

## Novel Approach of Isolation of Tannins from the Fruit Rind of *Terminalia chebula* and *Punica granatum* and its Synergistic Antidiabetic Activity against Streptozotocin Induced Diabetic Rats.

Sampath V<sup>1\*</sup>, Mani N<sup>2</sup>

<sup>1</sup>Department of Phytochemistry, The Himalaya Drug Company, Makali-562162, Bangalore, Karnataka, India.

<sup>2</sup>P.G.and Research Department of chemistry, A.V.V.M.SriPushpam College (Autonomous), Poondi-613503, Tamilnadu, India.

Available Online: 1<sup>st</sup> February, 2015

### ABSTRACT

To evaluate the synergistic effect of tannins from the fruit rind of *Terminalia chebula* and *Punica granatum* against streptozotocin (STZ) induced diabetes mellitus in Wistar rats. The equal blend of tannins enriched extract of HCP (From *Terminalia Chebula* and *Punica granatum* fruit rind) at doses of 250mg /kg bw and 500mg/kg bw were administered orally to streptozotocin (120mg/kg bw) induced diabetic rats for 28days. The various parameters were studied including fasting blood glucose, body weight, glycosylated hemoglobin (HbA1c), lactate dehydrogenase (LDH), creatine kinase levels (CK) and histochemical analysis of pancreas in normal, treated diabetic rats. The HCP (Tannins enriched extract) at diverse dosage (250 and 500mg/kg bw) showed a significant decrease in fasting blood glucose, lactate dehydrogenase, glycosylated hemoglobin and creatine kinase (CK) levels. The body weight also significantly increased upon treatment with HCP. Histopathology study exhibited that the  $\beta$ -cell damage in the pancreatic was prevented in the HCP treated diabetic rats. Acute toxicity study revealed that HCP was safer and non-toxic nature. From the compilation of study results, it was concluded that the HCP at dosage of 500mg/kg bw exhibit significant antidiabetic activity and it may be a promising antidiabetic agent for the treatment of diabetes mellitus. Also the HCP prevent the  $\beta$ -cell damage and induce the insulin secretion in the pancreatic.

**Key Words:** *Terminalia chebula*, *Punica granatum*, Tannins, Streptozotocin, Antidiabetic, Histopathological Study.

### INTRODUCTION

Diabetes mellitus is a chronic disease due to stress, heredity and environmental leads to the abnormal metabolism of carbohydrate, fat and protein in our body. Its increasing rapidly day by day and estimated to rise 366million people will suffer by 2030 according to the survey of international diabetes federation. Ancient time to till now there is no proper medicine to cure diabetes permanently. But synthetic drugs are available for the management of diabetes which is showing side effects also<sup>1</sup>. So finding the antidiabetic agent from the medicinal plant is an alternative and safer for the management of diabetes. Polyherbal therapy is one of the approach for the treatment of diabetes mellitus due to their synergistic effect through producing the higher therapeutic efficacy<sup>2</sup>.

*Terminalia chebula* Retz. (Combretaceae) is an important medicinal plant and its distributed throughout India. Fruit is a hard, greenish yellow in color and 3.5 to 4.0cm in length which has been traditionally used to treat against various diseases<sup>3</sup>. *Punica granatum* L., is a fruit-bearing deciduous small tree growing between 5–8 meters belongs to the puniceae family and native to afghanistan, iran and the himalayas in northern india<sup>4</sup>. Pomegranate fruit is both round and spherical shape with a tough exocarp and light yellowish to red in color. Fruit rind of both *terminalia*

*chebula* and pomegranate were exhibited the pharmacological activity such as antioxidant, antibacterial, cardioprotective, anti-inflammatory, antiulcer, wound healing, digestive and antihypoglycemic due to their major phytochemical such as tannins, flavonoids, glycoside<sup>5,6</sup>. Generally the crude extract is focused more attention for the screening of biological efficacy of medicinal plants than the specific phytochemical. So the aim of present study was screening of antidiabetic activity of equal blend of tannins enriched extract of fruit rind of *terminalia chebula* and pomegranate.

### MATERIALS AND METHOD

#### Plant material

The dried fruit rind of *terminalia chebula* and pomegranate were procured from the local market, thanjavur district, tamilnadu. The raw materials were authenticated by botanist, A.V.V.M. Sri.Pushpam college, poondi, thanjavur district. The plant materials were subjected to coarse powder using a mechanical grinder.

#### Chemicals

Streptozotocin (STZ) was obtained from sigma aldrich, St.Louis, USA. Gilbenclamide was obtained as gift sample from alken laboratory, Mumbai, India. Diagonistic instrument ACCU-CHECK active roche diagonistic

Table 1: Effect of HCP on Blood Glucose Levels during treatment

Groups	Treatment	Blood Glucose Levels					
		Day 0	Day 1	Day 5	Day 7	Day 14	Day 28
Group I	Normal CMC (0.5%)	83.00± 1.77	87.17± 1.62	85.67± 0.88	85.00± 1.69	82.33± 1.33	84.00± 1.15
Group II	Diabetic CMC (0.5%)	217.50±2.50	224.33±3.14	230.33±2.40	235.80±2.66	240.50±3.39	244.50±2.54
Group III	Standard Glibenclamide (5g/kg)	215.67±1.76	189.00±7.50	124.17±1.42	117.67±1.80	104.50±0.92	102.17±1.96
Group IV	HCP Extract (500 mg/kg)	226.00±1.98	219.50±3.14	208.50±3.88	193.50±1.91	189.83±2.07	173.83±1.56
Group V	HCP Extract (250 mg/kg)	218.30±1.93	212.30±2.23	213.50±2.81	203.50±2.17	213.60±3.6	194.00±5.8

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean ± S.E.M.  $n = 6$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$ .

Table 2: Effect of HCP on STZ induced changes on the body weight, blood glucose, HbA1c, CK and LDH.

Groups	Treatment	Body weight (g)	Blood Glucose (mg/dL)	HbA1c (%)	CK (IU/L)	LDH (IU/L)
Group I	Normal CMC (0.5%)	249.83±2.95	84.00± 1.15	1.35± 0.03	213.57±5.40	91.53± 1.31
Group II	Diabetic CMC (0.5%)	168.17±5.58	244.50±2.54	4.12± 0.08	287.00±5.60	168.25±2.38
Group III	Standard Glibenclamide (5 mg/kg)	247.33±2.91	102.17±1.96	2.57± 0.11	253.86±5.92	129.80±1.80
Group IV	HCP Extract (500 mg/kg)	230.17±4.00	173.83±1.56	3.36± 0.13	264.08±7.6	135.78±3.08
Group V	HCP Extract (250 mg/kg)	228.50±4.01	194.00±5.8	3.67± 0.13	274.86±4.13	137.73±2.48

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean ± S.E.M.  $n = 6$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$ .

(Germany) was used to determine the blood glucose level. All other chemicals used in the experiments were analytical grade (Merck, Rankem) procured from Mumbai.

#### Apparatus

High performance thin layer chromatography (HPTLC) equipped with a sample applicator linomat V, camag thin layer chromatography (TLC), scanner III, camag prostar III and win cats software were used. UV (Ultra violet) spectrophotometer – 2202, Systronics (India) was used for the estimation of tannins. High performance liquid chromatography (HPLC) system (Aligent 1260) equipped with a 1260 quaternary pump, photo diode array (PDA) visible detector and column luna 5UC<sub>18</sub> (2)100A (250×4.6mm) was used. Data were recorded and processed using EZ chrome software version A.0.1.04, Germany. Perkin-Elmer API 2000 (Applied bio systems MDS, SCIEX,canada) mass spectrometry equipped with ESI (Electron spray ionization) was employed and data were obtained using 1.5 version software.

#### Extraction method

Each 200g of fruit rind of *terminalia chebula* and pomegranate were extracted separately with 1.2 liters of demineralized water and adjusted the pH to 9 using 10% potassium hydroxide. Thereafter, it's allowed to stand for one hour at room temperature. Further it's filtered through 100mesh muslin cloth and adjusted the filtrate pH to 7 using 5% hydrochloric acid, during that period precipitate was obtained. To the same 4 liters of methanol were added with manual stirring and allowed it to stand for 30minutes. Then methanol soluble fraction was filtered through ordinary filter paper and concentrated using water bath at 90°C under fume hood. Thus the obtained extracts were randomly blent in equal ratio (1:1) and encoded as HCP with subject to screening of antidiabetic activity.

#### Preliminary phytochemical screening of HCP

About 500mg of HCP was dissolved in 10ml purified water and the solution was spotted to 10×4cm precoated silica gel 60 F<sub>254</sub>tlc plate. Then the plate was developed using different mobile phase and derivatized with various

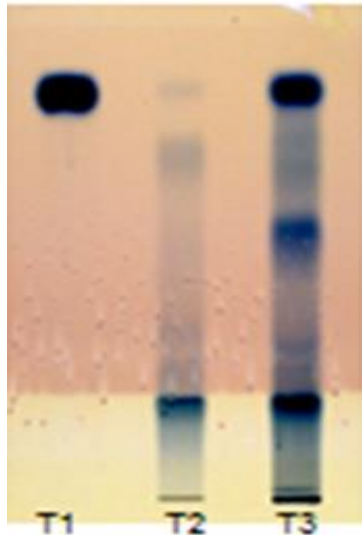


Figure 1. HPTLC chromatogram showed the presence of gallic acid and punicalin in the HCP. T1: Gallic acid (Standard); T2: Punicalin (standard); T3: HCP.

spraying reagents as the method described in plant drug analysis<sup>7</sup>.

#### Identification of gallic acid and punicalin in HCP by HPTLC

##### Preparation of standard solution

Each 2mg of Gallic acid and punicalin weighed separately into 5ml volumetric flask and made the volume up to the mark with purified water.

##### Sample preparation

Accurately weighed 10mg of HCP was dissolved in 10ml purified water. Then filtered through whatman filter paper and used for the spotting.

##### Chromatographic conditions

Chromatograph was performed on 10×10cm precoated silicagel 60F<sub>254</sub> tlc plate and applied 5µl of standard and test solution at 25mm distance to the tlc plate. There after the plate was developed using optimized solvent system Butanol: Formic acid: Water (10:3:3), as mobile phase in a glass chamber which is previously saturated for 10 minutes. The plate was developed up to 8cm and the development time was 50 minutes. Then the developed plate was dried at 60°C for 15 minutes and sprayed with 1% methanolic ferric chloride reagent<sup>8</sup>. Identification of gallic acid and punicalin in the HCP through comparison of *R<sub>f</sub>* value with respect to the standard.

#### Quantitative estimations of tannins in HCP by UV spectroscopy

##### Sample preparation

Accurately weighed 0.1g of HCP in 250ml flat bottomed flask. To that added 100ml of purified water and refluxed at 95°C using water bath for one hour. Then transferred the solution into 500ml volumetric flask and made the volume up to the mark with purified water. Thereafter, it's filtered through whatman paper and used for analysis.

##### Standard Preparation

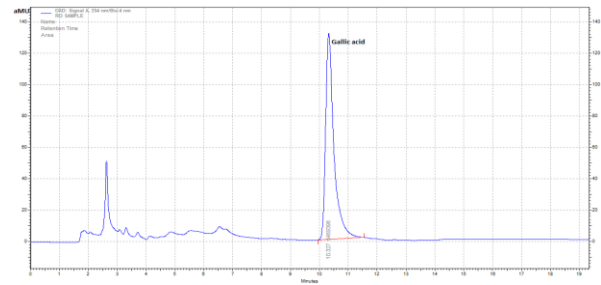


Figure 2. HPLC chromatogram of gallic acid present in the HCP and the peak was identified by comparison of retention time (10.32) with the standard.

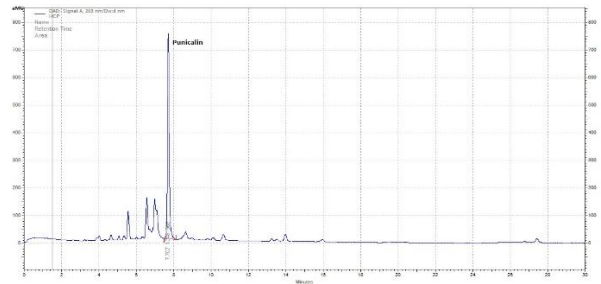


Figure 3. HPLC chromatogram of punicalin present in the HCP and the peak was identified by comparison retention time (7.70) with the standard.

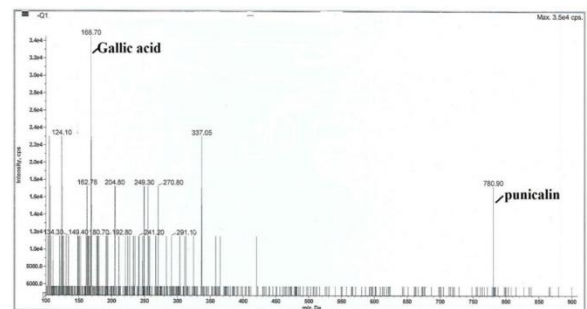


Figure 4. Mass spectrum of HCP confirmed the presence of gallic acid and punicalin in which correspond to M-H *m/z* 168.7 and 780.90 respectively.

Accurately weighed 0.1g of tannic acid in 100ml volumetric flask and made the volume up to the mark with purified water.

##### Reagents preparation

Prepared 1% solution of potassium ferric cyanide and ferric chloride in 100ml volumetric flask using purified water.

##### Preparation of reagent blank

Each 1ml of potassium ferric cyanide and ferric chloride were pipette out into 10ml volumetric flask and made the volume up to the mark with purified water.

##### Preparation of test solution and standard solution

Each 0.2ml of test and standard solution was pipette out separately into 10ml volumetric flask. To the same each 1ml of potassium ferric cyanide and ferric chloride were added and mixed well. Then made the volume up to the mark with purified water. Exactly after 30 minutes absorbance was measured at 720nm as per the method

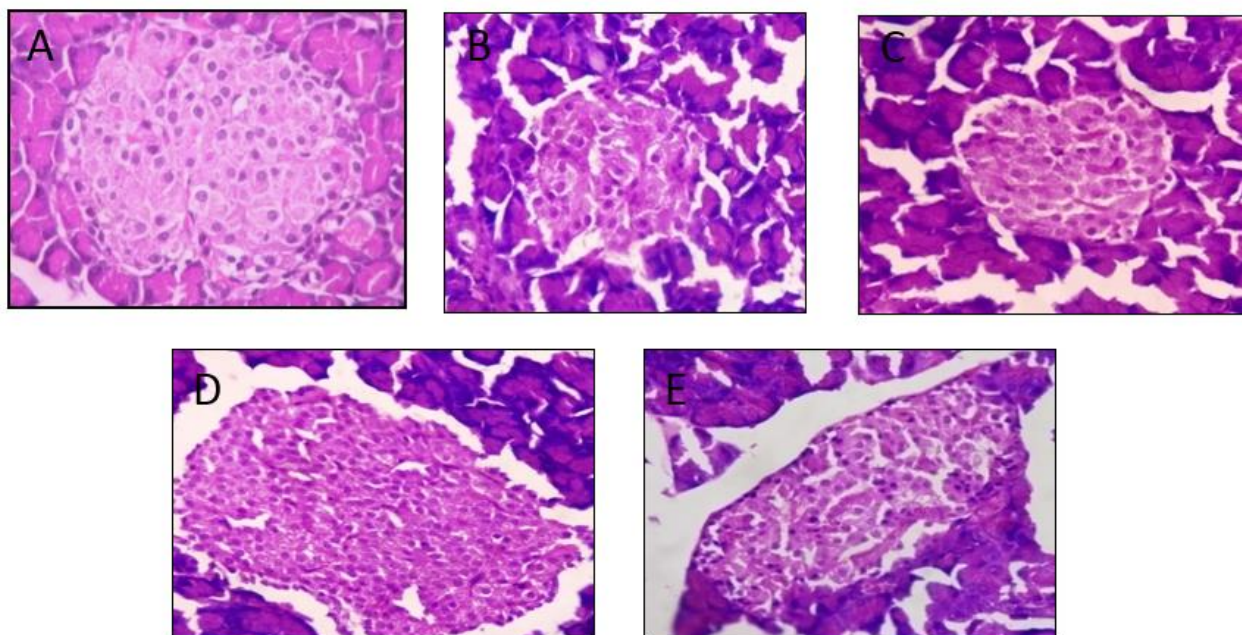


Figure 5. Histopathological observations of HCP and gilbenclamide treated pancreas of streptozotocin induced diabetic rats. A: Normal control (Normal islets of 75% beta cells and 20% of alpha cells). B: Diabetic control (Shrunkened islets of 35% of beta cells and 60% of alpha cells). C: Diabetic + HCP 250mg/kg bw (Moderate expansion of islets showing 55% of beta cells and 40% of alpha cells). D: Diabetic + HCP 500mg/kg bw (Absence of dilation and moderate expansion of pancreatic islets showing 70% of beta cells and 25% alpha cells). E: Diabetic + gilbenclamide 5mg/kg bw (Absence of dilation and moderate expansion of islets showing 65% of beta cells and 30% of alpha cells).

described earlier<sup>9</sup>. Tannins content in HCP was calculated from the sample and standard absorbance.

#### HPLC analysis of HCP

##### Quantitative estimation of gallic acid

About 100mg of standard gallic acid and HCP 103mg was weighed separately into 100ml volumetric flask and dissolved into 100ml purified water. The mixture was sonicated for 10minutes and filtered through 0.45 $\mu$ l syringe filter. Then 20 $\mu$ l of standard and sample solution was injected into HPLC and the column flow rate was 1ml per minute. Column mobile phase and chromatographic conditions were followed as per the method described earlier<sup>10</sup>. The data was collected at 254nm.

##### Quantitative estimation of punicalin

About 512mg of standard punicalin and HCP 102mg was weighed separately into 100ml volumetric flask and dissolved into 100ml purified water. The mixture was sonicated for 10minutes and filtered through 0.45 $\mu$ l syringe filter. Then 20 $\mu$ l of standard and sample solution was injected into HPLC and the column flow rate was 1ml per minutes. Column mobile phase and chromatographic conditions were followed as per the method described earlier<sup>11</sup>. The data was collected at 269 nm.

#### Mass analysis of HCP

The identification of gallic acid and punicalin in HCP was confirmed by mass spectrometry at the ion source temperature of 420 $^{\circ}$ c. The sample solution was prepared by 2mg/ml concentration with purified water and filtered through 0.45 $\mu$ l syringe filter. All the samples were injected by syringe pump at a flow rate of 20 $\mu$ l /min. The spectrum was scanned from 100-1000 in negative mode.

#### Animals

Healthy young adults non-pregnant female rats (150-200g) of Wistar strains were used for the acute toxicity and healthy young adult male wistar albino rats were used for the screening of antidiabetic activity of HCP. The animals were procured from M/S Sri venkateshwara enterprises, Bangalore, Karnataka. After randomization into various groups, all the animals were maintained at a standard room temperature of 27 $^{\circ}$ c $\pm$ 30 $^{\circ}$ c, relative humidity 65  $\pm$ 10% and 12 hours light/dark for one week. All the animals were fed with rodent pellet diet (Goldmohr,liptonindia ltd) and water was allowed ad-libitum under strict hygienic conditions. The study was approved by the Animal Ethical Committee of the Institute (JKKMMRFCP/IAECI2013/018).

#### Acute toxicity study

The acute toxicity study was performed as per Organization for Economic Co-operation and Development guide line<sup>12</sup>. The acute toxicity study of HCP was carried out using female rats 150 – 200g which were maintained under the standard conditions. Acute toxic class method of CPCSEA was adopted for toxicity studies. The acute toxic class method was set out as a stepwise procedure using 3 animals of single sex per step. Animals were administrated with HCP at the dose of 2000mg/kg bw was fixed on the basis of their mortality. Carboxyl methyl cellulose 5% was used as a vehicle to suspend the HCP and was administrated orally. Animals were observed individually after dosing at least once during the first 30minutes and periodically during the first 24 hours. The



special attention given for the first 4 hours and daily thereafter for a total of 14 days.

#### *Induction of diabetes mellitus in experimental rats*

Streptozotocin 120mg/kg was given by a single intraperitoneal injection to induce the diabetes in the rats. The fasting blood glucose levels were checked on 4<sup>th</sup> and 7<sup>th</sup> day using ACCU-Check blood glucose monitoring system. Rats with blood glucose more than 200mg/dl were used for study.

#### *Experimental design*

Adult healthy male albino rats of wistar strain with body weight of approximately 200-250g were used. The animals (30 rats) were divided into two groups and first group (6 rats) was normal control which received the saline solution intraperitoneally. A single intravenous dose of STZ at 120mg/kg bw was dissolved in normal saline injected to the second group (24 rats). After three days the blood samples were collected from the tail to confirm the diabetic condition. Drug treatment was started from the 8<sup>th</sup> day of STZ administration. Diabetic animals (24 rats) were further divided into four groups of 6rats each (Group II to V). Details of the experimental animals as follows:

Group 1: Normal control;

Group 2: Diabetic control;

Group 3: Diabetic rats treated with gilbenclamide 5mg/kg bw;

Group 4: Diabetic rats treated with HCP of 500mg/kg bw;

Group 5: Diabetic rats treated with HCP of 250 mg/kg bw.

Blood samples were collected from the tail tip of the rat and blood glucose level were checked on 0<sup>th</sup>, 1<sup>st</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day using ACCU-Check blood glucose monitoring systems.

#### *Evaluation of biochemical parameter*

At the end of the experiment (After 28 days) rats fasted overnight and anesthetized with sodium pentothal intraperitoneally. About 4 ml blood was withdrawn through the retro-orbital plexus using a glass capillary and plasma serum was separated. Thereafter the plasma serum was used for the estimation of glucose, glycosylated hemoglobin (HbA1c), lactate dehydrogenase (LDH) and creatine kinase (CK) using commercial kits (ERBA diagnostics).

#### *Histopathology study*

The animals were sacrificed and the whole pancreas from each animal was removed and collected in 10% formalin solution and immediately processed by paraffin for histopathological studies as per the method described earlier<sup>13</sup>. The sections were examined under microscope and photomicrographs were taken. Histopathological study was carried out in radiant research services private limited, Bangalore.

#### *Statistical analysis*

All the values of fasting blood glucose, body weight and biological estimation were expressed as mean  $\pm$  standard error of mean (S.E - M) for 6 animals in each group and statistical differences between groups were assessed by analysis of variance one way ANOVA followed by turkey's test. P values less than 0.05 were considered statistically significant.

## **RESULTS**

### *Phytochemical screening*

Preliminary phytochemical screening revealed that the phenolic compounds (tannins) are major in the HCP. Also HPTLC revealed that the presence of gallic acid and punicalin in HCP and it's confirmed by comparison of standard *Rf* values of 0.9 and 0.3 respectively (Figure 1).

### *Estimation of tannins in HCP*

Tannins content in the HCP was calculated from the standard (0.470) and sample absorbance (1.550) and the total tannins in the HCP was obtained 81.21%.

### *Purity and structural confirmation of gallic acid and punicalin in HCP by HPLC and mass spectroscopy.*

HPLC analysis of gallic acid in HCP was obtained 14.47% and it was calculated from the standard and sample peak area of 1913081, 2909961 respectively (figure-2). Punicalin content in HCP was obtained 63.95% and it was calculated from the standard and sample peak area of 2618425, 6065260 respectively (figure-3). The obtained mass spectrum of HCP (figure-4) where at negative mode (M-H) *m/z* 168.7 accounted for gallic acid as previously reported<sup>14</sup> and (M-H) *m/z* 780.9 accounted for punicalin<sup>15</sup>.

### *Acute toxicity study*

The acute toxicity study on female rats were revealed that there is no mortality at a dose of 2000mg /kg bw during the 14 days treatment of HCP and its showed that HCP is safer and non-toxic.

### *Effect on blood glucose level*

HCP 250mg and 500mg /kg bw were significantly reduced the blood glucose level in diabetic induced rats over a period of 28 days of treatment. However the HCP at 500mg/kg bw dose was similar to that of standard drug gilbenclamide for reducing the blood glucose level during the treatment (Table1).

### *Physical and biochemical study*

Table 2 showed the changes in body weight, glycosylated hemoglobin (HbA1c), lactate dehydrogenase (LDH) and creatinin kinase (CK) levels in normal and experimental rats. There was a significant decrease in body weight and increases HbA1c, LDH, CK levels in the diabetic rats. The administration of HCP (500mg/kg bw) and gilbenclamide (5mg/kg bw) to diabetic rats were restored the bodyweight, HbA1c, LDH and CK to near normal level.

### *Histopathological examination of pancreas*

Figure 5 revealed the histopathological observation of pancreas of both HCP (250mg and 500mg/kg bw) treated diabetic rats and different groups. Figure 5A showed the normal control islets which consist 75% of beta cells and 20% of alpha cells. Figure 5B showed the shrunken islets of diabetic rats which consist of 35% of beta cells and 60% of alpha cells. Figure 5C and 5D showed the moderate expansion of islets after treatment of diabetic rats with HCP 250mg/kg bw which consist of beta cells 55% and alpha cells 40% and 500mg/kg bw which consist of 70% of beta cells and 25% alpha cells respectively. Figure 5E showed the islets of gilbenclamide treated diabetic rats which consist of 65% beta cells and 30% of alpha cells.

## **DISCUSSION**

The present study was screening of a specific phytochemical tannins enhanced fraction (HCP) for antidiabetic activity against STZ induced diabetic rats. STZ induced free radical in pancreatic beta cell which causes diabetes by increasing of glucose level in the blood<sup>16</sup>. The blood glucose level in diabetic controlled rats were consistently increased during the study. But in the HCP treated diabetic rats blood glucose level was significantly decreased during the 28 days of treatment. Its revealed that HCP significantly controlled the glucose level towards normal in diabetic rats and its suggesting that the possible action probably preventing the beta cell damage from the STZ induced free radical in the pancreas. It has been reported that the STZ induced diabetes causes the weight loss in the animals<sup>17</sup>. In present study also showed that the diabetic controlled rats were lost their body weight over 28 days period of study. But the HCP treated diabetic rats were significantly gained the body weight as similar to normal control rats. High level of glycosylated hemoglobin is one of the sign associate with hyperglycemia complication which leads to diabetes<sup>18</sup>. In present study the diabetic controlled rats had shown higher level of glycosylated hemoglobin (HbA1C). But the HCP significantly decreased the HbA1c in the diabetic rats and its possible action probably through normalizes the insulin secretion by preventing the beta cell damage in the pancreas. Lactate dehydrogenase levels in the animals with diabetes mellitus were significantly higher when compared with normal control which has been already reported<sup>19</sup>. The present study also revealed that the LDH levels of diabetic rats were higher as compared to normal control rats. However, the LDH levels in the HCP treated diabetic rats were significantly decreased and suggesting that the HCP has potent to prevent the cell damage from the STZ induced free radical. It was reported that the creatine kinase enzyme activity was significantly decreased in patients with diabetes mellitus<sup>20</sup>. Free radicals are interfering the function of CK enzyme activity which leads to the enzyme inactivation<sup>21</sup>. The present study revealed that the creatine kinase enzyme level was significantly increased in the diabetic rats and it was controlled in the HCP treated diabetic rats. So the possible action of HCP was probably protecting the CK enzyme from the STZ induced free radical.

The beta cell concentration in the pancreas was significantly decreased in diabetic controlled rats which are exhibited from histopathological study. But there is no much difference was observed in beta cell concentration between normal control and HCP (500mg/kg bw) treated diabetic rats.

Phytochemical screening of HCP shows the presence of tannins which constitute of the major proportion of punicalin and gallic acid. These phytochemical are may be bioactive in HCP for the management of diabetes mellitus. Generally phenolic compounds are effective in diabetes due to their antioxidant property<sup>22</sup>. According to that, the mechanism of HCP in the diabetic rats were probably the free radical scavenging activity which protect the beta cell damage from STZ induced free radicals.

## CONCLUSION

From the compilation of study results, it was concluded that the antidiabetic activity of HCP (500mg/kg bw) against STZ induced diabetic rats were similar to that of standard drug gilbenclamide in restoring the levels of blood glucose, bodyweight, glycosylated hemoglobin, lactate dehydrogenase and creatine kinase towards normal levels. In addition, the synergistic effect of gallic acid and punicalin which are major in HCP (Tannins enhanced extract) probably responsible for the antidiabetic activity. The mechanism of HCP might be prevention of STZ induced free radical and its assist to normalize the body metabolism and insulin secretion in the pancreas.

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. Ashok, Radiant Research Services Pvt.Ltd, Bangalore, India for providing research facilities.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## REFERENCES

1. Ashok Kumar Balaraman, Jagadish Sing, Sasmita Dash, TapankumarMaity. Anti-hyperglycemic and hypolipidemic effects of Melothriamaderas patina and Cocciniaindica in streptozotocin induced diabetes in rats. Saudi Pharmaceutical Journal 2010; 18, 173-178.
2. HarshadShete, SadhanaSathaye. Anti-diabetic activity of polyherbal therapy in streptozotocin induced diabetes mellitus. Int J Res Pharma Sci. 2013; 4(1), 88-95.
3. Gupta AK, NeerajTandon, Madhu Sharma. Quality standards of Indian medicinal plants 2003; 3<sup>rd</sup> vol. New Delhi: Indian Council of Medical Research. 206.
4. Tariq Ismail, PieroSestili, Saeed Akhtar. Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-diabetic effects. Journal of Ethanopharmacology 2012; 143, 397-405.
5. Anwesh Bag, Subir Kumar Bhattacharya, Rabi RanjanChattopadhyay. The development of Terminaliachebula Retz. (Combretacea) in clinical research. Asian Pacific Journal of tropical biomedicine 2013; 3(3), 244-252.
6. SaleemBanihani, SamerSwedan, ZiyadAlguraan. Pomegranate and type 2 diabetes. Nutrition Research 2013; 33, 341-348.
7. Hildebert Wagner, Sabine Bladt. Plant Drug Analysis 1996; 2<sup>nd</sup> ed. Springer, Germany.
8. Yamuna Devi M, Wesely EG, Jhonson M. Chromatographic Studies on the Tannins of Aervalanata (L.) Juss. Ex Schultes. IOSR Journal of Pharmacy 2012; 2(1), 041-051.
9. Diaz AM, Ageger A. Analytical methods of tannins and their application to MyrtusCommunis seeds extracts. Fitoterapia 1986; 58(6), 395-407.
10. LaxmanSawant, BalaPrabhakar and Nancy Pandita. Quantitative HPLC analysis of Ascorbic Acid and Gallic Acid in *PhyllanthusEmblica*. Journal of Analytical & Bioanalytical Techniques 2010; 1, 111.

11. Yan Wang, Hongchao Zhang, Hao Liang, Qipeng Yuan. Purification, antioxidant activity and protein-precipitating capacity of punicalin from pomegranate husk. *Food chemistry* 2013; 138, 437-443.
12. OECD Guideline for Testing of Chemicals, 423. Acute oral Toxicity-Acute Toxic Class Method 2001; 1-14.
13. Nirmala A, Saroja S, Vasanthi HR, Lalitha G. Hypoglycemic effect of *Basellarubra* in Streptozotocin – induced diabetic rats. *JPharmacogPhytother.* 2009; 2, 25-30.
14. Sulaiman CT, Gopalakrishnan VK, Indira Balachandran. Reverse Phase Liquid Chromatography Coupled with Quadra pole-Time of Flight Mass Spectrometry for the Characterisation of Phenolics from *Acacia catechu* (L.f.) Willd. *International Journal of Phytomedicine* 2012; 4, 403-408.
15. Seeram N, Lee R, Hardy M & Heber D. Rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry. *Separation and purification technology* 2005; 41(1), 49-55.
16. Manjusha, Neha Aggarwal, Nitesh, Pankaj Gupta. Effect of petroleum ether extract of *Sesbania sesban* (Merr.) roots in streptozotocin (STZ) induced diabetes in mice. *Asian Pacific Journal of Tropical Biomedicine* 2012; S1254-S1260.
17. Rangachari Balamurugan, Savarimuthu Ignacimuthu. Antidiabetic and Hypolipidemic effect of methanol extract of *Lippianodiflora* L. in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 2011; S30-S36.
18. Rajiv Gandhi G, Sasikumar P. Antidiabetic effect of *Merremia emarginata* Burm.F. in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical bio medicine* 2012; 2(4), 281-286.
19. Ismail CELIK, Esref YEGIN, Fehmi ODABASOGLU. Effect of Experimental Diabetes Mellitus on plasma Lactate Dehydrogenase and Glutamic Oxaloacetic Transaminase Levels in Rabbits. *Turk J Biol.* 2002; 26, 151-154.
20. Jevric-Causevic A, Malenica M, Dujic T. Creatine Kinase Activity in patients with diabetes mellitus type I and type II. *Bosn J Basic Med Sci.* 2006; 6(3), 5-9.
21. Mahmoud H. Hadwan, Mufeed J. Ewadh, Oda M. Al-Zamily. The Key Role Correlation of Creatine Kinase Activity and Antioxidants Status in Diabetic Patients Type I and II. *National Journal of Chemistry* 2006; 23, 424-436.
22. Yeshwant Kumar A, Nandakumar K, Handral M, Sahil Talwar and Daniel Dhayabaran. Hypoglycemic and anti-diabetic activity of stem bark extracts *Erythrina indica* in normal and alloxan-induced diabetic rats. *Saudi Pharm J.* 2011; 19(1), 35-42.