JHER Vol. 30, No. 2, December 2023, pp. 163 - 175

Biochemical Effect of Aqueous Extracts of Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) and African Black Pepper *(Piper guineense)* on Blood Glucose Level of Alloxan Induced Diabetic Albino Wistar Rat

[1]Nnam Ngozi M., [2]Okoye Obiageli I.
[1]Department of Nutrition and Dietetics, University of Nigeria, Nsukka
[2]Department of Home Science and Management, University of Nigeria, Nsukka

Corresponding Author**:** obiageli.okoye@unn.edu.ng

Abstract

The study investigated biochemical effects of aqueous extracts of turmeric, ginger and black pepper on blood glucose levels of induced diabetic albino wistar rats. Specifically, it determined effects of aqueous extracts of turmeric, ginger and black pepper on fasting blood glucose level of diabetic albino rats and body weight of the rats. Sixty- six healthy rats (138-144 grams) were used. Rats were distributed into 11 groups (8 test and 3 control groups) of six rats. Blood glucose level of the rats were determined before diabetes induction. Rats underwent 16 hours overnight fasting prior to induction. They were induced with alloxan injection based on body weight. Diabetes was confirmed using glucometer. Rats with fasting blood glucose level ≥ 200gm/dl were considered diabetic. Various doses of the aqueous extracts were administered orally to different groups based on body weight. Blood glucose level was measured on day 1,7, 14, 21 and 28. Data were analysed as mean ± standard deviation. Analysis of variance (ANOVA) was used to test for differences among all the experimental groups. Duncan's New Multiple Range Test was used to separate and compare means for significant differences. A p – value of < 0.05 was considered statistically significant. The study revealed significant ($p < 0.05$) decrease in blood glucose level of treated rats compared to the untreated (diabetic control) rats. The group treated with combined extracts of turmeric and black pepper with ratios 80:20 (TBP) (80: 20) had the highest reduction in blood glucose level (35.93%) than the other test groups. The group treated with TBP (80:20) had the highest increase in bodyweight (4.08%) compared to the other groups. The findings showed antihyperglycemic activities of these extracts and suggest they may be useful in control of postprandial rise in blood glucose particularly in diabetic condition.

Key Words: Diabetes, Glucose, Alloxan, Aqueous, Extracts, Antihyperglycemic, Bodyweight.

Introduction

Diabetes mellitus is one of the most common endocrine diseases that can affect blood sugar. It is caused by a breakdown in the glucose metabolic process which can result in abnormal blood glucose fluctuations (American Diabetes Association, (ADA) 2020). This chronic metabolic disease occurs either when the pancreas does not produce enough of the hormone, insulin or when the body cannot effectively use the insulin it produces. This is associated with high blood sugar and usually with passage of sugar in the urine (Anita & Abraham, 2010). This impaired insulin secretion and variable degrees of peripheral insulin resistance can lead to hyperglycemia (Simon & Wittmann, 2019).

High blood sugar is an abnormal state for the body and creates specific symptoms and possible long term health problems. ADA (2018) stated that fasting blood sugar above 125 mg/dL (7 mmol/L) indicates that an individual is diabetic. Acute life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or non ketotic hyperosmolar syndrome. Oxidative stress plays pivotal role in progression and development of diabetes and its complications. Oxidative stress involved in type 2 diabetes mellitus harmfully affects the insulin activity (Ito, Sono & Ito, 2019; Rehman & Akash, 2017). This Oxidative stress is produced by an excess of the reactive oxygen species and could deteriorate the islets β-cells of the pancreas resulting in the reduced release of insulin (Rehman & Akash, 2017).

Unhealthy diet and lack of exercise could lead to diabetes mellitus. A diet high in fat, calories, and cholesterol can increase the risk of diabetes. Consumption of sugar sweetened drinks in excess, high-fat diet and high intakes of saturated fat raise risk of diabetes metabolic syndrome (Malik 2010 et al., 2010; Popkin, 2015). Inappropriate diet can contribute to oxidative stress and thus the risk of many adverse health conditions, including the world's major non-communicable diseases (NCDs) such as coronary heart disease (CHD), type 2 diabetes, breast and colon cancers which shortens life expectancy (ADA, 2020).

Many studies confirmed that medicinal spices and their extracts may have some positive biochemical effect on blood sugar level (Roy & Awasthi, 2017; Zhang, 2015). Biochemical effect can be defined as the response of an organism to chemical changes that may occur in the body (Walker et al., 2011). Aqueous extract can be defined as elicit obtained by separation of medicinally active soluble plant materials or secondary metabolites using water as the solvent or extraction medium. Studies have reported that aqueous extracts of plants are safe, economical and eco-friendly alternative to use in treatment of diseases (Zhu, et al, 2018; Kocaadam & Şanlier, 2017). Medicinal plants and their aqueous extracts are the key bioresources of bio-active compounds and are well endowed with a variety of phytochemicals (Ojo et al., 2020; Evuen, Okolie & Apiamu,

2022). These extracts are currently of considerable importance because of their special attributes as a large source of therapeutic phytochemicals. They played an essential role in human nutrition have been linked to a reduced incidence of chronic health conditions such as diabetes, cancer, cardiovascular diseases and other chronic diseases (Zhu, et al, 2018).

Many traditional plant treatments exist as hidden wealth of potentials. Spices are also rich sources of phytochemical compounds with antioxidant activity which influence nutrition through many pathways (Kocaadam & Şanlier, 2017). Antioxidants have been shown to improve insulin sensitivity and reduce fasting blood sugars. They are compounds that can decrease oxidative stress, scavenge free radicals from the body cells, prevent and eliminate the damage caused by oxidation. Plant foods are rich sources of antioxidants. Foods that are high in antioxidants may reduce the risk of many diseases, including heart disease and certain cancers (Duyff, 2017).

Turmeric is a spice plant of the ginger family known as *Zingiberaceae*. The rhizomes have been used from antiquity as a spice, condiment, a textile dye, medicine and stimulant. Ginger is an underground rhizome that belongs to the family *Zingiberaceae*. It is also a spice with medicinal properties. Black pepper is spice plant from the family *Piperaceae*. The seed is used as a spice, seasoning, bioenhancer and medicine. Bioenhancers are defined as substances that increase the bioavaliability and bioefficacy of active substances in foods or drugs (Patil, et al, 2011). Spices are functional foods that can be demonstrated to have a beneficial effects on certain target functions in the body beyond basic nutritional requirements (Lobo, et al, 2010). Turmeric, black pepper and ginger have been reported to exert anti-hyperglycemic, anti-inflammatory, antioxidant, antiproliferative and anti-angiogenic activities effect both in laboratory animals and human subjects (Yadav, et al, 2013; Yadav, et al, 2016).

Nair (2013) stressed that curcumin in tumeric lowers blood sugar in multiple ways such as stimulating insulin production, improving activity of pancreas cells, improving sensitivity to insulin and stimulating utilization of glucose by the body. *Piper guineense* possess anti-bacterial, antioxidant and bioenhancing properties. Zhu, et al, (2018) revealed that the antioxidant in ginger could be helpful against a variety of health conditions, can manage and may even prevent type 2 diabetes and hypertension. Many oral orthodox drugs are toxic, contain active constituents and have a number of serious adverse effects on health. They can cause metabolic alterations and other degenerative conditions. They often lower blood sugar within range but actually increase morbidity and mortality. Many traditional plant treatments may be more effective, economical, less toxic in the treatment and management of chronic health problems.

Medicinal spices and their extracts are therefore becoming more popular because of their potential efficacy, minimal or no side-effects and

synergistic actions (Panda et al. 2013). Most synthetic oral anti- diabetic agents are toxic, can cause some metabolic alterations and other degenerative conditions (Jackuliak, et al, 2019). Van and Scheen (2015) reported that some of these synthetic anti- diabetic agents may lead to patients becoming overweight or obese. Epidemiological studies have reported that intake of plants rich in various antioxidants and phytochemicals reduce the risk of various chronic diseases (Yadav, et al, 2013; Kocaadam & Şanlier, 2017).

Unfortunately, some of these medicinal spices and their aqueous extracts have not been scientifically validated. Clinical trials using animal models are needed to exploit their therapeutics potentials. This is important for their scientific validation and for use in prevention, treatment and management of diabetes. Animal models such as albino wistar rats are non human species that are usually used in nutrition research. These rat strains have some important roles to play in understanding the aetiology and pathophysiology of diseases, testing herbal efficacy and safety in humans (Clemens, Jansson, Portal, Riess & Nguyen, 2014). Thus, the study investigated the biochemical effects of turmeric, ginger and black pepper aqueous extracts on blood glucose levels of induced diabetic albino wistar rats.

Objectives of the Study

The general objective of the study was to investigate the biochemical effect of aqueous extracts of tumeric, ginger and African black pepper on blood glucose level of alloxan induced diabetic albino wistar rats. Specifically, the study determined:

1. effects of aqueous extract of turmeric, ginger and black pepper on the fasting blood glucose (mg/dl) among the diabetic albino rats
2. effect of aqueous extracts of turmeric, ginger and black pepper on body weight of the diabetic albino rats.

Materials and Methods

Plant materials Collection and identification of samples: Fresh Turmeric rhizome *(Curcuma longa*), black pepper seed (*Piper guineense*) and ginger rhizome (*Zingiber officanale*) were procured from Ogige market Nsukka and were identified in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Processing of samples: Two kilogrammes of each of the three samples (*Curcuma longa,* Zingiber officianale and Piper guineense) were sorted to remove debris and defects. The samples were, carefully washed with running tap water to remove dirt and sand and then allowed to drain in a plastic sieve. The samples were peeled, sliced and was dried in an oven at a temperature of 55°C for 24 hours. The pepper was oven-dried for 4 hours. Each of the three dried samples were ground separately into flour using a high speed electric blender (Soyona, Japan). Samples were then packaged in

plastic air tight containers, labeled and stored under refrigeration for analysis.

Preparation of the extracts: Four hundred grammes (400g) of each of the flour sample (*Curcuma longa, Piper guineense and Zingiber officanale*) were macerated with 800ml of distilled water with frequent shaking for 24 hours. They were filtered and the marc was again subjected to maceration with distilled water for complete extraction. The mixtures were filtered using Whatman No.42 filter paper to obtain the filtrate. After filtration, the aqueous extracts were concentrated with the help of an oven dryer at 50°C to obtain the crude extracts. The extracts were stored in an air tight container and were kept in a refrigerator prior to use. The desired consistency for feeding the rats was later reconstituted with distilled water for a known weight of the dried filtrate to give the required dose which was administered during the study. Water was added to the crude extracts of each sample (turmeric, ginger and African black pepper) in the ratio of 10: 1 (weight/volume). This provided 100mg/ml of each extract.

$$\text{Volume} = \frac{\text{dose (mg/kg) x weight (kg)}}{\text{stock concentration (mg/ml)}}.$$

Procurement and housing of rats: Sixty- six (66) healthy male albino rats with no prior drugs treatment weighing between 138–144 grams were used for the experimental study. The rats were purchased from Department of Veterinary Pathology University of Nigeria, Nsukka. The rats were randomly distributed into eleven (11) groups of six rats each based on body weight. They were allowed to acclimatize for 5 days and were fed with standard pellets. The rats had access to water and feed *ad libitum*.

Each group was allotted into metabolic and standardized rat cages equipped to separate feaces and urine of the animals during the rat study period. The study was carried out in the animal house (metabolic laboratory) of the Department of Nutrition and Dietetics, University of Nigeria Nsukka. They were maintained under standard environmental conditions (ambient temperature (25°C ± 2°C), humidity (45% ± 5%) and 12 hours light and 12 hours dark condition during the study.

Experimental Design: Pure experimental research design was adopted**.** The study was conducted for twenty- eight (28) days. This consisted of four days acclimatization period, 1 day for inducement of diabetes, two days for establishment of diabetes and 21 days of treatment with aqueous extracts. All rats were fed commercial pellet diet (rat chow) and water ad libitum throughout the period of experiment. The daily dose of the extracts that was administered to the rats was calculated from the result of acute toxicity test (LD_{50}). During the study period (28 days), blood samples of the rats were collected on day 1, 7,14, 21 and 28 to determine blood glucose level.

Inducement of diabetes: The blood glucose levels of the rats were determined before the inducement of diabetes. After the acclimatization period of four days, diabetes mellitus

were induced on the fifth day. The rats underwent 16 hours overnight fasting prior to induction of diabetes mellitus. The rats were induced of diabetes using single freshly prepared five percent aqueous solution of alloxan monohydrate powder (chemically called 2, 4, 5, 6-tetraoxopyrimidine). This was dissolved in normal saline solution (0.9g sodium chloride in 100ml). It was intraperitoneally injected to the rats at a single dose depending on body weight of the rats. The rats were allowed free access to 5% glucose solution for 48 hours to overcome the early hypoglycemic phase.

Formulation of aqueous extract of *Curcuma longa* and *Piper guineense* blend: Four different formulations of *Curcuma longa* and *Piper guineense* with different ratios were used to determine the dose response of different concentration *of Piper guineense* on *Curcuma longa*. These were obtained by combining two different concentrations of *Curcuma longa* and *Piper guineense.* Each blend was formulated by combining the two aqueous extracts in different ratios to obtain 100g of each of the samples. These combined extracts were administered based on body weight. The ratios were;

TBP90:10 = Turmeric and black pepper (90:10); TBP80:20 = Turmeric and black pepper (80:20)

TBP70:30 = Turmeric and black pepper (70:30) TBP60:40= Turmeric and black pepper (60:40)

Keys: T= Turmeric; BP= Black pepper

Feeding trial: Feeding trial commenced when diabetes had been established. Sixty-six (66) albino rats were used for the study. Rats that were diabetic were grouped and treated with the extract samples. They were grouped into 11 groups of six rats per group. The rats were fed normal rat chow and water ad libitum throughout the period of the experiment. The rat groups were treated by oral administration of different doses of the aqueous extracts at regular intervals of 12 hours. The treatments (different grams of various extracts, and antidiabetic drug dissolved in water) commenced the day the rats were confirmed diabetic. The treatments were given orally every day via a cannula attached to a syringe for 21 days.

Collection of Blood samples: Blood samples were collected from the animals before and after treatment from the animals. Blood samples were taken from the rats after 48 hours of inducement of diabetes to estimate the blood glucose level and to confirm diabetes. Blood glucose concentration was measured by a glucometer on a drop of blood from the tail. This was confirmed using accu-check glucometer and its test strip. Rats with fasting blood glucose level ≥ 200gm/dl were considered diabetic and were selected for the experiment (Mahmoud, 2013). When diabetes was established, treatment commenced with extracts. The dose of the extracts that were administered were based on mean lethality (LD_{50}) of the extracts.

At the end of the 28 days experimental period, the rats were anaesthetized using anaesthetic ether. The blood samples were used in the

determination of biochemical parameters such as blood glucose levels.

Body weight measurement: The initial individual body weights of the rats were taken at the beginning and at the end of the experiment for all animals to determine weight gain using the following equation. Body weight gain = final weight – initial. The initial and final weights of the rats were measured and recorded using an electronic weighing balance.

Biochemical analysis

Determination of Blood Glucose: Glucometer was used for estimation of blood glucose. Determination of blood glucose was measured by the Trinders principle using One Touch Basic glucometer (Lott & Turner, 1975). Rat's tail was cleaned and pricked with a sharp-pointed surgical instrument (lancet). 0.5 µl of blood was taken using a glucometer strip. The strip is then inserted into a glucometer. The test strip was inserted in the glucometer which automatically turns on. A small quantity of blood was dropped on the top white edge of the test strip. The blood glucose level in mg/dl was read on the meter and recorded.

Data Analysis Techniques: Data were analysed using mean ± standard deviation and analysis of variance (ANOVA). was used to test for differences among all the experimental groups. Duncan's New Multiple Range Test was used to separate and compare means for significant differences. A p – value of < 0.05 was considered statistically significant.

Results

Table 1: Effects of aqueous extract on the fasting blood glucose (mg/dl) of diabetic rats

Groups	Baseline	Endline	% Difference
T_{100}	259.40 ^{d} ± 11.72	237.00 ^{cd} ± 6.63	11.60
T_{200}	235.80 ^{bc} ± 10.85	204.80 ^{b} ± 14.39	14.15
G_{100}	243.80 ^{cd} ± 14.86	201.20 ^{b} ± 20.06	18.47
G_{200}	264.80 ^{d} ± 22.73	205.80 ^{b} ± 19.12	21.38
$TBP_{90:10}$	246.80 ^{c} ± 8.23	224.60 ^{c} ± 9.39	20.01
$TBP_{80:20}$	269.00 ^{d} ± 8.86	195.60 ^{b} ± 17.30	35.93
$TBP_{70:30}$	253.60 ^{c} ± 8.26	223.40 ^{c} ± 8.88	17.91
$TBP_{60:40}$	260.20 ^{d} ± 7.56	241.40 ^{cd} ± 10.48	14.23
Normal control	75.20 ^{a} ± 8.84	74.80 ^{a} ± 8.23	0.93
Standard control	233.60 ^{bc} ± 28.38	166.40 ^{ab} ± 8.14	40.77
-ve control	252.80 ^{c} ± 22.74	293.40 ^{e} ± 9.58	15.06

*n=3. Results expressed as means ±SD . value; %D = percentage difference; * = (P < 0.05); baseline = after induction; end-line = after treatment; increase = ; decrease =*

Keys: T100=rats treated with 100mg/kgBW of turmeric extract; T200= rats treated with 200mg/kgBW of turmeric extract;G100= rats treated with 100mg/kgBW of ginger extract; G200= rats treated with 200mg/kgBW of ginger extract; TBP90:10= rats treated with 90:10mg /kgBW of turmeric and black pepper extract; TBP80:20= rats treated with 80:20mg/kg BW of tumeric and black pepper extract ; TBP70:30= rats treated with 70:30mg/kg BW of tumeric and

black pepper extracts; TBP60:40= rats treated with 60:40mg/kg BW of tumeric and black pepper extracts; Normal Control= rats fed with normal rat chow + water ; standard control=- treated with standard drug (glibenclamide) 0.6mg/kg body weight; negative control= rats fed with normal rat chow and water(induced but not treated)

Table 1 shows the effect of aqueous extracts of turmeric, black pepper and ginger on the fasting blood glucose levels of hyperglycemic rats. The fasting blood glucose level of the rats after induction of diabetes ranged from 233.60mg/dl to 269.00mg/dl with the highest found in the group treated with combined aqueous extracts of turmeric and black pepper at ratio 80:20mg/kg (TBP80:20). (269.00mg/dl). It was observed that initial blood glucose level of group treated with 200mg/kg turmeric extract only (235.80mg/dl) and standard control (233.60mg/dl) were significantly similar at ($p < 0.05$). Moreso, groups treated with combined extract with ratio 70 :30 with bodyweight (253.60mg/dl) and negative control (252.80 mg/dl) were significantly similar at ($p < 0.05$). The final blood glucose level of the treated rats ranged from 166.40mg/dl to 241.40mg/dl. The diabetic control group (negative control) had the highest blood glucose level of 293.40mg/dl. The group that was treated with standard drug (standard control) had the highest percentage decrease (40.77%) when compared with other groups. Among the group that was treated with aqueous extract, group 6 which was the group treated with combined aqueous extract TBP80:20 had the highest percentage decrease of 35.93%. The diabetic control group recorded an increase (15.06%) in blood sugar level.

Table 2: The effect of the aqueous extracts on body weight of the hyperglycemic rats

Groups	Initial weight	Final weight	% Difference
T100	141.80 [a] ±6.69	141.98 [a] ±7.83	1.13↑
T200	139.20 [a] ±9.01	140.40 [a] ±9.10	1.58↑
G100	140.20 [a] ±6.53	140.80 [a] ±6.38	0.71↑
G200	139.00 [a] ±8.19	140.70 [a] ±8.94	1.44↑
TBP90:10	142.60 [a] ±6.84	143.80 [a] ±6.69	0.84↑
TBP80:20	138.00 [a] ±8.76	141.80 [a] ±9.76	4.08↑
TBP70:30	141.80 [a] ±6.69	143.40 [a] ±7.83	3.13↑
TBP60:40	140.20 [a] ±6.53	141.20 [a] ±6.38	1.51↑
Normal control	142.20 [a] ±6.53	146.00 [ab] ±9.63	5.08↑
Standard control	142.20 [a] ±7.40	145.00 [ab] ±6.67	4.09↑
-ve control	144.00 [a] ±9.76	140.00 [ab] ±9.75	5.04↓

n=3. Results expressed as means ± SD. % D = percentage difference; increase = ↑ ; decrease = ↓
Keys: T100 = rats treated with 100mg/kgBW of turmeric extract; T200 = rats treated with 200mg/kgBW of turmeric extract;G100 = rats treated with 100mg/kgBW of ginger extract; G200 = rats treated with 200mg/kgBW of ginger extract; TBP90:10 = rats treated with 90:10 mg/kgBW of turmeric and black pepper extract; TBP80:20 = rats treated with 80:20mg/kg BW of tumeric

and black pepper extract ; TBP70:30= rats treated with 70:30/mg/kg BW of tumeric and black pepper extracts; TBP60:40 = rats treated with 60:40mg/kg BW of tumeric and black pepper extracts; Normal Control = rats fed with normal rat chow + water; standard control =- treated with standard drug (glibenclamide) 0.6mg/kg body weight; negative control= rats fed with normal rat chow and water(induced but not treated)

Table 2 shows the effect of aqueous extracts on body weights of the experimental rats. There was no significant difference ($p < 0.05$) in the body weights of rats used before diabetes induction. The mean body weight before induction ranged from 138.00g to 144.00g. After treatment, the mean body weight of the rats showed no significant difference ($p < 0.05$). None of the extracts caused significant weight increase. The mean bodyweight of the rats treated with aqueous extracts ranged from 140.40g to 143.40g. Among the treatment groups, the group treated with combined extract of turmeric and black pepper TBP (80: 20) had the highest percentage increase in weight (4.08%) when compared with the other rats treated with the extracts. The normal and standard control groups had significant increase in body weight with 5.08% and 4.09% increase respectively. The group fed with combined extract 80:20 (Turmeric: Black pepper) had the highest percentage increase in weight (4.08%) while the group fed with 100mg/kg Ginger extract only had the lowest percent increase (0.71%) in body weight. The negative control group had significant decrease in body weight (-5.04%) when compared with the diabetic treated groups.

Discussion of Findings

The findings of the study showed the effect of black pepper, turmeric and ginger aqueous extracts on the fasting blood glucose level of hyperglycemic rats after treatment period. It showed that there was significant rise in blood glucose levels after induction with alloxan injection in all diabetic rats as compared to the normal control rats. The increase in blood glucose level in the diabetic animals compared to that of the control rats could be as a result of chemical exposure to diabetogenic agent called alloxan. The is in line with works by Jain and Arya (2011) who demonstrated that intraperitoneal injection of alloxan into the rats causes significant diabetogenic response in wistar rats with significant rise in the blood glucose level of the experimental rats. Behl et al. (2020) stated that alloxan induces diabetes through reactive oxygen species which results to a rapid destruction of pancreatic beta cells causing hyperglycemia. The mechanism of action of alloxan is selective destruction of the beta cells of the pancreas through the formation of reactive oxygen species (Jain & Arya, 2011). Fakhruddin, et al, (2017) stated that oxidative stress is the main factor for initiation of various degenerative and chronic diseases. Zhu, et al, (2018) reported that high blood sugar (hyperglycemia) could be attributed to deficiency or impairment in insulin secretion and / or metabolic consequences of insulin resistance

which can affect carbohydrate, protein and lipid metabolism.

All treatment groups treated with aqueous extracts showed decrease in blood glucose level when compared against the diabetic control group. However it was observed that group with combined aqueous extracts of turmeric and black pepper with ratios 80:20 (TBP 80:20) had the highest percentage decrease in blood glucose level compared to the other groups that were treated with aqueous extract. This is in line with the findings by Prasad, et al, (2014) which demonstrated in an animal study that effect of combining turmeric (100mg/kg body weight) with lower dose of black pepper(25mg/kg body weight). This is similar with Manodeep, et al, (2017) in a rat study which demonstrated that incorporation of black pepper with the doses of 25 mg/kg with turmeric 100mg/kg exhibited significant beneficial effect compared to turmeric alone-treated group. Similar observations were made by Sunmonu and Afolayan (2013) using combined aqueous extract of *Phyllantus amarus* and *Artemisia afra* in a rat study respectively, for the treatment of diabetes.

The positive effects of these aqueous extracts on blood glucose level may be attributed to presence of some bioactive constituents (alkaloids, flavonoid, terpenoid, saponin, phenols and other antioxidants vitamin) in the extracts which have dietary and medicinal properties. The anti-hyperglycemic action of the extracts may be attributed to improved insulin sensitivity or inhibition of endogenous glucose production (Nimse & Pal, 2015). The extract may have achieved this anti- hyperglycaemic property via increased insulin secretion, increased peripheral utilization of glucose, inhibition of endogenous glucose production or by inhibition of intestinal glucose absorption (Manodeep, et al, 2017).

The findings on Table 2 demonstrated the change in body weight of the experimental rats. There was slight increase in the body weight of alloxan-induced diabetic rats that were administered various doses of these aqueous extracts when compared with the diabetic control group. Progressive increase in body weight was observed in normal control group and diabetic treated rats respectively when compared with their initial values, whereas the diabetic control group (negative control) recorded progressive decrease in bodyweight. The normal control rats showed body weight gain throughout the treatment period, while the diabetic control rats showed significant ($p < 0.05$) weight loss. The increase in body weight observed in diabetic rats administered aqueous extracts could be an indicated that these aqueous extracts is not toxic and does not have any harmful effect on the physiological state of the rats at the various dose level. This supports the works by Sunmonu and Afolayan (2013) which stated that aqueous extracts are safer, more effective and less toxic compared to synthetic drugs.

A study by Fakhruddin, et al, (2017) showed a significant increase ($p < 0.05$) in the body weight of alloxan-induced diabetic rats that were administered

various doses of phenolic aqueous leaf extract of *V.doniana* when compared with the diabetic control group. The combined aqueous extracts with ratios 80:20 had the highest percentage increase in body weight (4.08%) compared to other groups treated with aqueous extracts. Otunola and Afolaya (2015) reported that oral administration of combined aqueous extract of garlic, ginger and cayenne pepper modulated the body and organ weights of diabetic rats, reduced hyperglycaemia, attenuated blood and cellular toxicity parameters.

Conclusion

The present study provided some information on the biochemical effects of aqueous extracts of turmeric, ginger and black pepper on blood glucose level of alloxan induced diabetic albino wistar rats. It demonstrated that oral administration of these aqueous extracts had positive effects on blood glucose level and body weight of the rats. It revealed that the rats treated with graded doses of these aqueous extracts gained slight weight and no adverse effect was observed. It revealed the synergistic effect of combined aqueous extracts of turmeric and black pepper on blood glucose level and body weight of the diabetic rats. The findings showed that these aqueous extracts possess antihyperglycemic properties in the treated diabetic rats. The study suggests that aqueous extracts of turmeric, ginger and black pepper might help prevent, reduce and manage hyperglycemia in diabetic patients.

Recommendations

(1) Extensive investigations of the active ingredients of these aqueous extracts on clinical trials with human are needed to exploit their therapeutics utility to cure many diseases.
(2) Further research should be carried out on:
    (i) effect of high dose consumption of black pepper extracts in humans and animal models.
    (ii) whole samples rather than aqueous extracts to determine their therapeutic potentials.
    (iii) antimicrobial assay of these aqueous extracts.

References

American Diabetes Association (2018). Lifestyle Management: Standards of Medical Care in Diabetes, *Diabetes Care.* ; 41(1): S38-S50

American Diabetes Association (2020). Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-,Diabetes Care. ;43*(1):S14-S31.

Anita, E.P., & Abraham, P. (2010). *Clinical Dietetics and Nutrition*. published in Oxford University Press. New Delhi 110018

Behl, T., Bungau, S., Kumar, K., Zengin, G., Khan, F., Kumar, A., Kaur, R., Venkatachalam, T., Tit, D.M. & Vesa, C.M. (2020). Pleotropic Effects of Polyphenols in Cardiovascular System. *Biomed. Pharmacother*, *130*, 110714.

Clemens, L. E., Jansson, E. K. H., Portal, E., Riess , O.& Nguyen, H. P. (2014).). A behavioral comparison of the common laboratory rat strains Lister Hooded, Lewis, Fischer 344 and Wistar in an

automated home cage system. *Genes Brain Behaviour*.13, 305-321

Duyff, R.L. (2017). *Academy of Nutrition and Dietetics Complete Food and Nutrition Guide, Fifth Edition*. Boston: Houghton Mifflin Harcourt

Evuen, U.F., Okolie N. P. & Apiamu, A. (2022). Evaluation of the mineral composition, phytochemical and proximate constituents of three culinary spices in Nigeria: a comparative study *Science Report*; 12(1): 20705.

Fakhruddin, S., Alanazi, W. & Jackson, K.E. (2017). Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. *Journal of Diabetes Research,* Corpus ID: DOI:10.1155/2017/8379327

Higbea, A.M., Duval, C. & Chastain, L. M. (2017). Weight effects of antidiabetic agents. *Expert Review of Endocrinology & Metabolism* ;12:441–449.

Ito, F.; Sono, Y.; Ito, T. (2019). Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation. *Antioxidants* , *8*,(1) 72.

Jackuliak P., Kužma M. & Payer J. (2019). Effect of Antidiabetic Treatment on Bone. *Physiology Resources*. 68: S107–S120.

Jain, D.K. & Arya, R. K. (2011). Anomalies in alloxan-induced diabetic model: It is better to standardize it first. Indian *Journal of Pharmacology*. 2(1);43:91.

Kocaadam, B., & Şanlier, N. (2017) Curcumin, an active component of turmeric (Curcuma longa), and its effects on health, *Critical Reviews in Food Science and Nutrition,* 57:13, 2889-2895.

Mahmoud, A.(2O13). Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. *Journal of Experimental and Clinical Science.* 12: 647–657.

Malik, V.S., Popkin, B.M., Bray, G.A., Després J.P., Willett, W.C., & Hu, F.B. (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care*. 33(1):2477–83.

Manodeep, C., Ananya,,B.,& Jagadish V.(2017).Cardioprotective effect of curcumin and piperine combination against cyclophosphamide-induced cardiotoxicity. *Indian J Pharmacol*.; 49(1): 65–70.

Nair, K.P. (2013). *The agronomy and economy of turmeric and ginger: the invaluable medicinal spice crops.* London: Elsevier; 2013.

Nimse, S. B. & Pal, D. (2015). "Free radicals, natural antioxidants, and their reaction mechanisms," *Royal Society of Chemistry,* volume 5, no. 35, pp. 27986–28006,.

Ojo, A. O., Osukoya O.A., Ekakitie L .I., Ajiboye B.A., Oyinloye B. E.,Agboinghale P.E. & Kappo, P. (2020). *Gongronema latifolium* leaf extract modulates hyperglycaemia, inhibits redox imbalance and inflammation in alloxan-induced diabetic nephropathy *Journal of Diabetes Metabolic Disorder*. 19(1):469-481.

Otunola, G.A. & Afolayan, A.J. (2015) Antidiabetic Effect of Combined Spices of *Allium sativum*, *Zingiber officinale* and *Capsicum frutescens* in Alloxan-Induced Diabetic Rats. *Frontiers in Life Science* ;-8 , 314-323.

Panda, S.K., Swain, M.A., Singh, S.& Ray R.C. (2013). Proximate compositions of a herbal purple sweet potato (*ipomoea batatas* l.) wine. *Journal of Food Processing and Preservation* 37;7: 596 - 604

Popkin, B. M. (2015). Nutrition Transition and the Global Diabetes Epidemic. *Current Diabetes Reports* , Sep; 15(9): 64.

Prasad S., Tyagi, A.K., & Aggarwal, B.B. (2014). Recent developments in

delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. *Cancer Research and treatment.* 46:2.

Rehman, K. & Akash, M.S.H.(2017). Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: How are they interlinked? *Journal of Cell Biochemistry.* 118, 3577–3585.

Roy, S., & Awasthi, H.I. (2017). Herbal medicines as neuroprotective agent: A mechanistic approach. *International Journal of Pharmacy and Pharmaceutical Sciences*, 9(1):1-7.

Simon, K. & Wittmann, I. (2019). Can blood glucose value really be referred to as a metabolic parameter? *Rev Endocr Metab Disord*. 20(2):151-160.

Sunmonu, T.O. & Afolayan, A.J. (2013) Evaluation of Antidiabetic Activity and Associated Toxicity of Artemisia afra Aqueous Extract in Wistar Rats. *Evidence-Based Complementary and Alternative Medicine,* Article ID: 929074.

Van G. & Scheen, A. (2015). Weight Management in Type 2 Diabetes: Current and Emerging Approaches to Treatment**.** *Diabetes Care* ;38(6):1161–1172

Walker, A.W., Ince, J., Duncan, S.H., Webster, L.M., Holtrop, G., Ze, X., Brown, D., Stares, M.D., Scott,.P & Bergerat, A. (2011). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 5:220–230.

Yadav, D., Yadav, S.K., Khar, R.K., Mujeeb, M., & Akhtar, M. (2013). Turmeric (Curcuma longa L.): A promising spice for phytochemical and pharmacological activities. *International Journal of Green Pharmology*; 7(1):85-9.

Yadav, M., Parle, M., Sharma, N., Ghimire, K., & Khare, N. (2016). Role of bioactive phytoconstituents from several traditional herbs as natural neuroprotective agents. *Inventi Rapid Planta Activa* ,4(2):1–4.

Zhang, Y.J. (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*, 20:21138-21156.

Zhu, J., Chen, H., & Song, Z. (2018). Effects of ginger (*Zingiber officinale* Roscoe) on type 2 diabetes mellitus and components of the metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. *Evidience Based Complement Alternate Medicine* https://doi.org/10.1155/2018/5692962