



Research Article

EXPERIMENTAL PHARMACOLOGICAL STUDY OF *SAPTACAKRA- SALACIA RETICULATA* WIGHT. AND *PUGA - ARECA CATECHU* LINN. (*ASHODITA AND SUDDHA*) IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

In present era of science, facts established by proofs derived after careful investigations, observations and experiments and supported by accurate data can convince the people about validity of the statement. Modern medical science achieved tremendous advance in past few centuries with the help of experimental research. An experimental pharmacological study of *Saptacakra* and *Puga* (*Ashodita* and *Asuddha*) in alloxan (120 mg/kg) induced diabetic rats was done at Pharmacology Laboratory of Sree Vidyanikkethan Pharmacy College, Tirupati. The diabetic rats were divided into six groups [Control group (NC), Diabetic control group (*ALX*), *Ashodita Puga* group (*ALX* + *ACP*), *Saptacakra* group (*ALX* + *SRP*), *Suddha Puga* group (*ALX* + *BACP*) and Combination of *Saptackara* and *Suddha Puga* group (*ALX* + *SRBACP*)] with 6 animals in each groups and treated with 450 mg/kg of respective drugs. Blood sugar level and the serum lipid profiles such as total cholesterol, Phospholipids, triglycerides, LDL, V-LDL and HDL investigations and histopathological studies on liver and pancreases tissues were carried out in normal and diabetic rats.

The combination of both *Saptacakra* and *Suddha Puga* (*SRBACP*) shows 47.02% decrease in blood sugar level. It is extremely significant ($p < 0.001$), though all the other groups show reduction in blood sugar level. Reduction in lipid profile was best noticed in *BACP* group; in particular reduction in triglycerides and improvement HDL was in combination group. Hepatoprotective action and regeneration of islets of langherans was best seen in combination group.

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INTRODUCTION

The experimental research are done on lower animals to study normal and abnormal conditions and for artificially producing diseases and then to study the effect of trial drugs, natural course of the disease, changes produced in the organs and tissues, etc. as a result of the actions of etiological factor or the action of the trial drug.

Pharmacology is a subsidiary subject of modern medicine. It deals with the action of drugs on the body. Dynamics of drug action is it's another name. That is why it has got a synonym "Pharmacodynamics". Special feature of basic pharmacology is that most of its studies are based on animal experiments. The ultimate object of course is to make an attempt to correlate the results obtained in basic pharmacological study with

therapeutics or remedial measure employed in the treatment of disease.

In the recent few years *Saptacakra* and *Puga* has drawn the attention of scientist on various activities in human body especially in the field of Diabetes Mellitus i.e., *Madhumeha*. So it was thought to do comprehensive literary study of these drugs in *Madhumeha* (Diabetes Mellitus). The literary review of the *Puga* and *Saptacakra* was started right from the Vedas up to recent research works to obtain thorough knowledge of drug. The literary reviews of the drugs are mentioned below.

Saptacakra is the main ingredient in *Yogas* like '*Nisakatakadikvatha*, *Katakakhadiradi kvatha*,

Niryadigitika etc., which are indicated in *Madhumeha*^[1, 2, 3],

Recent research studies have reported that it has Antihyperglycemic, antioxidant activity^[4], hypolipidemic, hepatoprotective^[5] and cholinergic activity (Contraction of uterus). While going through article by name 'Anti diabetic activity of roots of *Salacia macrosperma*', by Venkateswarulu et.al. of Kakathiya University, showed that the methanolic fractions followed by the residual fractions of alcoholic extracts exhibited significant anti-diabetic activity^[6].

The second drug *Puga* is mentioned in Vedas by the name *Kramuka* and in ancient *Ayurvedic* literature viz. *Brihatrayi*, *Laghutrayi*. There are many references in *Ayurvedic* literature where *Puga* is prescribed in *Prameha*, like

- *Susruta* in *Prameha Chikista* advised to use *Kadhira- Kramuka kasaya* in the treatment of *Madhumeha*^[7].
- In *Bhaishajya Ratnavali Kadara-Khadira-Puga Kvatha* is advised in *Kshoudrameha*^[8] and *Cakradatta* in *Prameha rogadhikra*^[9]
- *Yogaratanara* mentioned *Pugapaka* in *Prameharogadhikara*^[10].
- *Puga* is mentioned in *Salasaradi gana* of *Susruta Samhita* and *Asanadi gana* of *Astanga Hridayam*, these are *Ganas* are indicated in *Prameha* and have *Medo-Kaphahara karma* as *Kapha* and *Meda* are the causative factors of *Madhumeha*^[11,12].

Here the present study is undertaken to provide pharmacological basis and to support the clinical evidences and also to compare the efficacy of *Saptacakra* and *Puga* for pharmacological profiles and to ascertain whether administration of both *Saptacakra* and *Puga* in purified and raw form, singly or in combinations produces any adverse effects or not.

Aim and objectives

1. This study attempts to justify therapeutic application of the drugs used in AYUSH on the basis of efficacy and safety evaluation as per national and international norms.
2. To study the effect of trail drugs (*Saptacakra* and *Puga*) on blood sugar level and histopathological studies on Alloxan induced Diabetic Winstar strain albino rats.
3. To assures the efficacy of both *Suddha Puga* and *Ashodita Puga* as anti diabetic.

MATERIAL AND METHODS

A) Instruments used

One touch Glucometer with Ez Smart strips, weighing scale, needle, syringe, Rat Feeding Needles (No.18 & 20), mono pan balance, oral feeding needle, rubber catheter, mortar & pestle, surgical instruments,

refrigerator, sterilizer, pipette, glass slides and watch glass.

B) The trail drugs

The trail drugs taken for the studies are

1. *Ashodita Puga*
2. *Suddha Puga*
3. *Saptacakra*

Sharangadhara mentioned *Kramuka* as an example of *Vikasi guna* in the *karma Paribhasha* chapter^[13]. After going through the different *Nighantus*, it was found that *Vikasi, Ojonashaka* property seen only in *Apakva- Adrapuga*^[14,15] as it said to be *Visha samana*. *Acharyas* have indicated use of *Puga* in *Madhumeha*, which is one of the *Vataja prameha* manifestation due to *Shaithilya abaddhatva* of *Dhatus* and *Ojas kshaya*.

In *Bhavaprakash Nighantu* mentioned that *Puga* should be boiled in "*Choughar*"^[16] (*Decoction of Jambupatra and bark, Manjista Khadira sara, Raktachadana, Guda and Eranda taila*) for *Shodhana* purpose. Most of the drugs used for *Shodhana* of *Puga* are of *Kasaya rasa* and *Sita virya*, which will pacify the *Vikasi guna* of *Puga* and potentiate the *Puga gunas* for the treatment of *Madhumeha*. So the *Puga* was purified in *Choughar* before using for treatment.

The raw form of trail drugs *Saptacakra* and *Puga* were taken for the studies and were pounded to powder form in the department of *Dravyaguna*. *Ashuddha Puga* was purified in department of *Rasashastra*, T.T.D's S.V. Ayurvedic College, Tirupati.

C) Dose Selection^[17]

The dose selection plays vital role in animal studies, as it may sometimes increase mortality rate of rats.

Rat dose- 450 mg/kg was fixed

Here Rat dose = Human dose x 0.018 for 200gm rat weight.

i.e. 5 gm x 0.018 = 0.09 gm or 90mg.

Conversion to dose/kg body wt. = 90 X 5 = 450 mg/kg

D) Preparation of drug for rat dose

10 gms. of trail drug mixed in 100 ml of water and left over one night and next day morning it is filtered. From the filtrate 4.5 ml of aqueous solution is given to each rat (4.5 ml = 450 mg).

E) Route of Drug Administration

The trail drugs and vehicle to control were administered according to the body weight of the animals by oral route with the help of oral feeding needle.

F) Animals

Winstar strain albino rats of either sex, weighing between 170 – 280 g were obtained from the animal house attached to the Pharmacology Laboratory of Sree

Vidyanikkethan Pharmacy College, Tirupati. They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature and humidity and fed with *ad libitum* of Amrut brand rat pellet feed supplied by Nava Maharashtra Chakan oil mills and tap water.

EFFECT OF TRAIL DRUG IN THE ALLOXAN INDUCED DIABETIC RATS

a). Induction of Diabetes: Winstar Rats were taken for the study and weighed, then alloxan monohydrate (Loba Chemie, Bombay) mixed in normal saline at the dose of 120 mg/kg was administered I.P. twice at the

interval of 48 hrs to overnight fasted animals. After 96 hrs of the 1st dose of alloxan, the glucose level was estimated. The rats with blood glucose level ranging between 250 mg/dl to 600 mg/dl were selected for the experimental study.

b). Grouping: The diabetic rats were weighed, divided into five groups with 6 animals in each groups. The six animals were taken in each group to avoid death of animals after inducing diabetes by alloxan as it has high mortality rate (at least 3 to 4 rats may live, if remaining die).

Group I:	Control group (NC) was given tap water only
Group II :	Diabetic control group (ALX) Diabetic induced rats by alloxan Dose - 5 ml/kg.
Group III :	Ashodita Puga group (ALX + ACP) The aqueous solution of <i>Ashodita Puga curna</i> was given to alloxan induced diabetic rats. Dose - 450 mg/kg.
Group VI :	Saptacakra group (ALX + SRP) The aqueous solution of <i>Saptacakra curna</i> was given to alloxan induced diabetic rats. Dose - 450 mg/kg.
Group V:	Suddha Puga group (ALX + BACP) The aqueous solution of <i>Suddha Puga</i> was given to alloxan induced diabetic rats. Dose - 450 mg/kg.
Group VI :	Combination of Saptackara and Suddha Puga group (ALX + SRBACP) The aqueous solution of Combination of <i>Saptackara</i> and <i>Suddha Puga curna</i> was given to alloxan induced diabetic rats. Does - 450 mg/kg

c). Procedure: Before one hour of administration of the drugs, the initial readings were taken by puncturing retro-orbital plexus under ether anaesthesia or cutting tail. After 15 days of treatment the blood glucose level were measured by collecting blood from tail or by puncturing retro orbital plexus method. The blood glucose level was measured by using one touch glucometer.

BIOCHEMICAL ESTIMATIONS

Blood sugar level and the serum lipid profiles such as total cholesterol, Phospholipids, triglycerides, LDL, V-LDL and HDL (Folch and Dunn, 1973; Burstein and Scholnick, 1972), investigations were carried out in

normal and diabetic rats in the Bio-chemistry laboratory of Sree Vidhyanikethan, Tirupati.

STATISTICAL ANALYSIS

All quantitative measurements were expressed as means ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC (statistical package for social sciences, personal computer) and the group means were compared by Duncan’s Multiple Range Test (DMRT). The results were considered statistically significant if the p value was ≤ 0.05.

OBSERVATIONS AND RESULTS

Table 1: Effect of trail drugs on blood sugar level in all six groups

Group	Dose /kg.	Blood sugar (mg/dl) Mean ± SEM		% of change
		B.T	A.T	
Diabetic control	5 ml	289.50 ± 58.74	334.83 ± 70.34	15.65 ↑
ALX + ACP	450 mg/ kg	278.16 ± 47.72	248.33 ± 23.94	12.01 ↓
ALX+ SRP	450 mg/kg	269.80 ± 32.18	221.40 ± 53.00	17.93** ↓
ALX+ BACP	450 mg/ kg	395.20 ± 82.52	329.34 ± 105.16	16.66* ↓
ALX+ SRBACP	450 mg/ kg	423.60 ± 83.53	224.40 ± 53.39	47.02***↓

* P<0.05 ** P<0.01 *** P<0.001

Table 2: Effect of trail drugs on serum lipid profiles in ALX-induced diabetic and control rats

Groups	Total cholesterol (mg/dl)	Phospho-lipid (mg/dl)	Tri-glycerides (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Diabetic control	149.76± 14.56 ^a	196.75± 20.35 ^a	124.66± 11.83 ^a	16.76± 1.90 ^a	23.43± 0.22 ^a	97.76± 0.94 ^a
ALX+ACP	89.78 ± 8.18 ^{b,*}	117.67± 11.91 ^{b,*}	63.22 ± 5.98 ^{b,*}	35.08 ± 3.78 ^{b,*}	16.07 ± 0.17 ^{b,*}	49.17 ± 0.46 ^{b,*}
ALX+ SRP	112.45± 11.76 ^{b,**}	149.09± 14.53 ^{b,**}	77.44± 6.99 ^{b,*}	27.73± 2.97 ^{b,*}	19.24± 0.21 ^{b,*}	71.16± 0.71 ^{b,*}
ALX+ BACP	82.45± 11.76 ^{b,*}	117.09± 14.53 ^{b,*}	62.44± 6.99 ^{b,*}	36.89± 2.97 ^{b,*}	15.23± 0.21 ^{b,*}	46.58± 0.71 ^{b,*}
ALX+ SRBACP	83.76 ± 8.56	118.52 ± 10.77	61.56± 7.12	36.98 ± 3.87	15.94 ± 0.13	48.32 ± 0.43

Values are mean ± SEM, n=6; ALX-^a Alloxan induced diabetic group vs normal group, ^b trail drug treated group vs Alloxan induced diabetic group, #p <0.001; *p <0.05; **p <0.001.

Assessment of hypoglycaemic activity by GTT in normal healthy rats:

Glucose tolerance test is a standard procedure that addresses how quickly exogenous glucose can be cleared from blood. Specifically, uptake of glucose from the blood by cells is regulated by insulin. Impairment of glucose tolerance (i.e., longer time to clear given amount of glucose) indicates problems with maintenance of glucose homeostasis (insulin resistance, carbohydrate metabolism, diabetes, etc).

Thirty six normal rats fasted overnight were divided in to six groups of six rats each were same as

mentioned earlier expect that rats here are not induced Diabetes.

Group III, IV, V, VI received single oral doses extract of drug powders suspended in distilled water. The blood samples were collected from the tail vein of these groups and blood glucose levels were estimated after 120 min of treatment. The Blood glucose level value at 120 min was treated as '0'min value for GTT.

The animals were then orally administrated with 2 g/kg of glucose and their glucose tolerance was studied at 15 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively, blood collected from the tail vein. The values are noted and statistically analysed.

Table 3: Effect of trail drugs on oral glucose tolerance test (GTT) in normal rats

Groups	Serum glucose levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Control	140.00 ± 17.43 a #	184± 1.71 a#	258.60 ± 45.38 a#	156.60 ± 18.43a#	149.60± 6.53a#
Normal + glucose (2 g/kg)	82.64 ± 1.78	241.86± 1.35	184.46 ± 1.71	154.57 ± 1.89	90.34 ± 1.49
ACP + glucose (2 g/kg)	83.68 ± 1.57	180.25± 2.41 b *	152.46 ± 2.47 b *	133.86 ± 1.42 b *	91.78 ± 1.28
SRP + glucose (2 g/kg)	84.63 ± 1.83	161.65 ± 1.47 b **	144.33 ± 7.62 b **	124.68 ± 1.49 b **	86.29 ± 1.88
BACP + glucose (2 g/kg)	83.29 ± 1.95	155.93 ± 2.81 b **	148.06 ± 3.84 b **	110.44 ± 2.53 b **	88.26 ± 1.98
SRBACP + glucose (2 g/kg)	82.78 ± 1.22	173.25 ± 1.47 b **	151.49 ± 0.89 b **	131.26 ± 0.89 b **	87.59 ± 1.26

Values are given as means ± SEM for 6 rats in each group. a normal glucose group vs normal group,; b trail drug treated group vs normal glucose group, #p <0.001 *p <0.05; **p <0.001

Effect of trail drugs on histological findings of liver and pancreatic tissues

The effect of the trail drugs on tissues of the Liver and pancreas of normal and diabetic rats were evaluated by histological studies of tissue sections obtained from the animals. On day 15 of the experiment, one animal was randomly selected from the different groups and sacrificed by over-dose of ether anaesthesia. The whole Liver and pancreas from each animal was removed and placed in 10% formalin

in normal saline for histological studies and they were was placed in an automatic tissue processor for 24 hrs.

After 24 hrs, the tissues were solidified in molten wax and sectioned using automatic tissue sectioner. These tissue sections were then fixed on slides with haematoxylin and eosin and stained slides were fixed with mount, allowed to dry and viewed under the microscope (x400).

Table 4: Histopathological Changes in Liver

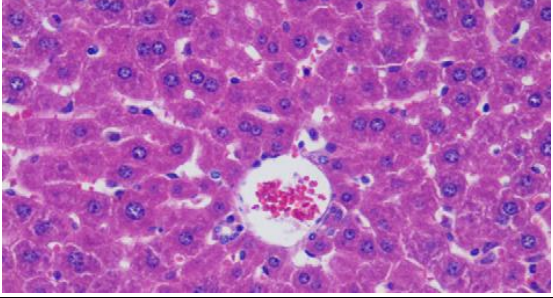
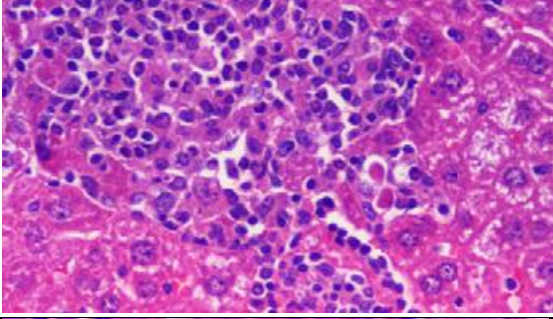
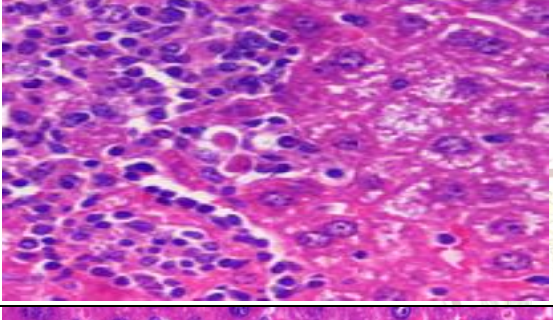
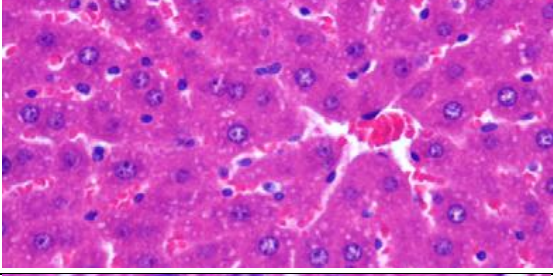
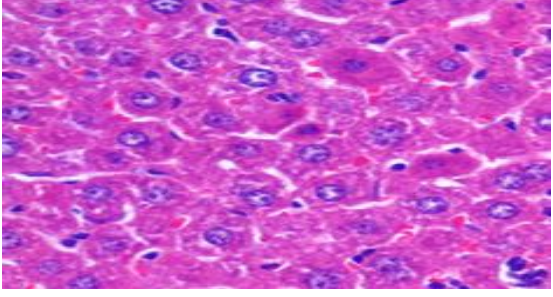
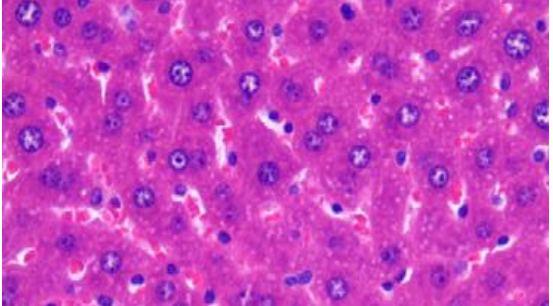
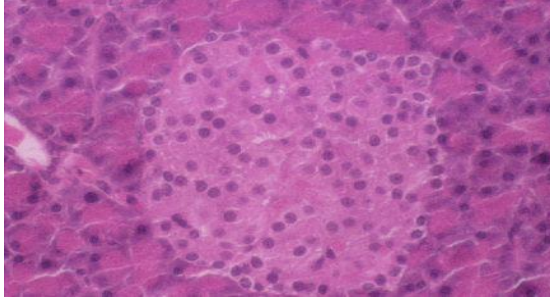
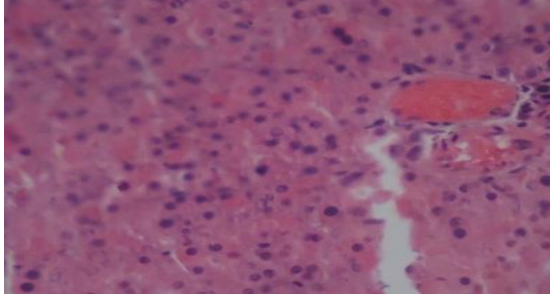
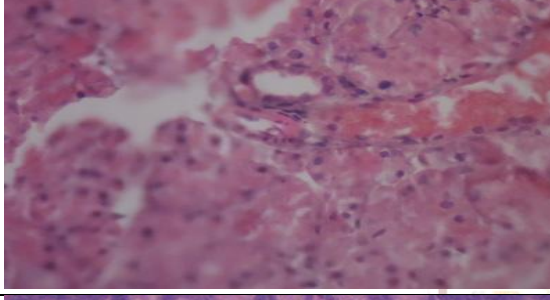
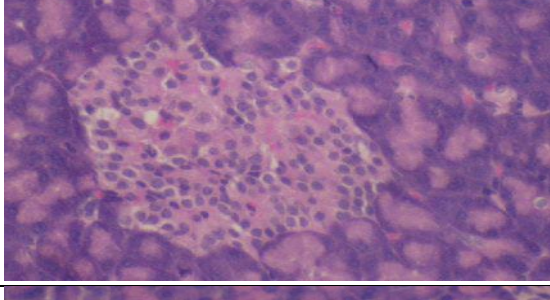
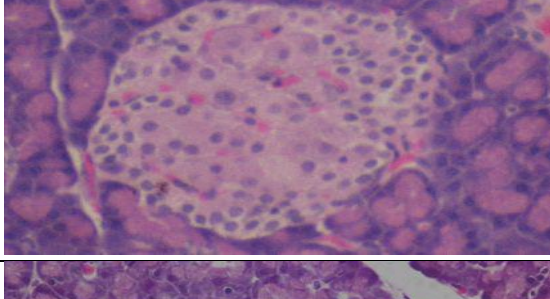
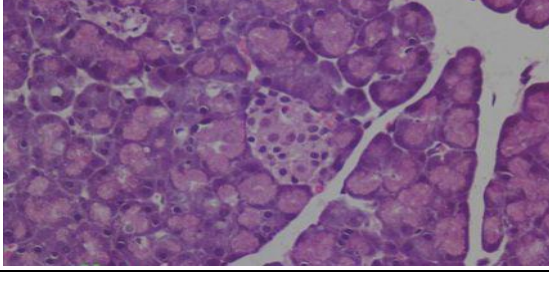
	<p>Control Group Histopathologically, liver structure and especially hepatocytes were in normal appearance and any degenerative, steatotic, fibrotic and inflammatory changes were not observed.</p>
	<p>Diabetic control Group Degenerative liver changes, characterized with very small intra cytoplasmic fat vacuoles at the periacinar and centrilobular areas, in various degrees were observed in alloxan treated group.</p>
	<p>ACP Group Degenerative liver changes, characterized with very small intra cytoplasmic fat vacuoles at the periacinar and centrilobular areas better than alloxan group.</p>
	<p>BACP Group In experimental groups, best liver conditions were seen in Ayurvedic formulation, with marked regeneration in mitochondria, nucleus and cytoplasmic structure in hepatocytes.</p>
	<p>SRP Group In experimental groups, best liver conditions were seen in Ayurvedic formulation. The mitochondrial structure in hepatocytes here seen normal.</p>
	<p>SRBACP Group In experimental groups, best liver conditions were seen in Ayurvedic formulation, with marked regeneration in mitochondria, nucleus and cytoplasmic structure in hepatocytes.</p>

Table 5: Histopathological Changes in Pancreas

	<p>Control Group The histology of pancreatic islet cells was normal in control group</p>
	<p>Diabetic Control Group In diabetic control group Histological examination of the pancreas shows the necrosis of the islet tissues with the alveolar cells moderately destroyed; there was also moderate congestion of the blood vessels.</p>
	<p>ACP Group In the group treated with ACP (450mg/kg.), the tissues of the pancreas appeared intact. The interlobular, intralobular and the alveolar granules were seen.</p>
	<p>BACP Group Group treatment with individual extracts of BACP (450mg/kg) showed initial regeneration of islets of langherans.</p>
	<p>SRP Group Group treatment with individual extracts of SRP (450mg/kg) showed initial regeneration of islets of langherans.</p>
	<p>SRBACP Group Group treatment with composite extracts of SRBACP (450mg/kg) in diabetic rats showed normal acini and islets that were comparable to that of normal control.</p>

CONCLUSION

The values mentioned under the word BT indicates initial blood sugar level after the injection of alloxan was given, the values increased 3 to 4 times than the normal blood sugar level. In control diabetic rats, after fifteen days of vehicle treatment, 15.65% increase in blood sugar level was observed. (Table- 1)

In *Ashodita Puga* group (ACP) 12.01% decrease in blood sugar was observed, it is not significant ($p > 0.05$), whereas in *Suddha Puga* group (BACP) 16.66% lowering of blood sugar level was observed which is significant ($p < 0.05$) and *Saptacakra* group (SRP) 17.93% decrease which is very significant ($p < 0.01$). In combination of both *Saptacakra* and *Suddha Puga* (SRBACP) 47.02% decrease in blood sugar level was observed, it is extremely significant ($p < 0.001$), though all the other groups show reduction in blood sugar level. Reduction in lipid profile was best noticed in BACP group; in particular reduction in triglycerides and improvement HDL was in combination group. (Table No- 1 and 2)

In oral glucose tolerance test, after 120 mins. the glucose level was reduced in all the groups, best seen in *Saptacakra* group (SRP) (Table No- 3). Best hepato protective action was observed in combination group, though *Puga* group also showed hepato protective activity and regeneration of islets of langherans was best seen in combination group. (Table No- 4 and 5)

Pharmacological experiments are always based on animal organs or tissues. As such it is not expected to find these experimental findings correlating with human therapeutics in exact manner. The action of a drug varies to a great extent on different animals becomes very apparent from the article "The evaluation of new drugs" by Goodwin and Rose (Vide Pharma. Jour. Sept. 27 1951, Page 223). They said "It is hardly necessary to be reminded of the classical example of the rabbit, which can live on belladonna leaves, because it is insensitive to the poisonous effect of atropine". They further say "It would not be satisfactory to use the rabbit alone as a guide to the safety of belladonna alkaloids in man". Notwithstanding the above observation - basic pharmacology has important place in the study of drug.

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Photographs of Drugs



Figure 1: *Areca catechu* plant



Figure 2: *Saptacakra* plant



Figure 3: *Puga* (Seeds of *Areca catechu* Linn.)



Figure 4: Roots of *Saptacakra*



Figure 4: *Suddha Puga Curnam*



Figure 5: *Saptacakra Curnam*