RESEARCH ARTICLE

HYPOGLYCAEMIC AND HYPOLIPIDEMIC EFFECTS OF POLYHERBAL FORMULATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Manuscript Info

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Abstract

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This study evaluated the effects of a polyherbal formula on the plasma lipid profile and blood glucose in Streptozotocin-induced diabetic rats. A single dose of Streptozotocin (STZ), (60 mg/kg body weight) in sodium citrate buffer at pH 4.5 was used to induce diabetes intraperitoneally in 20 adult Albino Wistar Strain Rats. 25 rats were put into 5 groups: Normal, Standard, Control, Test I and Test II respectively and were sacrificed after 7days of treatment. The weight and glucose baseline were taken prior to: (1) STZ administration (2) after a week of inducing diabetes (3) after the first, third and fifth day of treatment and (4) at sacrificial time. The body weight and blood glucose level of all animals in different groups varied depending on treatment. Blood glucose test showed that there was a significant increase in blood glucose level after induction. After treatment, the normal group blood glucose level increased by 19.12 % but not diabetic, and a decrease of 10.61 %, 61.64 %, 48.19 % and 77.16 % for the Control, Standard, Test I and Test II respectively. The body weight for normal group increased by 6.29 % while the Control group, Standard group, Test I and Test II emaciated by 7.91 %, 3.35 %, 6.9 % and 5.1 % respectively. The lipid profile test showed that Test I and Test II groups have lower lipid values compare with other study groups. Results from this study clearly indicated that the polyherbal formula had hypoglycaemic and hypolipidemic effects on Streptozotocin-induced diabetic rats.

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Introduction:-

Diabetes mellitus, often referred to as diabetes is a choric disease and one of the most common diseases in our world today, which has increased the mortality rate drastically. In times past, only aged men and women were diagnosed of diabetes, but now, both old and young are susceptible to diabetes. Diabetes mellitus is often called 'The silent killer', because it causes serious complications without serious symptoms and can affect many of major organs in the body (Papaspyros, 1964). Many authors have tried to define diabetes, but, going by the definition of the World Health Organization (WHO) on the basis of laboratory findings, diabetes mellitus is characterized by a fasting

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venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal (Nwanjo and Nwokoro, 2004). A study conducted by the World Health Organization reported that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million by 2030 (Wild *et al*., 2004).

The Diabetes Association (ADA, 1998) says that chronic hyperglycaemia is associated with microvascular and macrovascular complication that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, hyperlipidaemia, heart disease, and stroke. This implies that diabetic individuals are at the risk of developing series of other health problems apart from hyperglycaemia .There are two major types of diabetes: type I and type II diabetes (National Diabetes Fact Sheet, 2011).

There is an increased focus on plants in the search for appropriate hypoglycaemic and anti-hyperglycaemic agents for diabetic conditions (Ebong *et al.*, 2008). Not all plants prepared and administered locally have been tested and proven scientifically to justify their traditional uses and potencies. Diabetes can only be managed throughout an individual's life time (Londhe, *et al*., 2011; Uma *et al*., 2014).

The Wistar rat is an outbred albino rat that is used to mimic diabetes in humans. The most commonly used drug for this induction is Streptozotocin (STZ) because of its toxic effects on islet beta cells (Fadillioglu *et al*., 2008). (Rakieten *et al*., 1963; Ar'Rajab and Ahrén 1993; Gu *et al*., 1997). Co-morbid diseases with hyperglycaemia include high cholesterol, loss of body weight, loss of appetite etc. The plasma lipid profile consists of the total cholesterol, high density lipoproteins (HDL), the triglycerides and the low density lipoproteins (LDL).

Garlic, *Allium sativum,* is a member of the *Alliaceae* family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. The active constituents are sulfur-containing compounds that are rapidly absorbed and metabolized. Numerous studies on garlic have suggested that garlic lowers total cholesterol concentrations favorably altering HDL/LDL ratios (Singh and Singh, 2008, Bordia (1981).
Garlic contains at least 33 sulfur compounds, several enzymes and the minerals vitamins, fiber amino acids and water (Josling, 2005; Fenwick and Hanley, 1985).

Ginger, *Zingiber officinale,* is one of the most widely consumed tuberous spices worldwide. In time past it has been of use as herbal medicine for the treatment of a variety of ailments, including vomiting, pain, indigestion, and cold-induced syndromes, tumor (Wang and Wang, 2005; Shailah, 2010; Khadem *et al*., 2008). Ginger exists in quite a number of varieties. Nigerian ginger is darker in colour, minute size, and has a more pungent taste (Duke and Ayensu, 1985).

The major active ingredients in ginger oil are sesquiterpene, bisapolene, zingiberene, and zingiberol, glycolipids and oleoresin (Connell and Sutherland, 1969, Newall *et al*., 1996, Chrubasik *et al*., 2005). According to Firas (2013), polyherbal mixture has synergistic effect on regulating blood glucose than individual plants.

Bitter melon, *Mormodica charantia,* is a tropical and subtropical vine of the family *Cucurbitaceae*, widely grown in Asia, Africa and the Caribbean for its edible fruit. The fruit is among the most bitter of all fruits and is one of the well-known medicinal plants (Alamgir *et al*., 2012).

All parts of the plant taste bitter and it contains biologically active chemicals include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids ((Alamgir *et al*., 2012, Raman and Lau, 1996). It is used in traditional medicine to treat stomach pain, diabetes, fevers, colds, coughs, headaches, malaria, skin complaints, menstrual disorders, aches and pains, hypertension, infections, and as an aid in childbirth (Leslie, 2002).

Onion, *Allium cepa*, is one of the most important commercial condiment vegetable grown and consumed all over the world. Onions are perennials, where the fleshy bulb that grows below the ground is used medicinally as well as for food (Farooqui and Kumar, 2003).

The volatile oils and allyl propyl sulphide present in onion is responsible for the appealing flavour. There are three types of colour onions: white, yellow and red. Cutting onions causes irritation to the eyes due to the presence of a

phytonutrient called IsoAlliin.. Onion has been experimentally documented to possess anti-diabetic potential anti hyperlipidaemia and bone inhibitory effect (Muhlbauer *et al.*, 2002).

Grape fruit, Citrus paradise, belongs to the *Citrus* genus, in the family *Rutaceae* (Uysal *et al.*, 2011). Citrus contain a high amount of useful by-products which include essential oil. It is consumed fresh or used as raw materials for juice and wine (Schieber *et al.*, 2001). Grape is one of the citrus fruits cultivated and consumed in Nigeria (Odubanjo and Sangodoyin, 2002). They have been used medically for liver cleansing, boosting the immune system, treating respiration issues and to reduce hearth disease risk.

In this research, the effects of polyherbal mixture on blood glucose and the plasma lipid profile level of Streptozotocin-induced diabetic rats was investigated.

Materials and Methods:-
The experiment was carried out in the Chemistry laboratory, Biochemistry laboratory and the Animal house at Babcock University, Ilishan-Remo, Ogun state. Nigeria.

Plant materials, Drugs and Chemicals:-
Selected plants; ginger, garlic, onion, grape fruit, and bitter melon were obtained from a local market at Ikorodu, Lagos State, Nigeria. The standard drug glybenclamide (Daolin, Aventis Pharma, U.K.) and Accu-Chek glucometer (Accu-Chek, USA) and the lipid profile diagnostic kits (QCA Spain cholesterol Kit and SPINREACT triglycerides kit, Spain) were purchased from a Pharmacy in Lagos State Nigeria. Streptozotocin (Sigma, USA), Lithium Heparin bottles (BD, USA), plastic syringes were purchased from a chemical supplier in Lagos Nigeria.

Animals:-
Thirty male albino rats (Wistar strain) weighing between 150 – 200 g and their cages were purchased from an inbred colony, in the Animal Facility Unit, Babcock University, Ogun state, Nigeria. Animals were allowed to acclimatize for two weeks before commencement of study and were given commercial rat chow and water *ad libitum*. Animal experimental protocols were in conformity with the National Institute of Health/National Research Council Regulations on Laboratory Animal Care and Use Guidelines (NIH, 2010). Ethical clearance was obtained from Babcock University Health Research Ethical Committee (BUHREC699/16, January 2017). Glibenclamide was used as the standard drug for treating diabetes. The glucometer kit was bought at a pharmacy in Lagos state. EDTA bottles, Syringes and the lipid profile test kits were bought from a supplier in Ogun state.

Preparation of Polyherbal formula:-
The selected plants were gotten from a local market in Ikorodu, Lagos State Nigeria and were identified by a Professor of Botany in the Basic sciences Department of Babcock University. Bitter melon leaves, ginger, garlic, onion and grape were separately washed thoroughly with water in order to remove dirt and allowed to air dry. After 24 h, the mesocarp of each plant materials was completely peeled off, sliced into smaller parts to aid drying and placed in the oven at a temperature of 60°C for 24 h. Dried plant materials (100 g) were then pulverized using laboratory blender and soaked in warm water for 24 h. Subsequently, the suspension was then filtered using Whatman No.1 filter paper and the suspension was subsequently concentrated using a rotary evaporator (Eyela N-1001) at 40°C. The concentrate was stored in the refrigerator at 4°C until further use.

Induction of diabetes:-
Rats were fasted for 16 h before the induction of diabetes with Streptozotocin (STZ) (Sigma, USA). A freshly prepared solution of 60 mg/kg STZ in 0.1M cold citrate buffer, pH: 4.5, was injected intraperitoneally and the normal rats were injected with citrate buffer alone in accordance with the method described by Zafar *et al* (2009). In order to control the hypoglycaemia during the first day after the STZ administration, diabetic rat were given 5 % glucose solution orally. Hyperglycaemia was confirmed by the elevated fasting glucose level in the blood at 48 h and on day 6 after injection. Rats with diabetes exhibiting fasting blood glucose levels >240 mg/dL were selected for the study.

Treatment of diabetic rats:-
A week after the STZ induction, the diabetic rats were selected and put in four (4) groups of five (5) each. The control group was not given any treatment. The standard group was administered with 2.5 mg/ kg body weight

(B.W.) Glybenclamide. Test I and test II groups were administered with 50 mg/kg and 100 mg/kg body weight polyherbal formula respectively. The treatment period lasted for 7 days.

Collection of blood samples for plasma preparation:-
Blood samples were collected from the petroleum ether anesthetized rats by cardiac puncture using 5 ml hypothermal syringe and transferred into lithium heparin bottles. The blood was centrifuged at 3000 rpm for 10 min using a table top centrifuge. The clear supernatant (plasma) was used for the lipid profile assay in accordance with the method described by the QCA Spain cholesterol kits and SPINREACT Spain triglycerides kit. For the blood glucose assessment, each rat was held carefully while the tail tip was gently cut to collect few drops of blood. The glucometer strip was inserted into the glucometer (Accu-Chek Active) and the blood sample was dropped on the brown portion of the strip after 5 seconds of inserting the strip. The concentration of blood glucose was measured from the digital reading.

Result and Discussion:-
In this study, the effect of polyherbal formula extracts on Streptozotocin-induced diabetic rats was investigated. The plasma lipid profile and blood glucose in the diabetic rats were measured. Streptozotocin (STZ) (60 mg/ kg body weight (BW)) was injected once intraperitoneally to induce diabetic condition by signs and symptoms of diabetes ((Piyachaturawat *et al* 1991).

Table 1 shows how the rats were grouped and treated. Tables 2 and 3 and figure 1 show the average body weights of the rats before induction, after a week of induction and after a week of treatment with the polyherbal formula. The body weights of the normal group increased by ~ 2.9 % while control, standard, Test I, and Test II groups decreased by ~ 2.1, 2.1, 2.0 and 2.4 % respectively. The increase in the normal group body weight is an indicator of total wellbeing and good feeding. The decrease in body weight of other groups is a clinical symptom of the diabetic condition of the rats as a result of appetite loss and polyuria (White and Baxter, 1994). Figure 2 and data in Table 3 show that 50 and 100 mg/kg polyherbal formula and standard drug treated diabetic rats had significantly reduced ($P<0.05$) blood glucose concentrations when compared with untreated diabetic rats from day 3 to 7 post-induction of diabetes. In addition, the blood glucose level of the 100 mg/kg body weight (BW) polyherbal formula treated animals were significantly ($P<0.05$) reduced when compared with standard drug treated animals from day 3 to 7 post-induction of diabetes. This indicates that polyherbal formula possesses the capacity to reduce blood glucose level in diabetic condition. Previous studies (www.ncbi.nlm.nih.gov, *2007; www.diabetes.co.uk*) have shown that an herbal mixture that reduces blood glucose level possesses antidiabetic property. Glybenclamide elicits its antidiabetic effect by stimulating secretion of insulin. This also suggests that polyherbal formula may have reduced blood glucose level through stimulation of insulin secretion.

Evaluation of the effects of polyherbal formula on lipid profile of diabetic animals is shown in Tables 5, 6, and 7. The tables show that 50 and 100 mg/kg polyherbal formula treated diabetic rats had significantly reduced ($P<0.05$) LDL-cholesterol, total-cholesterol and triglycerides when compared with untreated diabetic rats in a dose dependent manner. This observation was comparable to standard drug treated group. Data indicated that polyherbal formula possesses hypocholesterolaemic property in diabetic condition. More so, 50 and 100 mg/kg polyherbal formula and standard drug treated diabetic rats had significantly elevated ($P<0.05$) HDL-cholesterol level when compared with untreated diabetic rats. From the results, it appeared that the efficacy of the plant extracts declined with time. So, it is suggested that its storage time should not be more than five (5) days. This justifies the use of these plants in traditional medicine for the management of high blood pressure and hyperglycaemia.

Conclusion:-
The result showed that the polyherbal formula was effective for the treatment of diabetes and for regulating the lipid profile. It is recommend that further research should be carried out on the polyherbal formula to ascertain its chemical composition and fully elucidate its antidiabetic mechanism of action.

Table 1:- Diabetic groups and detailed protocol and treatment.

Groups	Treatment protocol
Normal	Normal, neither STZ-induced nor treated.
Standard	STZ-induced diabetes and treatment with Glybenclamide (standard)

Control	STZ-induced diabetes without any treatment
Test I	STZ-induced diabetes and treatment with 50 mg plant extracts
Test II	STZ-induced diabetes and treatment with 100 mg plant extracts

Table 2:-Average body weights of rat groups before induction, after induction and after treatment.

Group	Body Weight(g) Before	Body Weight(g) After 7 Days of Induction	Body Weight(g) After 7 Days of Treatment
Normal	170±0.051	175±0.2	186±0.32
Control	142±0.045	139±0.45	128±0..45
Standard	183±0.018	179±0.08	173±0.32
Test I	148± 0.125	145± 0.125	134±0.5
Test II	161±0.102	157±0.2	149±0.23

Data are averages of two determinations

Table 3:-Post Induction Treatments of Diabetic Rat.

Group	After a week of Inducing Diabetes	Treatment of Diabetes			
		Day 1	Day 3	Day5	Day 7
Normal	68± 0.625 (not induced)	60± 0.03	59± 0.03	74± 0.03	81 ± 0.26
Control	490±0.745	494±0.05	491±0.05	475±0.05	438 ± 1.00
Standard	477±0.565	217±0.03	241±0.01	186±0.02	183 ±0.18
Test I	359±0.205	255±0.02	176±0.05	176±0.05	186 ± 0.82
Test II	464±0.16	245±0.01	182±0.02	114±0.06	106 ±1.00

Data are averages of two determinations,

Table 4:- Total Cholesterol Result for All Groups.

GROUPS	TOTAL CHOLESTEROL mg/dL	Benchmark (mg/dL)[a]
NORMAL	110±0.006	Desirable < 200
CONTROL	125±0.04	Borderline 200-239
STANDARD	106±0.02	Undesirable > 240
TEST I	105±0.2	
TEST II	102±0.04	

Data are averages of two determinations,   a= Ref 36

Table 5:- High Density Lipoprotein Result for All Groups.

GROUPS	HDL (mg/dL)	Benchmark (mg/dL)[a]
NORMAL	63±0.03	Desirable > 60
CONTROL	64±0.05	Undesirable ≤ 40 (men); ≤ ≤ 50 (women)
STANDARD	74±0.10	
TEST I	69±0.04	
TEST II	71±0.18	

Data are averages of two determinations, a = Ref 36

Table 6:- Triglyceride Result for All Groups.

GROUPS	TRIGLYCERIDE (mg/dL)	Benchmark (mg/dL)[a]
NORMAL	138±0.05	Desirable < 150
CONTROL	161±0.13	Borderline 150-199
STANDARD	130±0.04	Undesirable > 200
TEST I	129±0.12	
TEST II	127±0.23	

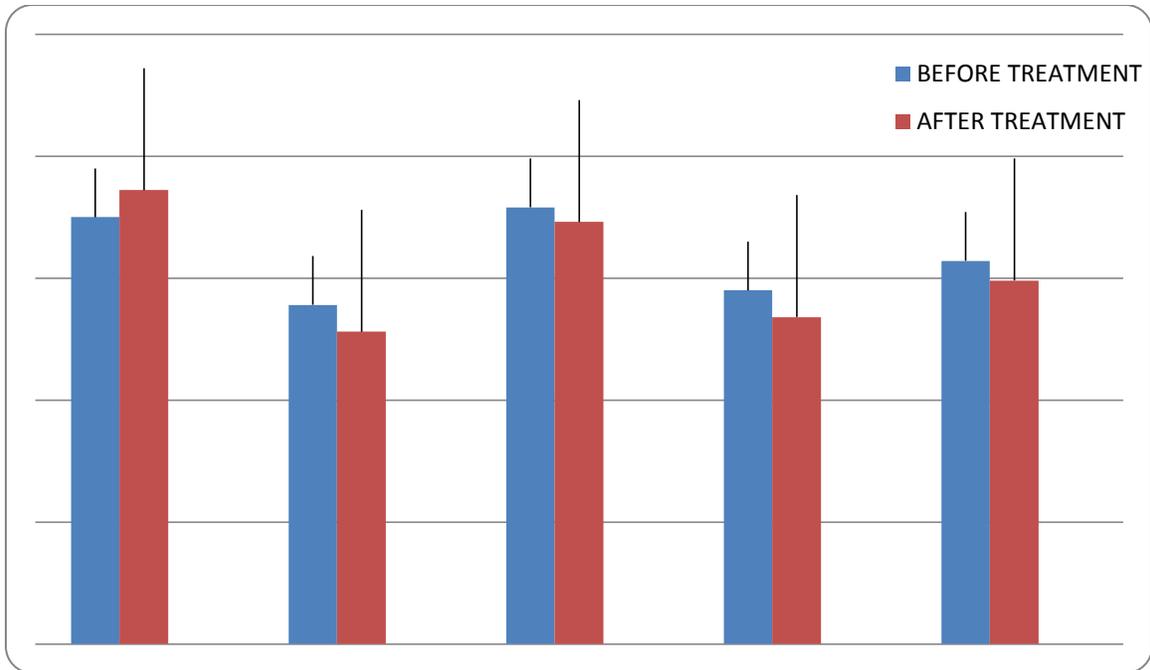Data are averages of two determinations, a = Ref 36

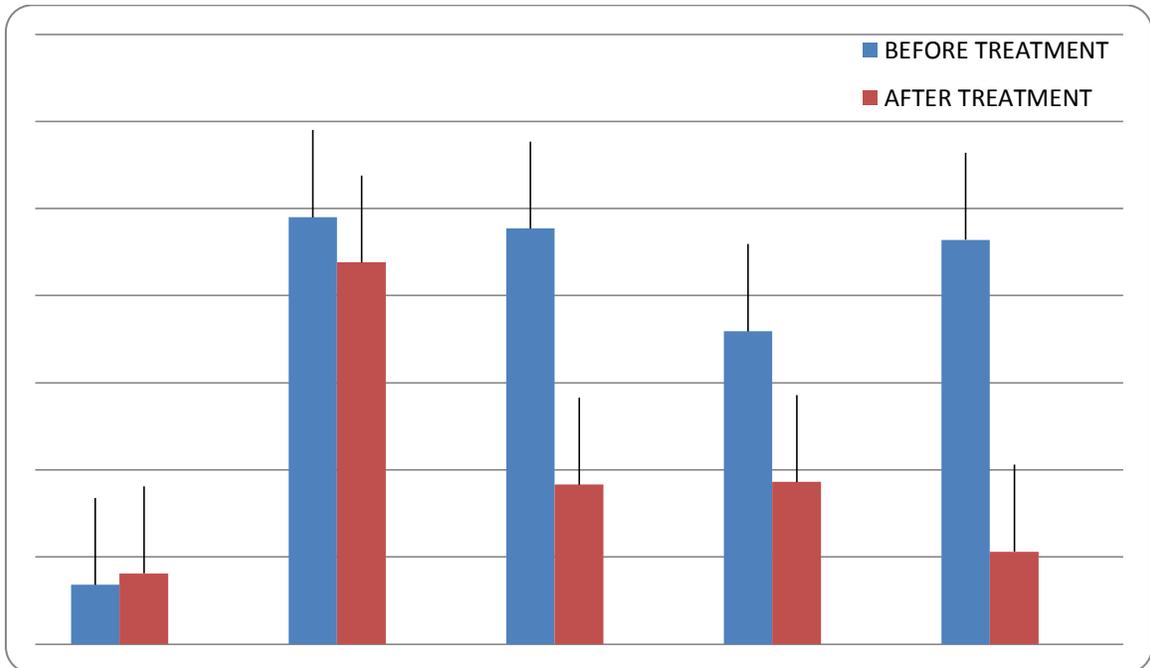**Fig. 1:-**Comparison Between Body Weights Before Treatment And After A Week Treatment.

Fig. 2:- Comparison between Blood Glucose Level Before Treatment And After A Week Treatment.

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