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Effect of root extract of *Salacia chinencis* (Linn) on dendritic arborization of frontal cortical neuron in early diabetic young rat experimental model- A preliminary investigation

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ABSTRACT

Neurological problems are the major complications generally recognized during the early childhood Type I Diabetes Mellitus (DM).Which causes potential physio-morphological changes on central nervous system. Its early adverse impact can't be ignored during the childhood cognitive behavior. The recognition of disease and initiation of early treatment can prevent the advanced complications in young diabetic children. The diabetes was induced in 22 days (postnatal) wistar rats by giving intraperitoneal injection of Streptozotocin at a dose of 60mg/kg body weight. After the confirmation of diabetes induction treatment with the alcoholic root extract of *Salacia chinencis* at a dose of 100mg/Kg body weight was started immediately. And it was continued for one month duration. At the end of 30 days treatment schedule the animals were scarified and the brain tissue was collected. The tissue was processed under rapid Golgi staining. Tissue sections of frontal cortical neurons were subjected to Camera Lucida drawings and later it was analyzed by considering the changes in dendritic arborization pattern. The alcoholic root extract of *Salacia chinencis* has shown significant changes in the dendritic arborization pattern in different groups as follows. The analysis of apical intersections has shown that normal control differs with treatment group (p=.014), diabetic control group differs with treatment group (p=.020). And in case of apical branching the normal control differs only with diabetic control (p=.029.) The basal branching points between the normal control differs with diabetic group (p=.013), diabetic control group differ with treatment group (p=.006). And in case of basal intersections none of them are statistically significant to compare.

Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, fat, and protein metabolism, due to relative or absolute deficiency of insulin which translates into impaired glucose utility resulting into hyperglycemia and glucose intolerance. Generally, the type I DM represents the absolute failure in the endocrine part of pancreas. It is seen in young children where the body fails to make use of glucose for its metabolic requirement normally[1]. It is well known fact that hyperglycemia causes multiple life threatening complications like cardiovascular diseases, cerebrovascular disease, renal disease, hypertension, neuropathy etc, in both Insulin Dependent Diabetes Mellitus (IDDM) and Non Insulin Dependent Diabetes Mellitus (NIDDM). The type I a DM is associated with presence of autoimmune antibodies and type I b DM is represents without the presence of autoimmune antibodies[2]. The long term impact of hyperglycemia on the peripheral nerve was well studied, but its effect on the central nervous system is comparatively less stressed in the existing literature. A holistic treatment approach is required to tackle this disease and its associated complications. These are highly appreciated by the principles of herbal medicine mentioned in medical science Ayurveda. It stresses equally on the preventive and curative aspect of number of ailments[3]. The Saptha chakra (*Salacia chinencis* Linn) it is a woody shrub with blackish branches. It is called by different names like Swarnamoola, saptharangi etc. Classical texts like sahasrayoga, Yogamrutam and Chikitsa manjari have quoted the use of this herb in the management of diabetic syndrome[4]. As per the modern botanical classification the herb *Salacia chinencis* Linn, belongs to family *Celastraceae*. It contains compounds like triterpenes, Q,T,U assigned as friedelan – 1,3 dione bearing 24-al, and 24-ol and 24 oic groups respectively[5]. The incidence of childhood diabetes is increasing yearly at the rate of 3-4% world wide and in India it is about 2% of overall incidence of diabetes mellitus[6]. Though at present an effective therapy is available in the field of modern medicine, this rapidly growing disease still needs a holistic, alternative, cost effective and easily approachable therapy to serve the people at large.

Materials and methods: Animals-Young Juvenile inbred 22 days old Wister rats (postnatal) of either sex were procured as per Resolution code, JNMC/ Institutional animal ethical committee (IAEC)/2/1/2008. The rats were housed in the central animal house & they were maintained in 12 hours day and 12 night cycle in a well ventilated rooms by giving standard food pellets and water. Proper care was taken to maintain hygienic environment by the experts.[7]

Drugs- Well grown *Salacia Chinencis* roots were collected from Western Ghats forest near Sirsi taluk of Uttara kannada district, Karnataka state, India. They were authenticated by the scientist and Botanist Dr. Harsha Hegde, from Indian Council of Medical Research (ICMR) Regional Centre Belgaum, India**.** By using the soxhlet extraction procedure first the coarse powder was defatted by using the petroleum ether, later the same was used for extraction by using absolute alcohol. The final extract was

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dried by using the hot water evaporation bath and it was collected in a clean porcelain container and later was stored in a refrigerator**.**

On the 22nd day overnight fasted rats were given intraperitoneal streptozotocin injection at a dose of 60mg/kg body weight. The glucose solution was given for next 48 hours to prevent the drastic adverse hypoglycemic effect. On the fifth day the Fasting blood sugar of rats was assessed by using Optimum Exceed Glucometer. The rats which were having the fasting blood sugar (FBS) between 200-400mg/100 ml were selected under different experimental groups and each experimental group was containing 6 rats [8,9]. The normal control (NC) groups were containing rats without any treatment. The Diabetic control (DC) rats were given with regular food without any treatment**.** Another diabetic rat groups were started with the treatment immediately after the confirmation of diabetes on the 5th day. The early or preventive treatment was started with *salacia chinencis* extract at dose of 100mg/Kg body weight (PCLT). The treatment was continued for 30 days i.e. 27 days (22days old rats+ 5 days diabetic attention period=27 days) + 30 days treatment = total 57 days[10].

At the end of 30 days treatment rats were transcardially perfused and they were sacrificed by using anesthetic ether followed by dissection immediately. The skull was decapitated and brain was collected. The brain was put into 10% Formalin fixative, later the brain tissue was processed for rapid Golgi staining by using the chemicals potassium dichromate and silver nitrate solution. Later the processed brain tissue was subjected to microtome cuttings and the sections were taken at the thickness of 50 microns and later it was mounted.

Cell counting- In each frontal cortical sections 200 micron length area was selected by using ocular micrometer and the coded slides were screened by using light microscope under 40 x objectives. Relatively isolated and randomly selected neurons were considered for counting. Ten sections from each rat were selected for its drawings [11,12]. These neurons were used for dendritic analysis by using template prepared by the standard protocol of camera Lucida drawings **Figure-(1)**[13-15]. After completion of the analysis the data was subjected to statistical analysis to find the significant difference between different experimental groups.

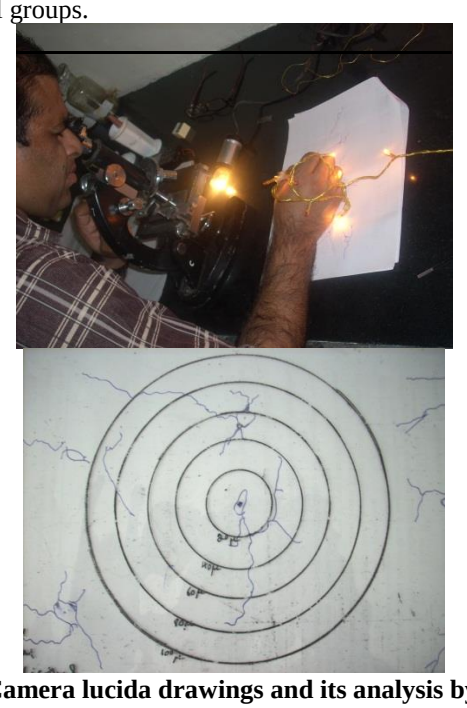

Figure 1. Camera lucida drawings and its analysis by using template

Treatment group: The neurons are showing their fair apical dendritic growth, when compare to basal dendrites **Figure (2).**

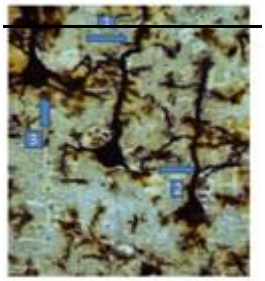

Figure 2. Showing neurons with apical and basal dendrites with enhanced growth, in drug treatment group, under Rapid Golgi stain. Most of the dendrites are also showing small hairy dendritic spines.

Diabetic control group: The neurons were showing stunted growth in both apical and basal dendrites with very short nerve fibers and cell body is small **Figure (3)**.

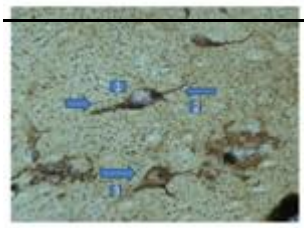

Figure 3. Showing stunted growth of apical and basal dendrites in diabetic control group, under Rapid Golgi stain

Normal control groups: Most of the neurons were seen with well defined dendritic growth pattern seen in its apical part **Figure (4)**.

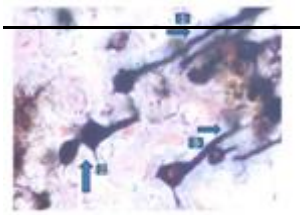

Figure 4. Showing normal pattern of apical and basal dendrites growth in normal control group under Rapid Golgi stain

Statistical analysis-The overall counting of numbers dendritic arborization points including dendritic intersections and branching at different circles were summarized by mean and standard deviation (SD). And the mean of NC, DB and PCLT were compared by using one way ANOVA and p value≤.05 was considered to be significant. To find the group differences Bonferroni multiple comparison tests was performed.

Results

Table 1. Analysis of apical dendritic intersection points

Range	1.Normal control Mean+/-SE	2. Diabetic control Mean+/-SE	3.PSLA Mean+/-SE		
0-20	14.1 +/-3.06	11.5+/-3.27	14.6+/-3.72	F=1.537	p=.247
20-40	5.6+/-2.73	6+/-4.69	9.1+/-3.7	F=1.554	p=.244
40-60	3.6+/-2.5	1.6+/-1.5	5+/-2.6	F=3.304	p=.065
60-80	0+/-0	.1+/-.4	3.5+/-3.14	F=6.970	p=.007
80-100	0+/-0	.1+/-.4	1.3+/-2.16	F=1.966	P=.175
In PSLA Treatment group on analysis of apical dendritic intersection points**,** on Multiple comparison test -1 didn't differ with 2, 1 differs with 3 p=.014, 2 differs with 3 p=.020					

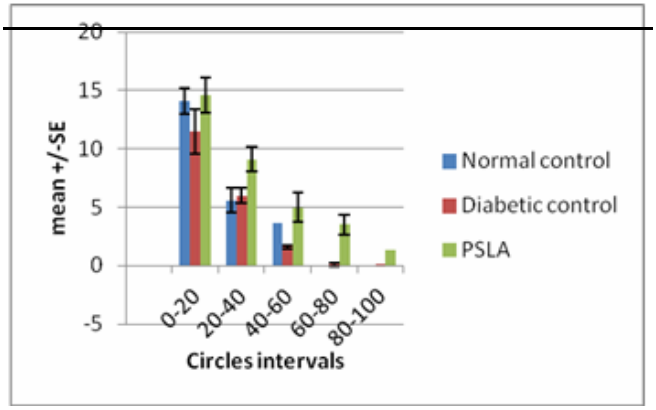

Figure 5. Showing analysis of apical dendritic intersection points

Table-2 Analysis of basal dendritic intersections					
Range	1.Normal control Mean+/-SE	2.Diabetic control Mean+/-SE	3.PSLA Mean+/-SE		
0-20	18.6+/-9.43	10.8+/-4.53	18.7+/-5.08	F=2.717	p=.098
20-40	2.8+/-2.71	.5+/-.54	4+/-3.74	F= 2.638	p=.104
40-60	.5+/-.54	0+/-0	2+/-2.44	F=3.095	p=.075
None of them are significant to compare					

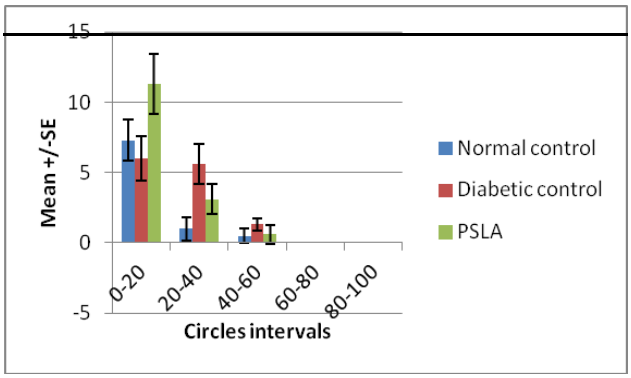

Figure 6. Analysis of basal dendritic intersections

Table 3. Analysis of Apical branching					
Range	1.Normal control Mean+/-SE	2.Diabetic control Mean+/-SE	3.PSLA Mean+/-SE		
0-20	7.3+/- 3.55	6+/-3.94	11.3+/-5.16	F=2.524	p=.114
20-40	1+/-2	5.6+/-3.4	3.1+/-2.56	F=4.376	p=.032
40-60	.5+/-1.22	1.3+/-1.03	.6+/-1.63	F=.669	p=.527
In case of PSLA treatment group Analysis of Apical branching on multiple comparison test 1 differs with 2 p=.029,1 didn't differ with 3 p=.570,2 didn't differ with 3 p=.402					

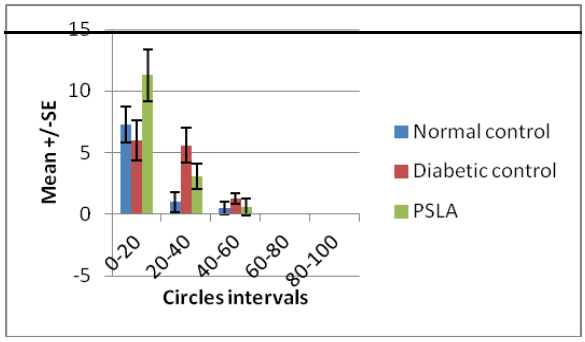

Figure 7. Analysis of apical branching

Table 4. Basal branching points					
Range	1.Normal control Mean+/-SE	2.Diabetic control Mean+/-SE	3.PSLA Mean+/-SE		
0-20	16+/-9.09	.3+/-.81	17.6+/-10.61	F=8.392	p=.004
20-40	2+/-3.63	0+/-0	3.6+/-8.04	F=.779	p=.477
In case of PSLA treatment group- The Basal branching points on multiple comparison- 1 differs with 2 p=.013,1 didn't differs with 3 p=1, 2 differs with 3 p=.006					

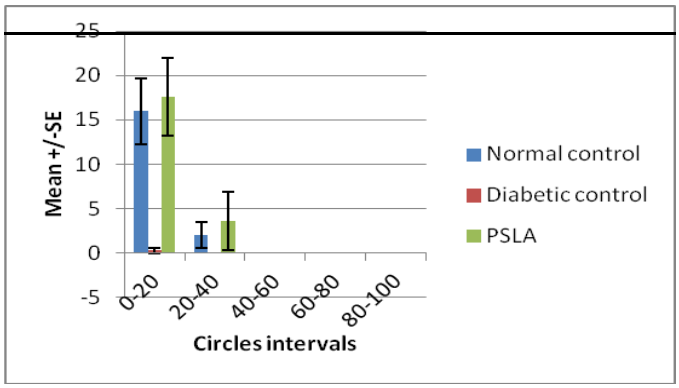

Figure 8. Analysis of Basal branching points

Discussions: Many factors are influencing the present generation. Where the shift in the tendency and incidence of diabetes is targeting the younger age groups when compared to adults. This influence is causing drastic impact which needs immediate attention towards young diabetic population where the brain becomes target of excess glucose metabolism. The same in adults can also lead to a number of adverse effects on the body from simple neuropathy to encephalopathy. It has been debated during the last decades whether asymptomatic, unrecognized diabetes or even a lesser degree of hyperglycemia in random samples shows the increased risk among the people of present population.Even though an effective modern therapy exists the contribution of alternative medicine for the disease free life is substantial and relevant, even by today. The advancement in the science and technology stands as a complimentary to carry out the research in a diversified manner where we can prove the rationality of traditional use of number of herbs as medicines. Generally the screening of herbal drug trial starts with the crude extract administration in animals & it is a base to continue the research further.

The *"Salacia"* species herb is a well known for its antidiabetic activity where one of its members ***Salacia chinencis.*** It is is an important drug used in many herbal medicinal formulations. Our experiment includes herbal root extract of *Salacia chinencis* used after its complete defating by using the petroleum ether. This defating process might have brought its influence on the efficacy of extracts by preventing undue interference of fat component present in the root part of this herb. Its advantage can be correlated by observing its influence on dendritic arborization changes in different groups.

The normal control group showed mild normal enhancement in growing pattern of dendritic arborization in all aspects. It probable represents regular growth enhancement happens normally. But in case of diabetic controls the same observations are witnessed with total stunted growth. This could be due to interference of excess load of glucose and its load handling effect on young developing neurons. In case of treatment group the apical and basal dendritic fibers have shown number of branching with considerable increase in their length. This observation supporting the use of this herb in diabetic

syndrome. But, here we are not able to co-relate the positive dendritic changes in treatment group in any way to its direct or indirect action on the glucose metabolism. These enhancing dendritic branching points may be connected with other layers of the cortical neurons. Such intensified arborization and increased synaptic contacts may influence higher functions of the brain. Such positive effects of this herbal root extract may influence the cognitive functions in many ways by enhancing the number of new synaptic contacts or by increased production of neurotransmitters or increased number of nerve growth factors etc. This influence is also could be due to effective control of excessively circulating glucose by reducing the undue glucose metabolic load on the brain cells. These factors directly or indirectly support the cognitive behavior in an individual by supporting stress free metabolic environment. This preliminary research is a base, which is answering the rationality of traditional use of herbal root extract of *Salacia chinencis* in diabetes. By observing its encouraging results in our experiment this herbal drug can be pursued for advanced research.

Conclusion- The herbs are natural, cheap and easily available sources. They are used by the human beings since thousands of years. Life on earth blends with close interaction with the nature & it has conceived a strong base for evolution of different systems of medical science around the world. By considering the limitation and side effects of modern medicine, the scope for the alternative medicine research should be stressed with respect to preventive and curative aspect of many prevailing diseases of present population. Though this experiment needs further detailed drug evaluation on its mechanism of action, but the outcome of present experiment has shown the relevance of using alcoholic root extract of *Salacia chinencis* in early childhood diabetes mellitus.

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