which may invite bacteria and fungi to grow fastly and destroy the leave components as observed in the packaging where sennosides yield decreased [10]. Therefore, the powder and tablets forms of *Senna* may not be beneficial due to these infections. Therefore, hot water preparation of *Senna* leaves in our present study may be beneficial in two ways, firstly hot water destroys bacteria and fungus which infected *Senna* leaves and secondly, the diabetics do not take other contents of *Senna* leaves which remained in the leaves itself after boiling in water. In our experiments, *senna* could not develop any type of toxicity in constipated diabetics after six months of treatment observing other diabetic complications as their blood glucose remained between 80-170 mg/mg, a potential range of controlling glucose. But before the treatment, it ranged from 80-240 mg/dl. It may be concluded that in diabetics the glucose level beyond 170 may be injurious and will develop diabetic complications in a long duration of time. Instead, diabetics developed a strong vigour to perform their daily routine work. Precaution should be taken to determine the dose of *Senna* which varies from person to person so that additional effects of *Senna* should not be observed like stomach pain during defecation. *Senna* may be indirectly adding beneficial effects after controlling constipation. In spite of relieving from constipation, *Senna* leaves also corrected the skin infection, liver disorder and hence, increasing the defence system, improving the neurological disorders like legs pain as peripheral neuropathy [1] and physiological disorders like swelling of legs and cheek. This improves the wellness of diabetics with full of vigour and strength. Constipation in diabetes may be more injurious to tissues due to gases formation and holding of stools in the intestine for a longer time [19]. Interestingly, the improvement of raised glycosylated haemoglobin to normalisation was the biggest change in determining the long-term management of diabetes after the treatment.

As reported earlier, the defensive enzyme, the glutathione peroxidase decreases in diabetes along with a decreased level of reduced glutathione [20,21]. In constipated diabetic persons, the decreased levels of GPx and GSH have not been reverted even

after controlling the blood glucose levels by antidiabetic drugs as observed in our present study. The long-term diabetics with constipation represented a low defence mechanism of red blood cells which may lead to weaknesses and other diabetic complications. But the *Senna* leaves treatment to diabetics normalised the GPx and GSH levels indicating that constipation may be generating more free radicals and their products [22] which have been neutralized on the sacrifice of GPx and GSH.

AlaAT activity which was slightly higher in diabetics whereas AsAT level unchanged in red blood cells showing some kind of liver disorder which may not be defined but indigestion observed in constipated diabetics, may be due to liver disorder. The *Senna* treatment to diabetics well defined the proper digestion with proper hungriness showing a healthy liver.

Conclusion

Senna leaves treatment to diabetics not only corrects the defence of red blood cells but also corrects skin infection, liver disorder, neurological disorder (pain in legs), physiological disorder (swelling in legs) and found to be a better alternative for a long-term management of diabetes.

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