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Original Research Article

Antidiabetic potentials of herbal content of 1960 drink and effect on liver enzymes in streptozotocin-induced diabetic Wistar rats

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Most herbal formulation are preserved and served in alcohol concentrations now named bitters. Whether the resultant effect is herbal dependent or alcoholic, remains an issue of concern. The aim of this study therefore, was to find out the effect of 1960 herbal alcoholic consumption on blood glucose level, glucose 6-phosphate dehydrogenase (G6PD) activity, lactate dehydrogenase (LDH) and serum liver enzymes levels. Twenty (20) male Wistar rats weighing between 180g-300g were randomly selected into four groups containing five rats each. Group 1(Control) received normal rat feed and water. Group 2 (Diabetic untreated) received intraperitoneal injection of 60mg/kg body weight of streptozotocin. Group 3 (Diabetic treated) group received 3mg/kg body weight of 1960 herbal drink extract while Group 4 received 3mg/kg body weight of 1960 herbal drink extract only. Treatment lasted for 28 days. Phytochemical study of 1960 drink revealed the presence of flavonoids, alkaloids, terpenoid, saponins, and cardiac glycosides. There was a significant ($p<0.01$) decrease in G6PD, increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine phosphatase (ALP) and lactate dehydrogenase (LDH) in the diabetics. The 1960 herbal alcoholic drink significantly ($p<0.05$) reduced blood glucose level, serum AST, ALT and ALP and increased albumin concentration and body weight when compared with the non-diabetic groups. The spectrum of alterations from these results is an indication that 1960 herbal drink contains hypoglycemic agents which enhances its antidiabetic effect and protects the functional integrity of the liver.

Key words: Hyperglycemia, streptozotocin, 1960 herbal drink, Glucose-6-phosphate dehydrogenase, lactate dehydrogenase, liver enzymes**.**

INTRODUCTION

The desire for man to cure or look for a more result oriented and sustainable management of metabolic disorders such as Diabetes mellitus and associated cardiovascular problems has led to heightened curiosity in the quest for herbal derivatives or products to sustain life. Sometimes, depending on the life style and hereditary factors, chronic metabolic syndrome may result (Jayakar and Suresh 2003). In such conditions characterized by hyperglycemia and insulin deficiency, Diabetes mellitus may result. Hyperglycemia may however, perpetrate bodily dysfunction beyond just insulinaemia nephropathy and retinopathy reported in other literature.

In experimental diabetes where the use of streptozotocin and alloxan is common, various reports have shown that these diabetogenic agents cause activation of stress-sensitive intracellular pathways and potentiate hyperglycemia and glucotoxicity, formation of free radicals such as reactive oxygen species and nitrogen species, that may progressively alter the balance in body immune system and cause cellular damage (Brownlee, 2001; Rosen et al., 2001; Evans et al., 2002; Bahorun et al., 2006; Halliwell, 2007). Research has also implicated hyperglycemia in the suppression of G6PD activity via activation of cAMP/PKA signaling pathway which can lead to the inhibition of the production of nicotinamide adenine dinucleotide phosphate reductase(NADPH) (Kamal et al., 1998; Zhang et al., 2000; Xu et al., 2005; carette et al., 2011). Such G6PD-induced reduction in NADPH results in oxidative stress (Xu et al., 2005) and a distorted antioxidant system.

In the treatment of diabetes, recently attention has shifted to the use of herbal medicine because of the chemical constituents they contain that produce efficacy in physiological systems (Edeoga et al., 2005) to compliment the orthodox medicine that has over the years gained recognition. Most of the herbal formulations are taken in the form of alcoholic beverages or bitters. The use of bitters can be traced back to ancient Egypt who mixed herbs in jars of wine and later in the 19th century, when there was a renaissance in pharmacognosy, increased the concentration of the herbal bitters and tonic preparations and used it as preventive medicine(*"Ancient Remedy: Bitter Herbs and Sweet Wine". 2013).*

In most parts of Nigeria, the 1960 herbal alcoholic drink is consumed as a social drink and ignorant of its efficacy either as a causative agent to some physiological dysfunctions or whether it may potentiate beneficial bodily reactions. Both the healthy and the physiologically challenged (Diabetics & hypertensives) consume these bitters in good quantity. This form of bitters are also available as digestive or cocktail bitters in Ghana as "Alomo"; in Italy it is sold as "Campari" and "Amaro Lucano", in Germany it is St. Vitus in USA it sold as "Calisaya" and "Tubi 60" in Isreal ("*A Brief history of Bitters". smithsonianmag.com 2013*). There is lack of information on how the herbal drink affects the physiological state of man. The interest in this study therefore, is to find out the pharmacological effects of this herbal drink (1960) on glycemic control and liver enzymes as well as the integrity of the pancreas and the liver.

MATERIALS AND METHODS

Herbal Drink and Extraction of Content

1960 herbal alcoholic beverage was purchased from the provision store within Okuku,Village in Yala Local Government Area, Cross River State, Nigeria. About 1,400ml of the herbalized alcoholic drink was poured in two 1000ml beaker and subjected to concentration in a water bath regulated at 35℃ . The slurry was then subjected to phytochemical screening to know the bioactive constituents in the herbal drink.

Phytochemical screening

Chemical tests were carried out on the alcoholic mixture of 1960 herbal drink using standard procedures to identify the constituents as described by Trease et al. (1989) and Sofowara, (1993).

The phytochemicals tested for include flavonoids, glycosides, tannins, alkaloids, saponins, steroids and terpinoids) using the method described by Van-Burden and Robinson (1981); Trease and Evans (1989).

Quantitative determination of the phytoconstituents

The alkaloid was quantitatively determined by the method of Van-Burden and Robinson (1981) tannins and saponins and flavonoid were determined by the method described by Boham and Kocipai-Abyazan (1974) and Obadoni and Ochuko (2001).

Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ, Sigma, USA) in citrate buffer (0.1 M, pH 4.5) at a dosage of 60 mg/kg body weight. Diabetes was confirmed by stable hyperglycemia above 140mg/dl, in the tail blood glucose after three days of STZ injection using a glucometer (Accu-Chek, Roche, Germany).

Biochemical assays

The serum aspartate aminotransferase (AST) otherwise known as serum glutamic oxaloacetic transaminase (SGOT) was determined by the method described by Kind and King, (1972), serum ALP was also assayed with a Randox kit using the methods of Reitman and Frankel (1957). Determination of G6PD and LDH was done using the manufacturer's instruction.

Experimental design

Twenty adult male Wistar rats, weighing between 180g–250g used in this study were obtained from the animal House, Physiology Department, Cross River University of Technology, Calabar, Nigeria.

The animals were housed in plastic cages and kept in room temperature of 30°C ± 4°C with 12 h light/dark cycle. The animals were divided into 4 groups containing 5 rats each. Group 1(Control) received normal rat feed and water. Group 2 (Diabetic untreated) received intraperitoneal injection of 60mg/kg body weight of streptozotocin. Group

Table 1. Qualitative and quantitative analysis of 1960 herbal alcoholic beverage

Sample	Variable	Presence/absence	Percentage (%)concentration
	Alkaloid	-	0.2%
	Saponin	+++	10.8%
	Tannin	-	-
1960 herbal	Flavonoid	+	3.4%
alcoholic beverage	Steroid	-	
(HAB)	Terpenoid	+	0.4%
	Cardiac glycoside	+	-
	Carbohydrate	++	-
	Reducing sugar	+	-
	Protein	-	-

Key: + (Trace/Mildly positive); ++ (moderately positive); +++ (abundantly positive;- (negative)

3 (Diabetic treated) group received 3mg/kg body weight of 1960 herbal drink extract while Group 4 received 3mg/kg body weight of 1960 herbal drink extract only. The dose of 3mg/kg body weight was chosen based on our preliminary investigation. This was a more effective dose among the doses (1mg, 2mg, and 3mg/kg bwt) investigated in our preliminary study. HAB was administered by gastric gavage daily for a period of 28 days. At the end of the treatment period, the rats were weighed and then anaesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). Blood samples were collected via cardiac puncture into EDTA -containing tubes. Blood samples were centrifuged at 3000rpm for 10 min at 4^{o}C and serum collected for biochemical analysis. The liver and pancreas were dissected, rinsed in cold phosphate buffered saline (10 mM, pH 7.2) and preserved for histology work. The handling of the experimental animals in this study followed the principles of the declaration of Helsinki.

Histology

Pancreas and liver tissues were fixed in 10% (v: v) neutral buffered formalin and embedded in paraffin wax. Fixed tissues were cut into 5µm slices. After being the removal of paraffin using xylene and ethanol dilutions and rehydration, tissue sections were stained with hematoxylin and eosin (H&E). Mounted slides were examined in a masked fashion under a light microscope and photomicrograph taken using a digital camera.

Statistical analysis

All data collected were expressed as mean ± standard error of mean (Mean±SEM). The Graphpad version 5 statistical package was used for this analysis. Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA). The Bonferronis multiple comparison test was used. Differences were considered significant at $p<0.01$.

RESULTS

In this study, we carried out the phytochemical analysis of the sample to find out the presence of some relevant components. Our result revealed the presence of medicinally active substances such as Saponins, flavonoids, terpenoid, cardiac glycosides, carbohydrates and reducing sugar as shown in Table 1. Alkaloids, tanins, steroids and proteins were minimal.

Quantitative and qualitative analysis of the 1960 alcoholic beverage extract is summarized in Table 1. The percentage availability of Saponin was as high as 10.8%, Flavonoid was 3.4% while tannins and alkaloids were 0.3 and 0.2 % respectively.

Effect of herbal alcoholic beverage (1960 drink) on blood glucose level (mg/dl)

The result of blood glucose level following a single dose injection of streptozotocin (60mg/kg body weight) and subsequent treatment with 1960 herbal drink extract (3mg/kg) is shown in Figure 1. Blood glucose level in the diabetic untreated was significantly ($p<0.01$) increased compared to all other groups. Treatment with 1960 herbal extract significantly ($p<0.01$) lowered the glucose concentration. Blood glucose concentration in the HAB group only had similar glucose level with control.

Effect of herbal alcoholic beverage (1960 herbal drink) on Glucose-6-phosphate dehydrogenase of diabetic and nondiabetic rats

In this study, glucose-6-phosphate dehydrogenase (G6PD) activity was significantly ($p<0.01$) reduced in the diabetic untreated groups compared to control. This result is shown in Figure 2. Treatment with 1960 herbal extract (DM+HAB) significantly($p<0.05$) lowered G6PD.

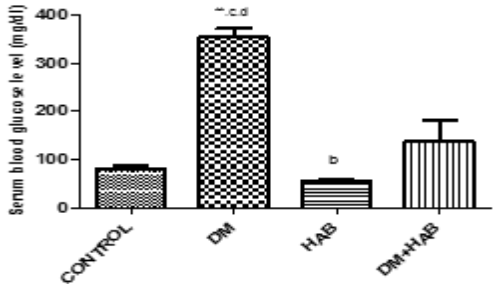

Figure 1: Effect of herbal alcoholic beverage (1960 drink) on blood glucose level (mg/dl)

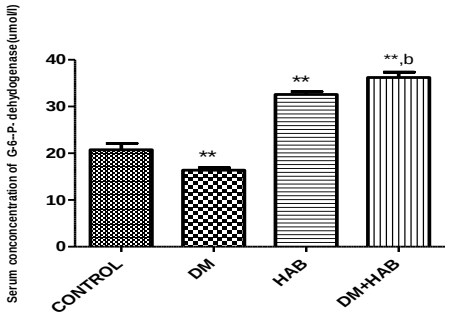

Figure 2: Effect of herbal alcoholic beverage (1960 herbal drink) on Glucose-6-phosphate

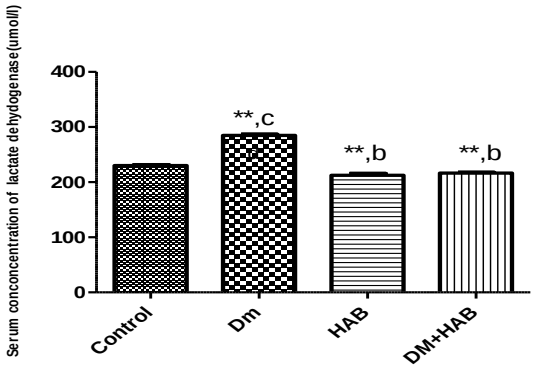

Figure 3: Effect of herbal alcoholic beverage (1960 herbal drink) on lactate dehydrogenase (LDH) of diabetic and non-diabetic rats.

Effect of herbal alcoholic beverage (1960 herbal drink) on lactate dehydrogenase (LDH) of diabetic and non-diabetic rats

The effect of herbal alcoholic beverage (1960 herbal drink) on lactate dehydrogenase(LDH) activity is represented in Figure 3. The LDH activity was significantly ($p<0.01$) increased in the diabetic untreated (Dm) compared to control.

LDH level became reversed in the treatment group

Effect of herbal alcoholic beverage (1960 drink) on average body weight changes (every 5 days) in STZ-induced Diabetic rats

Average body weight changes in the diabetic and non-diabetic rats is shown in Figure 4. Mean body weight of the diabetic untreated rats at the end of 28 days was drastically

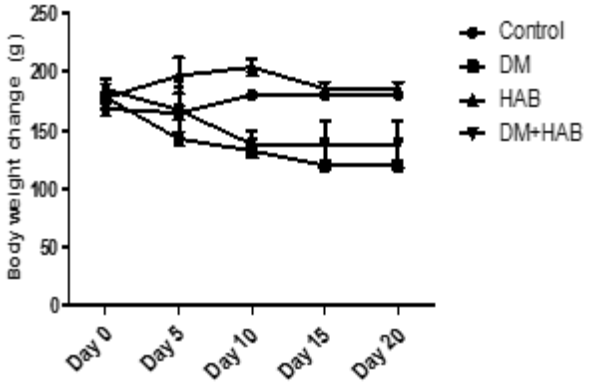

Figure 4: Effect of herbal alcoholic beverage (1960 drink) on average body weight changes (every 5 days) in STZ-induced Diabetic rats.

Table 2. showing effect of herbalized alcoholic beverage (HAB)- 1960 herbal alcoholic drink, on liver enzymes in streptozotocin induced diabetic rats.

Variable	Control	Dm	Dm+HAB	HAB
AST(mmol/l)	29.40±0.67	47.20±2.70a,c,d	31.40±1.07b	29.60±1.36b
ALT (mmol/l)	28.80±2.85	39.20±1.06a	28.00±1.64b	26.60±0.67b
ALP (mmol/l)	57.40±0.97	68.40±0.74a,c,d	59.40±1.66b	56.40±1.93b
ALBUMIN(g/l)	25.60±0.87	23.00±0.31c,d	33.80±0.58b	38.00±0.63b,c

Values are expressed in Mean±SEM, n=5, a=p<0.05vs control; b=p<0.05 vs Dm; c=p<0.05 vs diabetics treated(Dm+HAB); d=p<0.05 vs HAB

reduced (p<0.01). Supplementation with 1960 herbal alcoholic extracts significantly (p<0.01) increased rats body weight.

Effect of 1960 herbal drink extract liver enzymes of diabetic and nondiabetic rats

The effect of 1960 herbal drink on serum AST, ALT, ALP is shown in Table 2. There was a significant (p<0.01) increase in AST, ALT, ALP concentration in the diabetic group when compared with control. AST and ALT were lowered significantly (p<0.01) in the diabetic treated group (DM+HAB). Albumin was significantly (p<0.01) increased in the DM+HAB and the normal control.

Pancreas and Liver histology

Figures 5 and 6 shows the histology of the pancreas and liver in STZ-induced diabetic rats and supplementation with Herbal alcoholic beverage (1960 drink). In Figure 5, Diabetic untreated pancreatic cells showed reduced cytoplasm, condensed nuclei and altered acinar cells with dilated pancreatic islet. The diabetic treated (DM+HAB) rats showed poorly differentiated parenchyma with high grade morphology, dark and patchy cytoplasm.

In Figure 6, Diabetic untreated liver cells showed enlarged central vein (ECV) surrounded by numerous hepatic cells (H), and sinusoidal block. Diabetic treated (DM+ HAB) showed numerous dilated sinusoids, marked dilation of sinusoidal blood channels and dilated central vein. HAB treatment alone did not display any pathological lesion.

DISCUSSION

Diabetes mellitus is a debilitating endocrine disorder in both developed and developing countries that has over the years attracted concern. Various experimental reports involving the use of streptozotocin and alloxan as diabetogenic agents have shown that this substances potentiate hyperglycemia, lipotoxicity, and release of free radicals (Reitman and Frankel, 1957) all of which contribute immensely to the breakdown in the antioxidant defense system and progressively promote the degeneration of such vital organs such as the liver, pancreas, kidney and muscles as a result of generated reactive oxygen species (Ďuračková et al., 2010 ; Oyenihi et al., 2014).

In this study, we investigated the effect 1960 herbal alcoholic drink consumption on blood glucose level, glucose 6-phosphate dehydrogenase, lactate dehydrogenase and

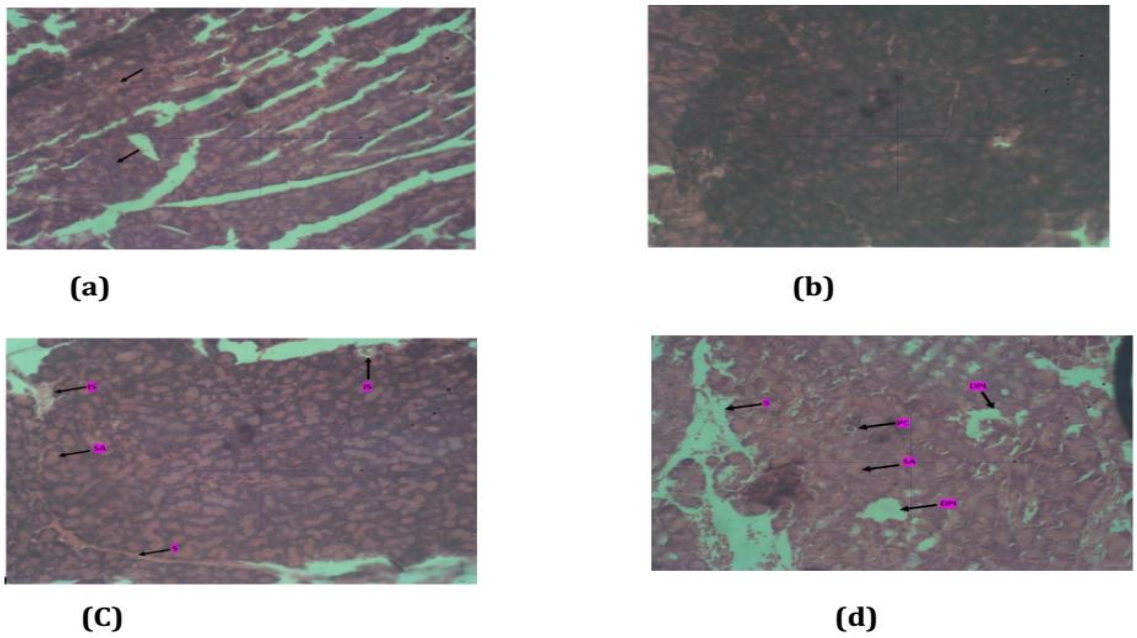

Figure 5: Showing photomicrogragh of the pancreas in STZ- induced diabetic rats and supplementation with 1960 herbal drink in (A) control; (B) Diabetic rats showing parenchyma with dark and patchy cytoplasm, altered acinar cells and dilated pancreatic islet (DPI) (C) HAB only showing Islets with numerous serous secretory acini (SA) no pathology. (D) DM+HAB rats showing slightly reduced serous secretory acini. Haematoxylin-eosin: 100x**.**

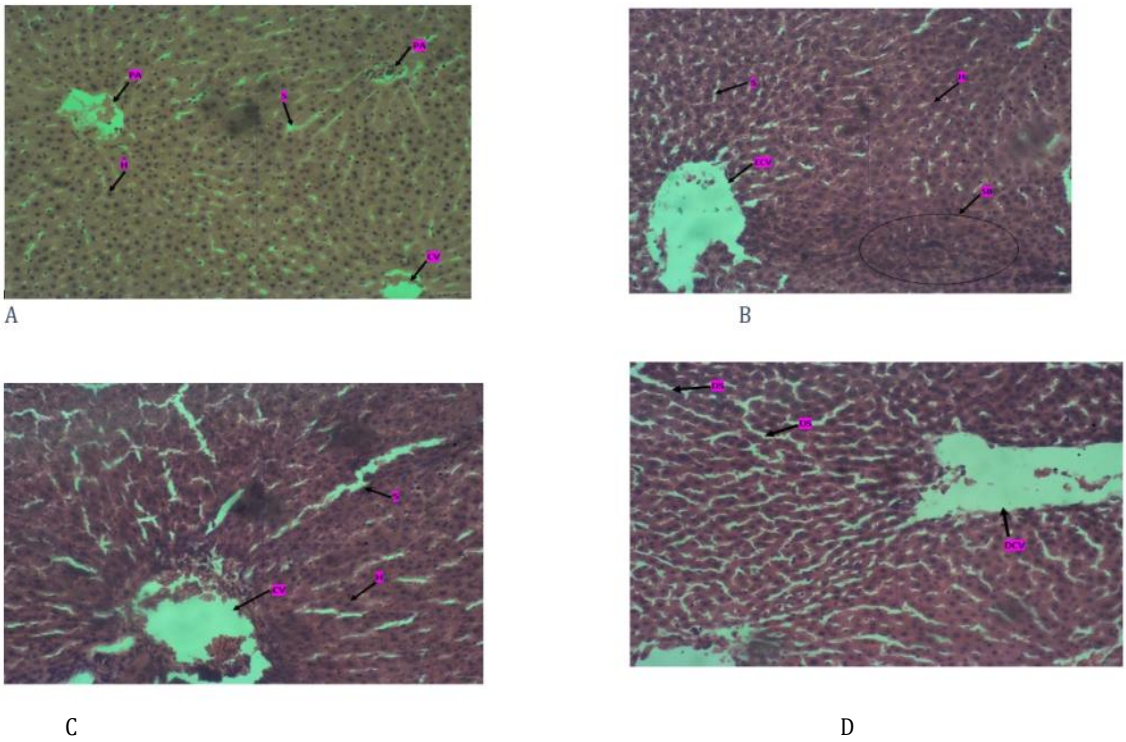

Figure 6: Showing photomicrogragh of the Liver in STZ induced diabetic rats and supplementation with 1960 herbal drink in (A) Control rats (B) Diabetic, rats with enlarged central vein (ECV) surrounded by numerous hepatic cells (H), and sinusoidal Block (SB) (C) HAB rats with numerous hepatic cells (H),and sinusoidal channels (S), no pathology. (D) DM+ HAB rats showing numerous parenchymal hepatic cells, minimal dilation of sinusoidal blood channels and central vein (DCV). X160. H

liver enzymes.

Recently, interest in orthodox medicine has shifted partially to the use of natural plant products as antidiabetic agents because of their low side effect and varied physiologic actions (Karasu et al., 2010). In this study, we recorded a significant ($P<0.01$) increase in blood glucose level in the untreated diabetic group compared to control as has earlier being reported by many research works (Weir et al., 1981; Nagappa et al., 2008). Amazingly, treatment with herbal alcoholic beverage (1960 herbal drink) extract lowered the blood glucose level. From our phytochemical analysis of the herbal content of the 1960 drink, we noted the presence of some constituents like saponins, flavonoid, cardiac glycosides, reducing sugar and minimal quantity of tannins. The physiological impact of these bioactive components have been widely implicated in the management of health problems and disease (Nagappa et al., 2003; Etah et al., 2018)

For instance, the activities of these bioactive constituents from various herbal formulation have been reported to be involved in glycemic control(Etah et al., 2018) by stimulating the action of glycolytic and glycogenic enzymes or by inhibiting glucose-6-phosphatase resulting in increased glucose uptake into the cells and reducing the rate of glycogen breakdown (Naik et al., 1999; Waltner-Law et al., 2002; Sarkhail et al., 2007). The presence of flavonoid and appreciable percentage of saponin in the herbal formulation of 1960 alcoholic drink, may be responsible for the hypoglycemic effect.

Furthermore, our results showed increase in concentrations of aspartate aminotransferase(AST), alanine aminotransferase(ALT) and alanine phosphatase(ALP) in the streptozotocin-induced diabetic rats. Usually, increase in serum level of these enzymes is often used as a measure of possible compromise of the structural and functional integrity of the liver [Dasofunjo, 2013]. Treatment with the herbal content of the 1960 drink significantly reduced the serum concentration of the liver enzymes.

Various experimental reports have also shown that hyperglycemia can lead to a decrease in Glucose-6-phosphate dehydrogenase (a rate limiting enzyme of the pentose phosphate pathway which can lead to the formation of ribose-5-phosphate as well as nicotinamide adenine dinucleotide phosphate) or that its deficiency could be a risk factor for the occurrence of diabetes (Carette, 2011).

In hyperglycemia, G6PD activity in experimental animal models is reduced and this inhibition may occurs via phosphorylation as a result of hyperglycemic-induced cAMP-PKA activation Kamal et al., 1998; Zhang et al., 2000; Xu et al., 2005; Leopold et al., 2001) Such reduction in G6PD activity lowers NADPH production and therefore increase oxidative stress(Yizhen et al., 2005). NADPH is pivotal to the maintenance of a relatively balanced antioxidative system and acts a co-factor in catalase, a strong scavenger of free radicals and protector of hydrogen peroxide toxicity (Kirkman et al., 1999).

In this study, Glucose-6-phosphate dehydrogenase activity was significantly higher in the diabetics treated with herbalized alcoholic beverage extract and HAB (in normoglycemics) compared to control and diabetic untreated rats. Our results supports the argument that hyperglycernia reduced G6PD activity when compared to the nondiabetic groups (Rashidi et al., 2009). Lactate dehydrogenase enzymes, a common marker of structural integrity or injuries to the pancreas and disease such as heart failure and myocardial infarction was lowered by the herbal constituent of 1960 thus presenting a hepatoprotective picture.

CONCLUSION

The spectrum of alterations from these results is an indication that 1960 herbal drink contains hypoglycemic agents which enhances its antidiabetic effect and maintains the functional integrity of the liver.

Disclosure Statement

The authors report no conflict of interest.

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