Reactive oxygen species linked diabetes mellitus management by natural products: A Pre-clinical study in rat

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Abstract

The environment has a great impact on health maintenance. Environmental stress factors generate reactive oxygen species (ROS) in our bodies. As a result, pro-oxidant status of our body is dominated over the antioxidant status that induces health disorders. Diabetes mellitus (DM), a common metabolic cum lifestyle disease, is now considered as X syndrome. Environmental stressors through ROS generation can impose point mutation for the production of mutant enzymes, leading to cellular metabolic disorders, especially carbohydrate metabolism, an important cause of diabetes. Moreover, free radicals through cellular signaling system down-regulate gene expression of antioxidant enzymes that produce high levels of cellular free radicals in metabolic organs, which may lead to DM.

Natural products with strong antioxidant activities have a major role in managing diabetes. Nutraceuticals in *E. jumbulana, Camellia sinensis, H. antidysenterica* majorly contribute to such management by increasing gene expression of antioxidant enzymes and carbohydrate metabolic enzymes that favor glucose utilization in cell. It has also been established that such nutraceuticals can generate β cells from hepatic stem cells, so plasma insulin and c-peptide levels are elevated. Insulin receptor gene expression is also corrected by such nutraceuticals in diabetic model animals. The efficacy of such nutraceuticals is comparable with antidiabetic gold standard drugs. Such studies have been confirmed by genomics and proteomics studies using real-time PCR followed by western blotting studies. This field of research unfolds a new domain known as 'Neutrogenomics' in health science.

It is concluded that natural products may be the parallel management process of modern therapy as per the guideline of WHO.

Keywords: Diabetes, Natural products, Oxidative stress, Gene expression.

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Introduction

Diabetes mellitus is a well known and very prevalent disease affecting the citizens of both developed and developing countries. One-fourth of the world's population is affected by this disease. Oxidative stress, among all the reasons of diabetes, plays a remarkable role. Oxidative stress produced in the body by physiological and biochemical catabolic processes in cells and also by the stressful working environment tend to generate free radicals and other reactive oxygen species leading to oxidative stress.[1] Chronic hyperglycemia-induced oxidative stress is associated with dysfunction and apoptosis of several cells, including pancreatic beta cells.[2] Production of Reactive Oxygen Species (ROS) has resulted from oxidative stress and insufficient antioxidant capacity that causes cellular dysfunction and apoptosis.

Currently, glibenclamide, insulin and metformin (antihyperglycemic) and aminoguanidine (anti-glycation) are used in diabetes management.[3] Antidiabetic drug use is limited due to loss of efficacy and side effects (i.e. hepatotoxicity, nephrotoxicity and depletion of antioxidants) on long term administration.[4] Natural extracts from several medicinal plant species are considered as an alternative source for antioxidant and antidiabetic molecules. 'World Health Organization (WHO) also give special emphasis on herbal drug development against diseases as alternative medicine.[5]

The ethnobotanical reports about 800 plants that may possess antidiabetic potential.[6] Several herbs have shown antidiabetic activity when examined in experimental ways. This article focused on mainly three herbal plants *Eugenia jambolana*, *Camellia sinensis* (Green tea), *Holarrhena antidysenterica* about their antidiabetic and scavenging role of free radicals.

Hence, the present study was conducted to explore the molecular mechanism of action of an effective fraction of *E. jambolana* (seeds), *H. antidysenterica* (seed), and *C. sinensis* (leaves) in STZ-induced diabetic rat at genomic, and proteomic levels.

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Materials and Methods

Collection of plant materials

The leaves of *C. sinesis* were purchased from Subodh Brothers Pvt. Ltd, Kolkata and preserved in airtight glass container. The

seeds of *E. jambolana* and *H. antidysenterica* were collected from a local market in Paschim Midnapur town and these pant parts were authenticated by taxonomists in the Department of Botany and Forestry, Vidyasagar University, Midnapore.

Preparation of solvent fractions from different extract of sample plant part

Preparation of crude extract of leaves of *C. sinensis* using – methanol, for*E. Jambolana* using hydro-methanol (2:3) and in case of *H. antidysenterica,* the crude extract is prepared using hydro-methanol (2:3). Fractionation was carried out using polarity grade solvents i.e. n-hexane, ethyl acetate and chloroform, n-butanol. These lyophilized fractions were collected, labeled and stored at 4°C until use. The ethyl acetate fraction of was dissolved in distilled water and administered orally to experimental diabetic rats for this experiment at a specific dose.[7]

Chemicals

Streptozotocin (STZ) was purchased from Sigma, Aldrich (USA). All other chemicals like nicotinamide adenine dinucleotide phosphste (NADP), adenosine triphosphate (ATP), 2-[4-(2- Hydroxyethyl) 1- piperazinyl] ethane sulphonic acid (HEPES) were purchased from Sigma- Aldrich Diagnostic Ltd. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities assessment kits were purchased from 'Span Diagnostic Ltd. Surat, India'.

Selection of animals and their maintenance

Thirty-six matured normoglycemic wistar strain male albino rats of 3 month of age, weight about 120 ± 10 g with well-ventilated light controlled (12 h light:12 h dark cycle) animal house. Rats were provided protein-rich standard feed and water ad libitum; six animals were kept in each clean and dry polypropylene-made cage. The principle of animal care and directions for experimentation were maintained throughout the experiment, which have been given by our 'Institutional Animal Ethic Committee' (IAEC) which is in conformity with the guidelines of the Committee for Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Govt. of India.

Induction of diabetes mellitus in rats

Rats were made diabetic by single intramuscular injection of STZ (Sigma Aldrich, USA) at a dose of 4 mg/100 g body weight. Streptozotocin was dissolved in 0.1M citrate buffer (pH 4.5). After 7 days of injection, diabetes was confirmed by determining the fasting blood glucose (FBG) level. The rats with FBG levels of 300-350 mg/dl were selected for the experiment as diabetic animal.[8]

Experimental design

Group I (Vehicle-treated control group): This group's rats were orally fed through gavage with distilled water at the volume of 0.5 mL/100 g body weight/day for 28 days.

Group II (Vehicle treated diabetic group): Rats were made diabetic by a single intramuscular injection of STZ at a dose of 4 mg/0.1mL citrate buffer/100 g body weight. This group was oral fed through gavage with distilled water at the volume of 0.5 mL/100 g body weight/day for 28 days.

Group III (Mixture of the fraction treated group): From 7th day of STZ injection, the diabetic rats of this group were forcefully fed by gavage with mixture of the fraction was used for treatment at the ratio of 1:2:1 of *E. jambolana* : *H. antidysenterica*: and *C. sinensis* and the total amount 160 mg /kg body weight per 2 mL distilled water /day for 28 days. Ethyl-acetate fraction of hydro-methanol extract of seeds of *H. antidysenterica* and *E. jambolana*at and methanolic extract of *C. sinensis* were used for the preparation of polyherbal mixture.

Group IV (Parallel treated group): After the development of diabetes, the rats of this group were treated with a mixture of fraction and the antidiabetic drug. The quantity of the fraction was 50% of the therapeutic dose that is 80 mg and one-third part of the prescribed dose of gold standard that is 7 mg. So, the total quantity of the drug was 87 mg/kg body weight/day.

Group V (Antidiabetic gold standard drug treated diabetic group): After 7 days of STZ injection, diabetic rats of this group were treated with glibenclamide at the dose of 20 mg/kg body weight/day.

The duration of entire experiment was 28 days. At 09:00 AM, the oral doses of above-mentioned fractions were given to experimental animals before 2 hours of supplying animal feed. At 7:00 PM, the feeds were supplied again after cleaning the feed box. Fraction treatment was started from the 7th day of STZ injection to the diabetic rats. Level of FBG was measured by using single touch glucometer in all the groups on every 7th day.[9] After completion of 28 days of drug treatment, the animals were maintained in fasting condition for overnight and finally taking the body weight on 29th day of post drug treatment period, and then all the animals were sacrificed by decapitation using carbon-dioxide gas for euthanasia. From dorsal aorta, blood was collected by syringe and separates the serum by centrifugation at 3,000 x g for assessment of insulin. Liver, kidney and pancreas were dissected from each animal, washed in normal saline, soaked in blotting paper and stored in deep freezer (-20°C) to measure different relevant biomarkers, genomic and histological sensors in this concern.

Measurement of fasting blood glucose (FBG) level

A single touch glucometer measured fasting blood glucose level. After every six days of treatment (on every 7th day), fasting blood glucose level was recorded in all the groups' animals.

Assessment of serum insulin, insulin receptor and plasma C-peptide

The level of serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) kit for rat (Boehringer Mannheim Diagnostic, Mannheim, Germany). Level of insulin was expressed in μ IU/mL[10]

The quantitative measurement of insulin receptor in hepatic and skeletal muscle, the ELISA kit (Elabscience, USA) was used as per standard procedure.[11] The optical density was measured 450 nm. Insulin receptor in hepatic and skeletal muscle was measured in ng/mL.

The level of plasma C-peptide was measured by an enzyme-linked immunosorbent assay (ELISA) kit for rat (RayBiotech, US).

Evaluation of carbohydrate metabolic enzyme marker

Activities of the key enzyme of carbohydrate metabolism i.e., hepatic and skeletal muscle hexokinase,[12] glucose-6-phosphatase[13] were measured biochemically. Absorbance was measured by a spectrophotometer at 340 nm wavelength.

Evaluation of antioxidant enzyme markers

The assessment of catalase,[14] superoxide dismutase[15] activities in liver and kidney were measured biochemically according to standard protocol, the absorbent was measured at 240 nm in a spectrophotometer.

Analysis of gene expression of Bax, Bcl-2, Catalase, Superoxide dismutase by qRT PCR

For total RNA extraction, we used the kit (Roch Diagnostic, Germany); for synthesis of the cDNA, we used the transcriptor first strand cDNA synthesis kit.[16] For gene expression of Bax, Bcl-2, catalase, and superoxide dismutase of hepatic tissue, we used Light cycler 480 II (Roch Diagnostic, Germany).

Protein expression study by Western blot

According to standard protocol, the western blot technique conducted the protein expression of bax, bcl-2, Hex-l protein. The hepatic tissues were centrifuged for 20 min at 15,000 × g after liquefying in ice cold RIPA (radio-immuno precipitation buffer) and 100 µl protease inhibitor and then the supernatant was collected. The Bradford method was followed (Bradford, 1976) to assess the quantity of protein. After that pre-stained protein marker (5 mL) was used for electrophoresis with protein sample (100 mg/well) on 14% resolving gel for 2 hours at 80 V. Then to check the protein profile, gel was transferred to 'Coomassie Brilliant Blue' stain. After that, the resolved protein was transferred to the PVDF membrane from the gel. With the help of Tris buffer saline (TBS; pH 7.5), the blot was rinsed and blocking was done by 5% non-fat dry milk in Tris buffer saline with Tween 20 (TBST; pH 7.5) overnight. Next day the blot was incubated with primary antibody for bax (20 KD; 1:500 dilutionin TBST) and for bcl-2 (26 KD; 1:400 dilution in TBST) for overnight incubation at 4°C.The rabbit polyclonal antibody anti β-actin (A2066; 42kDa; 1:6000 dilution in TBST) was used to maintain the equal loading of protein. After the incubation period the blot was washed and incubated with horseradish peroxidase (HRP) linked goat anti-mouse, anti-rabbit secondary antibody at the ratio (1:2000) followed by washing with TBST and Tris buffer saline (TBS), and by the help of DAB system, the labeled protein bands were visualized using Lab Works image analysis software (Ver 4.0, UVP Inc., Upland, CA, USA), the density of protein was analyzed.[17]

Evaluation of beta cell population of the pancreas

In the purpose of isolated islet cell population study, flow cytometry has been employed. A standardized procedure did population study of beta cells. After collagenase digestion of pancreas, that tissue was collected and isolated, dissociated and an enzymatic procedure was adopted for beta cell identification by the help of flow cytometer. The islets were separated in HEPES buffered Earle's- Hepes medium (EH) during this process. This medium was supplemented with 2.5% (w/v) bovine serum albumin. The total process was carried out for the separation of beta cells.[18]

Statistical Analysis

The result were analyzed by calculating the mean and standard error of all sensors of different groups. The difference between the mean values were evaluated by Multiple Comparison student's two tail '*t*' test.[19]

Results

Measurement of fasting blood glucose level

FBG levels were significantly increased in STZ-induced diabetic rats in comparison to the control group. After mixture fraction treatment, parallel treatment and treatment by gold standard a significant ($p<0.05$) decrease was noted in FBG levels in respect to the untreated diabetic animals. There was no significant ($p>0.05$) difference was noted in the levels of FBG between glibenclamide-treated and parallel treated groups but a significant ($p<0.05$) differences was noted when compared this two groups with mixture of fraction treated group (Figure 1).

Estimation of serum insulin, insulin receptor and plasma c-peptide

Serum insulin level and insulin receptor level was significantly ($p<0.05$) diminished in STZ induced diabetic group in compare to the vehicle-treated control group. Treatment with the ethyl-acetate fractions mixture, standard drug and parallel drug, serum level of this hormone and its receptor in hepatic tissue were significantly restored towards the vehicle-

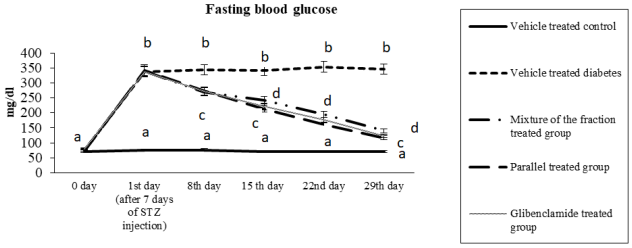

Figure 1: Ameliorative effect of ethyl-acetate fraction mixture of Es, Ha and Cs on fasting blood glucose level in STZ induced diabetic male albino rat in comparison with standard antidiabetic drug, glibenclamide treated group and parallel treated group. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Points on lines with different superscripts (a, b, c, d) differ from each other significantly, $p<0.05$.

treated control level. No significant (p>0.05) difference of this parameter was observed in-between the parallel group and glibenclamide treated groups. But significant (p<0.05) differences was noted in between fraction mixture and parallel group (Figure 2).

In case of plasma c-peptide, the recovery was also noted after treatment of triple-treated groups. But non-significant (*p*>0.05) differences was noted in between the parallel group and glibenclamide treated group but differences was noted when compared with these two groups with the fraction mixture group (Figure 3)

Carbohydrate metabolic enzyme activities in hepatic and skeletal muscle

A reduction in the activity of hexokinase was observed significantly (p<0.05) in vehicle treated diabetic rats in respect to the vehicle-treated control rats. On the other hand, the activity of glucose-6-phosphatase was increased significantly (p<0.05) in vehicle-treated diabetic rats compared to the vehicle-treated control rats. After treatment with a fraction mixture or standard antidiabetic drug i,e glibenclamide or the parallel drug to the diabetic animals, the resettlement was noted in the activities of the said enzymes towards the control at significant (*p*<0.05) level. No significant (*p*>0.05) distinction

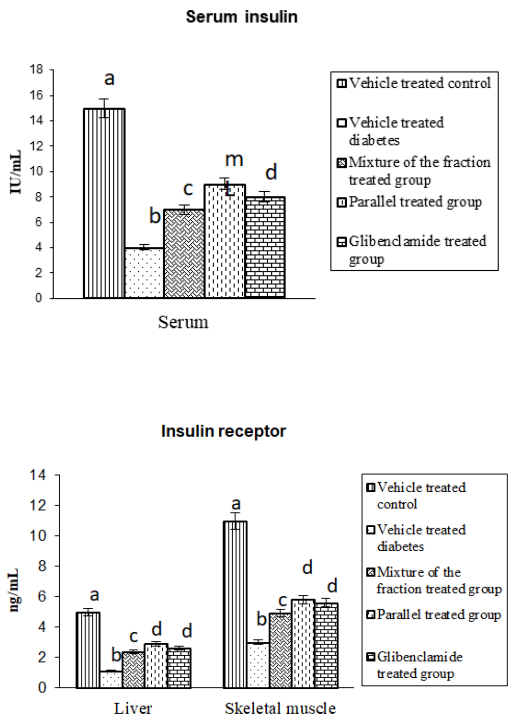

Figure 2: Resettlement in the levels of serum insulin and insulin receptor after treatment with ethyl-acetate fraction mixture of Es, Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d) differ from each other significantly, p<0.05.

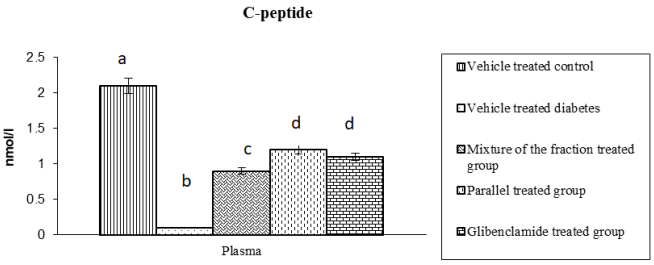

Figure 3: Resettlement in the levels of plasma C-peptide after treatment with ethyl-acetate fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6, ANOVA followed by'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

was observed in the levels of improvement of these parameters in between parallel group and glibenclamide treated group diabetic groups (Figure 4).

Assessment of antioxidant enzyme markers

The activities of antioxidant enzymes i.e. catalase and superoxide dismutase in liver and renal tissues were significantly (*p*<0.05) diminished in the vehicle-treated diabetic rats in respect to the vehicle-treated control rats. After treatment with an ethyl-acetate fraction of *H. antidysenterica* or glibenclamide, a significant recovery (*p*<0.05) was noted to the direction of the vehicle-treated control rats. There was no significant (*p*>0.05) differences in the activities of these enzymes in among the fraction-treated, parallel treated and glibenclamide treated groups (Figure 5)

Transcription of apoptotic genes and antioxidative gene by qRT-PCR study

The levels of transcription of pro-apoptotic gene i,e. bax was upregulated. But bcl-2, the anti-apoptotic gene and antioxidative enzymes' gene i,e. catalase and superoxide dismutase were downregulated in hepatic tissue in the vehicle-treated diabetic group compared with the vehicle-treated control group. A significant (*p*<0.05) resettlement in above gene expression of above genes was noted to the direction of the vehicle-treated control after treatment with fraction mixture or synthetic drug or parallel drug. There was no significant (p>0.05) differences in the recovery of above gene expression was noted in between parallel treated and fraction mixture treated group. But glibenclamide treated group showed less recovery than fraction mixture and parallel group (Figure 6).

Expression of proteins in hepatic tissues

Western blot densitometric analysis of protein of the carbohydrate biomarker Hex-I and anti –apoptotic marker Bcl-2 also indicated decreased level but the pro-apoptotic enzyme Bax in the diabetic rats when compared to control rats. A significant (p<0.05) remedial activity was noted after fraction mixture or glibenclamide or parallel drug on protein

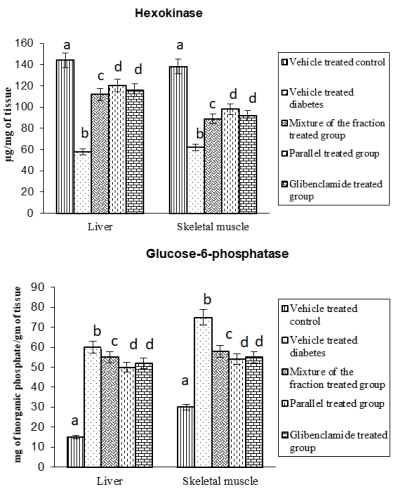

Figure 4: Resettlement in the activities of hexokinase and glucose-6-phosphatase in hepatic tissue and skeletal muscle after treatment with fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6. ANOVA followed by 'Multiple comparison Student's two tail-'t'-test' Bars with different superscripts (a, b, c, d) differ from each other significantly, $p < 0.05$.

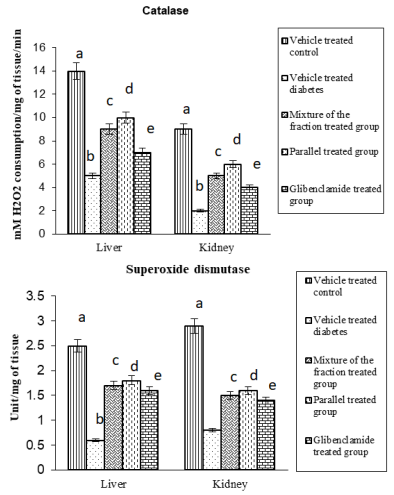

Figure 5: Amelioration in the activities of catalase and superoxide dismutase in hepatic and renal tissue after treatment with fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, $p<0.05$.

level in the target tissue to the diabetic animals (Figures 7, and 8).

β cells population study

The number of pancreatic β cells in percentage was significantly ($p<0.05$) diminished in vehicle treated diabetic group compare with the vehicle treated control group. After treatment with different kinds of drug a positive result towards control was noted. Non- significant ($p>0.05$) differences were noted in between parallel treated group

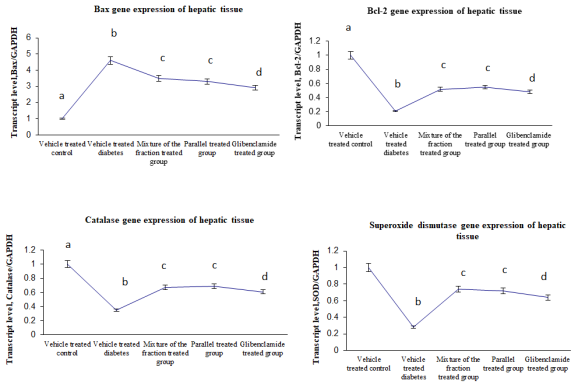

Figure 6: expression pattern of bax, bcl-2, catalase, superoxide dismutase in hepatic tissue after oral administration of ethyl-acetate fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d) differ from each other significantly, $p <0.05$.

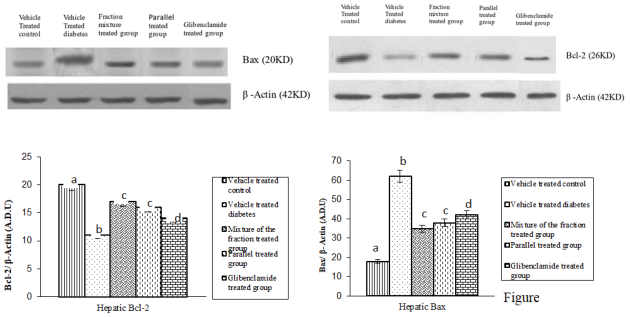

Figure 7: Western blot analysis of bax and bcl-2 protein in hepatic tissue of vehicle treated control, vehicle treated diabetic and ethyl-acetate fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats were quantified by western blot. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d) differ from each other significantly, $p<0.05$.

and glibenclamide treated group but there was slightly differences was noted in between fraction mixture and other two groups (Figure 9).

Discussion

The most toxic reagent to β-cells is STZ which is used widely to induce diabetes in animals.[20] Streptozotocin-induced diabetes has been proved by significant elevation of FBG level and diminished level of serum insulin.[21] Due to low level of insulin, the glucose can't metabolize in the cells and the FBG level increased drastically in diabetic rats. In this experiment, the increased FBG level was recovered after ethyl-acetate fraction and standard drug treatment towards vehicle-treated control, this recovery has been occurred due to direct stimulatory effect of above said fraction on pancreatic β-cells to release insulin.[22]

Glibenclamide is a synthetic antidiabetic drug. It is used wildly that helps in glucose metabolism and manage the

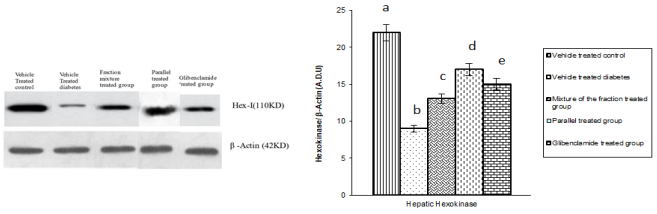

Figure 8: Western blot analysis of hex-I protein in hepatic tissue of vehicle treated control, vehicle treated diabetic and ethyl-acetate fraction mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats were quantified by western blot. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparison Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p<0.05.

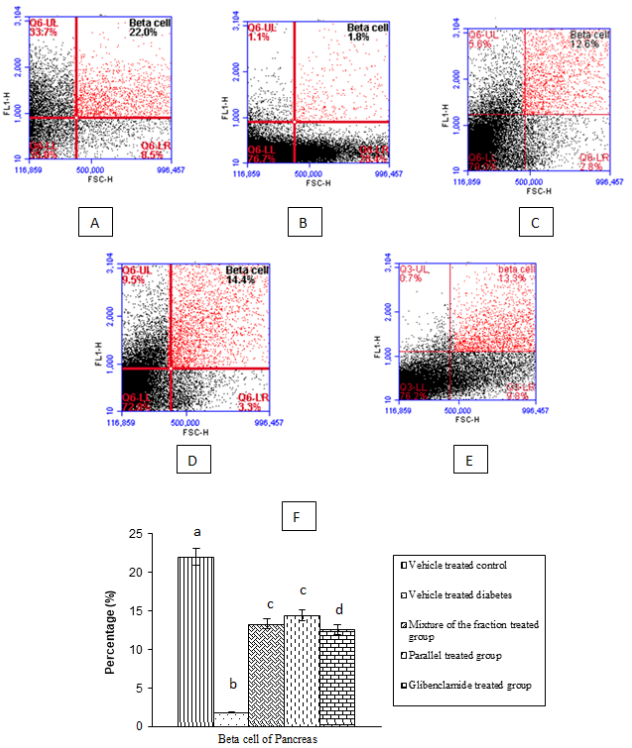

Figure 9: Comparative analysis of β cells population in (A) vehicle treated control, (B) vehicle treated diabetic, (C) ethyl-acetate fractions mixture treated group (D) Parallel treated group (E) glibenclamide treated group (F) Bar diagram of β cells in vehicle treated control, vehicle treated diabetic, mixture of Ha and Cs or standard antidiabetic drug, glibenclamide or parallel drug to diabetic rats. Values were expressed as Mean ± SEM, n=6, ANOVA followed by 'Multiple comparisons Student's two tail-'t'-test'. Bars with different superscripts (a, b, c, d) differ from each other significantly, p<0.05.

complications related with diabetes. The mode of action of this drug is several such as by inhibition of ATP-sensitive K+ channels, which leads to depolarization of the cells and insulin secretion.[23] The same mechanism of action are also exerted in extra pancreatic action of drug at liver, skeletal muscle, heart muscle and smooth muscle sites. This study was further supported by the elevation in the numbers of insulin receptors, which helps transport more free glucose from blood to liver after glibenclamide treatment compared with untreated diabetic condition.

In type 1 diabetes state, insulin production is insufficient and for this reason the sufficient amount of C- peptide is not present in plasma But after treatment with above said different parts of antidiabetic plants the recovery in serum insulin and insulin receptor in hepatic and skeletal muscle and c-peptide levels were observed. The reason of this resettlement in above said sensors towards control may be due to elevation in the numbers of β cells. Ethyl-acetate fraction of *C. sinensis*, *E. jambolana and H. antidysenterica* treatment, have either insulinotropic activity or can stimulate the generation of β cells from stem cells or maystimulate the β cells to release the insulin.[22]

In diabetic state, the most important enzymes for carbohydrate metabolism i,e hepatic hexokinase activity was decreased and glucose-6-phosphatase activity was increased, but after treatment with herbal fractions or synthetic drug to the diabetic rats, the improvement was noted in both of the cases towards the vehicle treated control. The possible reason of this, the above said plant parts contain active phytomolecules with antidiabetic properties and the insulinotropic activity by which the glucose transportation was going on from blood to muscle for metabolism.[25,26] The improvement of hexokinase activity level was further supported by its genomic and proteomic study

To manage the free radicals-induced oxidative stress and cellular damage, the antioxidative enzymes i,e catalase and superoxide dismutase play an indisputable role. But in diabetic state, the levels of stress are elevated extremely in the cellular part of the body and the antioxidant enzyme system unable to prevent the cellular damage. For this reason in oxidative stress induced diabetes, the activity level of these antioxidative enzymes were downstream. In the diabetic state, the cellular apoptosis was also increased, possibly due to up ward expression of pro-apoptotic gene i,e bax and downward expression of anti-apoptotic gene bcl-2. Here hepatic cell apoptosis has been considered from the viewpoint of hepatic stem mother cells for the generation of β cells in the pancreas, which is very much relevant for managing diabetes.[27] After treatment with the mixture of said fractions or standard drug or parallel drug, the recovery in gene expression of antioxidant enzymes and pro and anti-apoptotic genes were noted towards the control state. The proteomic study of this pro and anti-apoptotic protein also supported this result.

The important part of this investigation was the parallel treatment regimen as per WHO directive to find out the therapeutic efficacy of the sub-therapeutic dose of the drug. It has been noted that when one-third dose of glibenclamide given with half of the effective dose of fraction mixture, the results of all the sensors did not differ significantly than the therapeutic dose of glibenclamide. This suggests that this may be due to the synergistic effects of this drug and the phytomolecule(s) present there. This strategy also unfolds the possibilities of delaying drug resistance as well as the drug toxicity and side effects in normal and hyperactive individuals. It also lowers the bio-burden on liver and kidney.

Conclusion

After evaluation of the results, it may be stated that, this herbal fraction i,e ethyl-acetate fraction of *E. jambolana,C. sinensis* and *H. antidysenterica* has more or less same antidiabetic characteristics like a synthetic drug for the management of complications associated with diabetes without producing any side effects. The efficacy of this herbal drug mixture was enhanced when prescribed parallely with the synthetic drug that delay the drug resistance.

Acknowledgement

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Conflict of Interest

We have no conflict of interest.

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