Study No : ITC/ PC/ HBCS/17/02	Test Item: Herbally Blended cane Sugar

STUDY REPORT

EFFICACY STUDY OF HERBAL BLENDED CANE SUGAR (HBCS) IN ALLOXAN INDUCED DIABETIC RATS

STUDY NO: ITC/PC/BS/17/02

(Version 00)

TEST FACILITY	SPONSOR
Biological & Preclinical Lab **Interstellar testing centre pvt. ltd** Plot No: 86 Phase I, Industrial Area Panchkula Haryana Contact No. 0172-2561543, 2565825	**Dr.C.K Nandgopalan** Inventor & Director R&D **Natural Life Speciality Pvt Ltd** Ragvilas Society, Lane C, North Main Road, Koregaon Park, Pune-1; Maharastra, India

Study No : ITC/ PC/ HBCS/17/02	Test Item: Herbally Blended cane Sugar

QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled "**EFFICACY STUDY OF HERBAL BLENDED CANE SUGAR (HBCS) IN ALLOXAN INDUCED DIABETIC RATS"** has been audited and examined with respect to the research base protocol. The report provides true and accurate record of results obtained.

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Phases of Study	Date of Inspection	Date of Reporting
Protocol	04-09-2017	05-09-2017
Randomization of animal	22-09-2017	30-09-2017
Dosing of animals	01-10-2017	21-10-2017
Report	24-10-2017	26-10-2017

Quality Head

QA (HEAD)

Dr. Parkash Kaur

Date: 27-10-2017

Study No : ITC/ PC/ HBCS/17/02	Test Item: Herbally Blended cane Sugar

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled **"EFFICACY STUDY OF HERBAL BLENDED CANE SUGAR (HBCS) IN ALLOXAN INDUCED DIABETIC RATS"** has been audited and examined with respect to the research base protocol. The report provides true and accurate record of results obtained.

All the data /results reflect in the report are true as per obtained in the present study.

Study Director

STUDY DIRECTOR

Dr. Vivek Kumar Dwivedi

Date: 27.10.2017

Test Facility Management

TEST FACILITY MANAGEMENT (TF)

Mr. Anataryami Nayak

Date: 27.10.2017

Study No : ITC/ PC/ HBCS/17/02	Test Item: Herbally Blended cane Sugar

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ABBREVIATION USED

Abbreviations	Description
g	gram
Kg	Kilo gram
b.w	Body weight
No	Number
%	percentage
DRS	Data recording sheet
mg	Milligram
ml	Millilitre
HBCS	Herbally Blended cane Sugar
p.o	Oral route
W/V	Weight/Volume
SD	Study Director
QA	Quality Assurance
TFM	Test Facility Management
BBDU	Babu Banarsi Das University
SD	Standard Deviation
SGOT	Serum glutamate oxalo transminase
TG & TC	Triglyceride & Total Cholesterol
SGPT	Serum glutamate pyrotransminase

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SUMMARY OF STUDY

The objective of present study was to evaluate the efficacy study of Herbelly blended Cane sugar in alloxan induced diabetes rats. Total 40 male Sprague dawley rats were used in the present study. Out of 40 animals, seven animals transferred into other cage and serve as control group while 33 animals were used for diabetes induction. The diabetes was induced in animal *via* intraperitoneal route at dose level 150mg/kg. Diabetes was confirmed by more than 200 mg/dl blood glucose level. Out of 33 animal, 19 animals were showed raised blood glucose level used for diabetes model. Total 26 animals were used in the study. Group I (n=7) was control whereas group II(n=7) was diabetes induced and group III& Group IV animal (n=6) were treated with HBCS and NS treated groups for 21 days through oral route. The results of present investigation showed that there were gradually increased body weight and feed intake level in all groups from initial to 21 days treatment. These parameters were found reduced in diabetes group when compared to control group. No any mortality and clinical sign of test item related toxicity were found in treated group. The serum hepatic enzymes and lipid profile were found high in diabetes treated group as compared with control group. After treatment with respective test item, there biochemical parameters were improved in HBCS treated group as compared with diabetes induced group and normal sugar (NS) treated group and come almost near to control group. The serum insulin level was also significantly ($P<0.0001$) in HBCS treated group in comparison to NS treated group. These above parameters were found statistically significant in HBCS treated group than NS treated group. So conclusion of this study that HBCS test item is most effective for improvement of hepatic problem along with improve the lipid profile level and manage the insulin level during diabetes conduction .

Study No : ITC/ PC/ HBCS/17/02	Test Item: Herbally Blended cane Sugar

1. **STUDY DETAILS**		
Study Title	:	**Efficacy Study of Herbal Blended Cane Sugar (HBCS) in Alloxan Induced Diabetic rats**
Study No.	:	**ITC/ PC/ HBCS/17/02**
Test Item	:	Herbally blended Cane Sugar (HBCS)
Sponsor Representative	:	Mr. Ajay K P George
Duration of Study	:	35 Days
Sponsor	:	Natural Life Speciality Pvt Ltd Ragvilas Society, Lane C, North Main Road, Koregaon Park, Pune-1; Maharastra, India
Study Director	:	**Dr. Vivek Kumar Dwivedi**
Study Scientists	:	**1.** Mr. Amit Sharma (M.Sc.) **2.** Ms. Jaspreet Kaur (M.Sc)
STUDY SCHEDULES	:	
Study Initiation Date	:	22-09-2017
Experiment Start Date	:	01-10-2017
Experiment Completion Date	:	22-10-2017
Study Completion Date	:	26-10-2017

2. STUDY PERSONNEL

The following personnel were participated in the conduct of the study

Name	Responsibility	Signature and Date
Dr. Vivek Kumar Dwivedi	Over all In-charge for planning , conduct the experiment and check the data recording sheet and report preparation of the study.	26.10.2017
Mr. Amit Sharma	Assist in the study	26-10-2017
Ms. Jaspreet Kaur	Compile the data and statistical report preparation	26.10.17

3. OBJECTIVE: To evaluate the efficacy study of Herbal Blended Cane Sugar (HBCS) in Alloxan Induced Diabetic rats

4. SAFETY PRECAUTIONS

Personal protection equipments like gloves, masks, aprons, footwear were used as per requirement while handling the test item and test system.

5.0 MATERIALS AND METHODS

TEST ITEM

5.1 Test item information

Name of test item :	Alloxan Monohydrate
Description :	White Crystalline powder turn to pink
Strength :	25g
Batch No.:	70312
Manufacture Date :	01-03-2016
Expiry Date:	31-02-2019

5.2 Test item information (HBCS)

Name of test item :	Herbally Blended Cane Sugar(HBCS)
Description :	White granule cane sugar
Strength :	1 Kg.
Lot No.:	2
Manufacture Date :	26-07-2017
Expiry Date:	25-07-2019

5.3 Test item information (Normal Sugar)

Name of test item :	Normal sugar
Description :	White granule cane sugar
Strength :	1 Kg.
Lot No.:	4/1
Manufacture Date :	07-09-2017
Expiry Date:	06-09-2017

5.4Test item Analysis

Analysis for the identity and purity of the test item to be not conducted as part of this study. It is responsibility of sponsor.

6.0 TEST SYSTEM

Species	*Rattus norvegicus*
Strain	*Albino rats*
Source	Animal House, BBD
Sex	Male
Number of Animals	10 animals X 4 groups = 40
Number of Animals/ Cage	10 animals / cage
Acclimatization	5-7 days
Identification of Animals	**Pre randomization :** Animals were Tail marking and **Post randomization :** The animal was identified by Ear marking, **Cage Labeling :** Label the cage with study details
Randomization	Animals were randomized on the basis of body weight

7.0 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

Adult male Sex Sprague dawley rats was selected and used as the Test System as it is commonly reported in various scientific literature [1].

8.0 ANIMAL HUSBANDRY

Test Room	Experimental Room No.2
Temperature	22±3°C
Relative humidity	30 to 70%
Housing	Standard polypropylene cages with stainless steel top grill supplied by M/s Vishnu Taders, Lucknow, Uttar Pradesh, India was used to house the animals. The cages were washed, clean paddy husk was used as the bedding material.
Sanitization	Bedding material was changed on alternate days
Light/ Dark cycle	12 hour
No. of Animals Per Cage	6-7 animal each in 4 cages
Feed & Water	Standard pelleted feed was supplied by M/s Ashirwad Food Industries,Ropar, Punjab. Filtered water was provided *ad libitum*.

9.0 PREPARATION OF TEST ITEMS

Test item/ material hebally blended cane sugar (HBCS) and normal sugar were white clean granular form and calculated amount of test item "HBCS" and normal sugar were used according to individual animal body weight administered to animals *via* oral route (p.o). The test item was administered to each animal at the dose level of 10 ml/kg body weight whereas control group was received normal distilled water at the same dose lvel.

10.0 JUSTIFICATION FOR SELECTION OF ROUTE OF ADMINISTRATION

Oral route was the selected for test item as suggested by the sponsor on the basis of intended use of formulation.

11.0 DESCRIPTION OF TEST PROCEDURE

In the present study, total 40 male Sprague dawley (SD) rats with weighing (130-135g) were used. All animals were acclimatized Standard animal house environment for at least 5 to 7 days before initiation of experiment. After the acclimatization, animals were randomized into groups on the basis of body weight in each cage. After randomization, Seven animals were transferred into other cage which serve as control group (G1) whereas rest 33 animals were overnight fasted for induction of diabetes. These animals were received alloxan monohydrate at dose level 150mg/kg through intraperitoneal route. The test item alloxan monohydrate was dissolved into normal saline solution. After 72 hours, the blood glucose levels were measured in all animals (n=33). The animals which showed more than 200 mg/dL blood glucose level, it was considered as "diabetes". Out of 33 number of animals, 19 animals were showed high blood glucose level (more than 200mg/dL). On the basis of blood glucose response, total 19 animals were divided into three groups and 7 animals were used as control group (G1). These groups were followed

Groups	No of animals/ Sex	Dose(mg/kg body weight)	Test Item
I (control without diabetes)	07/M	10ml/kg	Distilled water
II (Diabetes induced group)	07/M	10ml /kg	Distilled water
III (Diabetes induced + HBCS treated group	06/M	2000 mg/kg	HBCS
IV (Diabetes induced +HBCS treated	06/M	2000 mg/kg	Normal sugar

After induction of diabetes, the group III and group IV animals were received twice daily test item "herbal blended cane sugar (HBCS)" and normal sugar at dose levels 2000mg/kg for 21 days treatment whereas group I and group II animals were received distilled water at dose level 10 ml/kg for same treatment days . All groups of animals were monitored after post dosing for any mortality and clinical sign of test items related toxicity.

12.0 OBSERVATION

12.1 Physiological parameters

12.1.1 All groups of animals were recorded body weight and food consumption weekly basis during the periods of study

12.1.2 Mortality and clinical sign of toxicity were recorded daily after post dosing for 21 days treatment periods in all groups

12.2 Blood Collection & Biochemical parameters analysis

At the end of experiment, blood samples were collected from tail vein to measured the blood glucose levels from all groups. After the measurement of blood glucose levels, all groups of animals were anaesthetised for collection of blood sample to measure the serum glutamate oxalotransminase (SGOT), serum glutamate pyuro transminase (SGPT) Triglyceride (TG) , total cholesterol (TC) and insulin levels after 21 days treatment.

12.2.1 Blood glucose level was determined before and after treatment by placing a drop of blood from the tail tip on strip of digital ACCU-CHEK advantage II glucose meter (Roche

diagnostic, Germany).

12.2.2 Lipid profile: Total cholesterol and triglyceride were determined by enzymatic (cholesterol oxidase) and (colorimeter) methods respectively in the serum sample

12.2.3 SGOT, and SGPT parameters were measured in the serum by using the reans

12.2.4 Serum Insulin parameters was measured by using ELISA kit as per set protocol of manufacturer.

Statistical analysis: Resulting data are expressed in mean ±SD . Newman Kaul test was performed for statistical significant

13.0 RESULTS

13.1 Effect On the body weight

In the present study there was significantly (P<0.0001) increased in all groups from initial day to 21 days . The body weight was found 28%, 12% 24% and 16% increased respectively. The body weight was slightly increased on 7^{th} , 14^{th} and 21^{th} days in diabetes group in comparison to control group. In the case of test item treated groups, the body weight was found increased on 14^{th} and 21^{th} days in comparison to diabetic group The body weight was found high increased in HCBS treated group in comparison to normal sugar treated group and level was almost near to control group. The result is presented in table 1.

Status of body weight (g) in diabetic group and treated groups

Groups	0 Day	7^{th} Day	14^{th} Day	21^{th} day	0-21^{th} day % ▲/▼
Group I (control)	133.8 ±8.51	151.2 ±4.02	166.2±3.87	171.20±3.97***	28%▲
Group II (Diabetic group)	133.6±5.27	142.2 ±2.70	145.4 ±3.98	149.4±4.52***	12%▲
Group III HBCS treated	133.8±4.27	144.8 ±2.68	156.2±3.71	165.6±3.36***	24%▲
Group IV NS treated	132.8±11.78	139.4 ±12.97	148.8 ±12.28	154.0±3.81***	16%▲

Table 1: All data are mean ±SD of 6-7 animals in each groups. The percentage parenthesis was compared between 0 to 21^{th} days treatment. Where *** is highly significant (P<0.0001)

13.2 Effect on Feed intake

The quantity of food consumed by animals in each group was recorded from the day of commencement of treatment and daily thereafter. Food intake per group was calculated using the amount of food offered to and left in each cage, and the number of rats surviving in each cage. There was slightly 28.2% ($P<0.0001$) reduced food intake in diabetes induced group in comparison to control group After treatment with respective test items, the body weight was significantly increased 23.86%; 13.0% in HBCS treated group in comparison to diabetes and normal sugar treated group. When the feed intake was compared to from 1st day to 21 days, the feed intake levels was increased 152%, 72%, 97% and 74% in all groups respectively. The data are presented in table 2.

Status of Feed intake (g) in diabetic group and treated groups

Groups	1 Day	7th Day	14th Day	21th day	0-21th day % ▲/▼
Group I (control)	45.2±5.32	86.47 ±6.11	94.89±4.58	114.1±7.14***	152%▲
Group II (Diabetic group)	47.61±6.40	58.31 ±4.78	75.44 ±6.11	81.97±8.04***	72%▲
Group III HBCS treated	51.41±4.27	74.94 ±5.87	89.55±4.08	101.53±7.41***	97%▲
Group IV NS treated	53.08±5.10	67.28 ±6.94	77.39±6.41	92.47±5.89**	74%▲

Table 2: All data are mean ±SD of 6-7 animals in each groups. The percentage parenthesis was compared between 0 to 21th days treatment. Where *** is highly significant ($P<0.0001$), ** is $P<0.001$(significant).

13.3 Effect on Clinical sign of toxicity and Mortality

There was no any mortality and no clinical sign of test item related toxicity were found in all groups. All the animals were showed the normal behaviour during the periods of experiment. The diabetic animals group II show increased blood glucose level.. The results are presented in table 3.

Status of Mortality and Clinical sign of Toxicity in treated group

Groups	No. of animals	Mortality	Clinical sign of toxicity
Control (GI)	N=7	0/7	Nil
Diabetes group (GII)	N=7	0/7	Elevated Blood Glucose
HBCS treated group (GIII)	N=6	0/6	Nil
NS treated group (GIV)	N=6	0/6	Nil

Table 3: Where N is number of animals, Nil ; No any test item related toxicity, 0; no any mortality.

13.4 Status of Blood Glucose Level

In the present study there was significant ($P<0.001$) increased the blood glucose level in group II to group IV as compared to control group before treatment (Table.4) . After treatment with test item HBCS and N S for 21 days, the blood glucose level was found highly significant lowered in HBCS treated group whereas in case of NS treated group, the blood glucose was decreased but non-significant (NS) as compared with diabetes induced group. When compared between both treated group, the blood glucose level was found significant ($P<0.05$) lowered in HBCS treated group and come near to control group. When blood glucose level was compared only in diabetes group from induction day to after treatment 21 days, the level was found non significant. The results are presented in table 4.

Status of Blood glucose level in all groups before and after treatement with Test Item

Groups / No. of animals	Before Induction	After Induction	After treatment
Group I /Control (N=7)	94±11.09	94±11.09	71±7.44
Group II /Diabetic induced (N=7)	71±26.00	268.33±32.32***	272.16±26.84Ns
Group III /HBCS treated (N=6)	89.43±30.29	237.3±35.42***	154.27±21.82***
Group IV /NS treated (N=6)	78.20±18.39	240.0±34.28***	204.55.±39.15Ns

Table 4: All data are mean ± SD of 6-7 animals in each group. Where *** is highly significant (P<0.0001); ** is significant (P<0.001); NS mean (P>0.05) non significant .

13.5: **Effect On hepatic enzymes (SGOT, SGPT) and lipid profile (TG and TC)**

In the present study there were significant (P<0.05; P<0.001) elevated the SGOT, SGPT , total cholestrol and triglyceride (TG) levels in diabetes induced group as compared with control group. After treatment with respective test item for 21 days, the hepatic enzymes SGOT and SGPT were found statistically lowered (P<0.05) in HBCS treated group and non significant (P>0.05) lowered in NS treated group as compared with diabetes induced group. When compared between both treated group the SGOT, SGPT level was found non significant decreased in HBCS treated group after 21 days treatment. The total Cholesterol

(TC) and triglyceride (TG) were statistically significant (P<0.0001) increased in diabetic induced group as compared with control group. After treatment with respective test items these parameters were significantly (P<0.0001) lowered in to both treated groups as compared with diabetes induced group. When compared between both treated groups, these parameters were significantly reduced (P<0.001, P<0.01) in HBCS treated groups and come almost near to control group (Figure 1)

Status of hepatic and Lipid profiles parameters in diabetes and treated groups

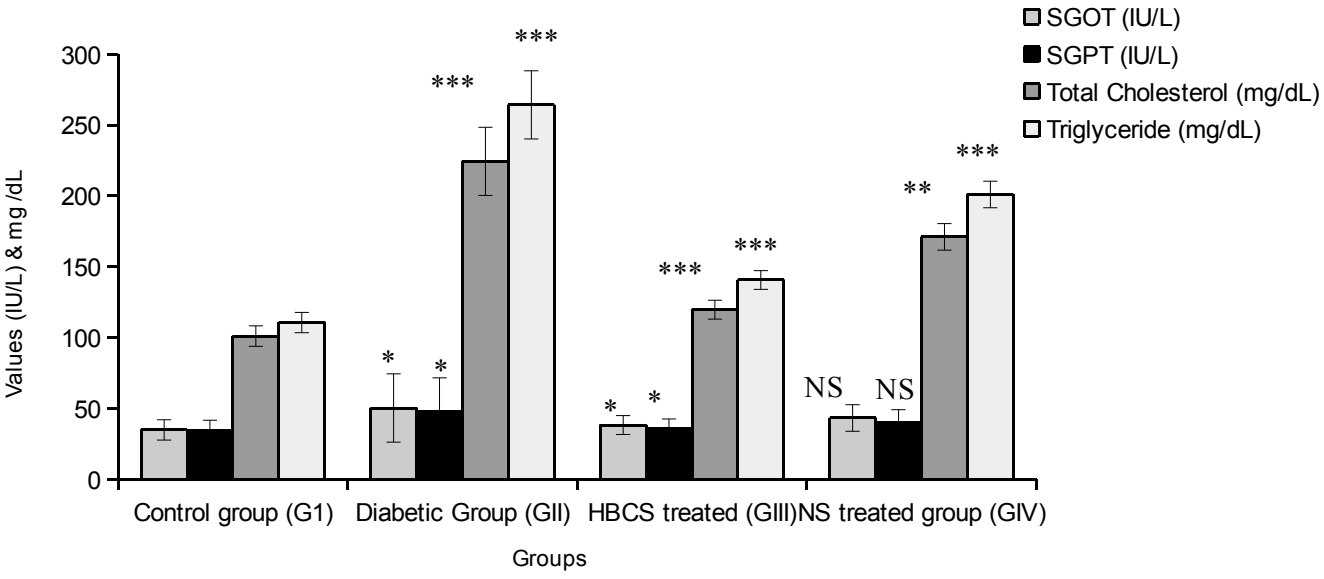

Figure 1: All data are mean ± SD of 6-7 animals in each group. Where *** is highly significant (P<0.0001); ** is significant (P<0.001); NS mean (P>0.05) non significant .

13.6: **Effect on Serum Insulin level**

In the present study, the serum insulin level was found significantly (P<0.00l) lowered in the diabetes induced group when compared with control group. When after treatment with HBCS and NS test item for 21 days, the serum insulin level was found significantly (P<0.001) increased in HBCS and NS treated group . When compared between both treated group, the serum insulin level was found high (P<0.001) in HBCS treated group and the level was found almost near to control group. The resulting data present in Figure2.

Status of Serum Insulin level in diabetes and treated groups

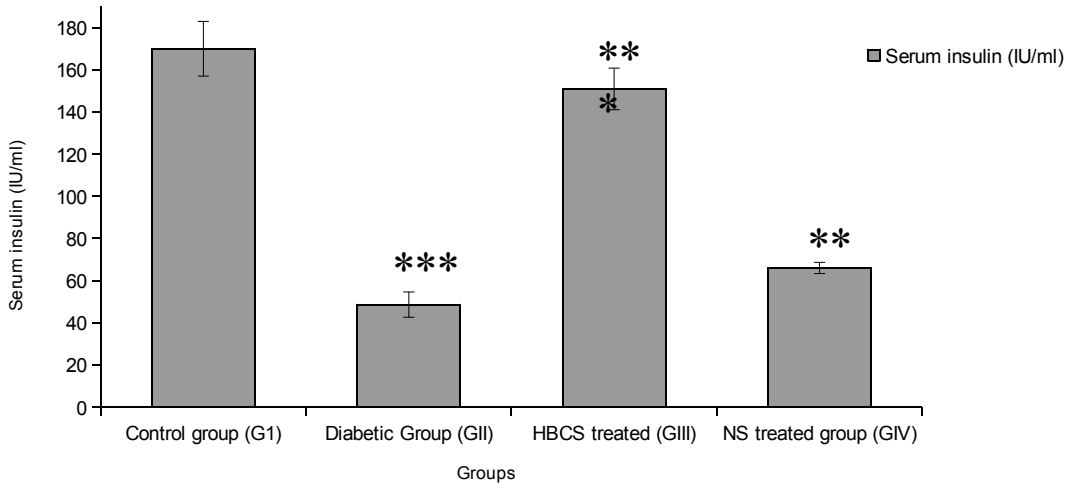

Figure 2: All data are mean ± SD of 6-7 animals in each group. Where *** is highly significant (P<0.0001); ** is significant (P<0.001); NS mean (P>0.05) non significant .

14.0 CONCLUSION

On the basis of above finding it is clear indicate that Herbelly blended cane sugar (HBCS) is very effective test item which prevent the diabetes along with improve the lipid profile (TG and TC) and prevent the hepatic injury along with manage the insulin level during diabetic condition

15.0 REFERENCES

12.1 Akindele O Adeyi, Babatunde A Idowu, Chiedu F Mafiana, Samuel A Oluwalana, and Oluseyi A Akinloye. Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology. **Int J Physiol Pathophysiol Pharmacol. 2012; 4(1): 51–58.**

12.2 Saeed Shirali,Shouresh Babaali,Hasti Babaali A Comparative Study on the Effects of Incretin and Metformin on sugar Profile and Insulin Resistance in STZ -induced Diabetic Wistar Rats. 2016; RJPBCS 7 (5) 1921 -29.