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RESEARCH ARTICLE

EARLY CONSUMPTION OF HIBISCUS SABDARIFA ATTENUATES THE SEVERITY OF TYPE 2 DIABETES IN A WISTAR RAT MODEL

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Abstract

Diabetes constitute a serious challenge for many health system and families due to the cost of its care. Alternatively, traditional plants offer a huge potential for health care. Thus, many plants have been used in form beverage including the use of Hibiscus sabdarifa (HS) as tea. Recent data suggested the beneficial effect of HS cardiometabolic diseases models. In this study, we evaluated the preventive and curative effects of crude extract of HS in a type 2 diabetes rat model. Through in vitro complexation and/ or precipitation reactions, we qualitatively assessed the phytochemical composition of the crude extract of HS for different groups of secondary metabolites. The antiradical scavenging activity was assessed through hydroxyl radical test. Type 2 diabetes was induced by high fat diet (HFD) and single dose streptozotocine (STZ) injection. Body weight change and blood biochemical analysis were carried out. Data were statistically analyzed. HS contains different phytochemical polyphenolic compounds such as tanins and flavonoids and presented an interesting antiradical scavenging activity. Early intake from experimental day (ED1) of crude extract of HS significantly prevented gain in body weight ($P < 0.05$), reduced T2D induced elevated glucose ($P < 0.01$) and lipids ($P < 0.01$) and has better outcome as compared to late intake (from ED14). These findings confirm and supports the use of HS as tea and may offer protective effect to consumers by regulating blood sugar and lipid profile.

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Introduction:-

Diabetes is a metabolic disorder marked by elevated circulating glucose and impaired metabolism of lipids and glucose. Diabetes was projected to affect 177 million people worldwide in 2000 and to increase to 300 million by 2025 (Porter and Barret, 2005). Two types of diabetes are well documented, which type 1 or insulin-dependent and type 2 marked by insulin-resistance and well known to be related to nutritional status. As important burden for health system, diabetes concerns both developed and developing countries to date, diabetes patients are registered in

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all health care providing centers and their number is dramatically increasing year by year even in rural areas. In addition, although effective in some limits, many antidiabetic drugs are still not affordable for most people in rural communities. This rises some interest in seeking for the most available resource for them that constitute the medicinal plants (Zarei et al., 2015). Herbal medicines remain one of the source to which populations in developing countries refer for their health needs and provide first-line and basic health service due to its availability, affordability to the people living in poor areas (Tijani et al., 2012). Unfortunately, the proper use of these resources for the beneficial health effect remains debated and requires scientific investigation not only for their safety but also for biological effects since the widespread use and increasing popularity of herbal medicines does not guarantee their efficacy and safety (Shafaei, 2011). The herbal medicines contain the secondary metabolites acting as antioxidants in the biological system by neutralizing free radicals and may reduce or even help to prevent some of the damage caused by reactive oxygen species (ROS) (Gad, 2013).

Hibiscus sabdarifa Linn., a member of the Malvaceae family, is one of more than 300 species of *Hibiscus* around the world. *Hibiscus sabdariffa* is used in many countries as a food, wine, jam, cold and hot beverages, and ice cream, chocolates, as a flavoring agent in the food industry, and as a medicinal herb (Wahabi et al., 2010). *Hibiscus sabdariffa* beverages have different names such as bissap, roselle, agua de Jamaica, *Hibiscus* tea, sour tea, red sorrel, Lo-Shen, Sudan tea, or karkade (McKay et al., 2009). Its protective properties for many body systems in experimental and clinical studies, such as antihypertensive and hypolipidaemic activities, increased its use worldwide (Joven, 2014). Studies also show its anti-hyperglycemic and lipidemia (Aziz et al., 2013) effect in rat at different doses. However, to the best of our knowledge, there is no data on the ability of *Hibiscus sabdariffa* crude extract of type 2 diabetes model comparing its preventive effects. Most studies focused on its use as remedy. Therefore, in this work, we strived to assess the preventive effect of *Hibiscus sabdariffa* crude extract of type 2 diabetes model.

Material And Methods:-

Animals and study design

For the purpose of this study, male Wistar strain albino rats (180 to 200g) of 10 weeks old obtained from our animal house (Laboratory of Animal Physiology and experimental pharmacology, FAST/UAC) have been used. Animals had free access to standard rat chow and drinking water ad libitum and were housed in clean plastic cages under controlled light and dark cycle (12/12h). To reduce changes due to the experimental procedures, animals were familiarized to the experimental conditions, including handling, blood sampling, labelling by permanent marker pen, prior to the experiment. To assess the effect of *Hibiscus S.* consumption before the challenge of type 2 diabetes, two (02) groups of rats were set and treated with either 200mg/kg of body weight of crude extract of *Hibiscus S.* or vehicle (clean water) for two weeks with or without a high fat diet (HFD) and 30 mg/kg (Skovsø, 2014). Crude extract or tap water was administered orally as 1mL solution. Study groups are as follow:

Tableau 1:- Study groups.

	N	N +HS	T2D	T2D + HS	T2D + LHS
HS	-	+	-	+	+
HFD	-	-	+	+	+
STZ	-	-	+	+	+

N: Control group fed with normal diet; **N + HS:** Animals were fed with normal diet and administered with crude extract of *Hibiscus S.* from ED1; **T2D:** Type 2 diabetes model fed with high fat diet ED1 à ED28 and injected with STZ on ED14. **T2D + HS:** Type 2 diabetes model fed with high fat diet ED1 à ED28 and injected with STZ on ED14 then administered with crude extract of *Hibiscus S.* from ED1. **T2D + LHS:** Type 2 diabetes model fed with high fat diet ED1 à ED28 and injected with STZ on ED14 then administered lately with crude extract of *Hibiscus S.* from ED14.

Plant material

The calyces of *Hibiscus S.* were harvested from the garden for lower plants of the Laboratory of Animal Physiology and Experimental Pharmacology. The collected material was dried in laboratory temperature conditions protecting it from sunshine for 10 days. The dried calyces were reduced in powder, sealed and stored in a dried and cold place. Phytochemical analysis and crude extract preparation were conducted using the powder.

Preparation of the extract

To prepare crude extract from material powder, 200g was macerated in 1L of warm (45°C) distilled water, kept on orbital shaker and filtered after 24 hours. Residues are then re-macerated for another 24 hours. Water from filtrate was evaporated using a lab oven set at 60° for one week to condense the extract. The weight of extract was determined. The dried extract was weighted, packed and capped in glass bottle and kept in the fridge until used. In addition, we determined the yield (P) of the extraction as follows:

$$P = \left(\frac{W_{ext}}{W_{prd}} \right) * 100, \text{ West} = \text{weight of the dried concentrated extract. Wprd} = \text{weight of the powdered leaves.}$$

Phytochemical screening

The qualitative phytochemical analysis was conducted on crude extract/or powder of Hibiscus S. to identify the secondary metabolite present in the calices using precipitation and coloration reactions as described by Houghton and Raman (1998). The analysis consists of a variety of in vitro tests such as Shinoda test and magnesium powder for Flavonoids, picric acid test for cyanogenic derivatives, test index foam for saponins, Mayer's test for alkaloids, stiasny test for cathetic tannins, test of absolute alcohol for mucilage, test with ether and ammonia for coumarins, ferric chloride test after saturation with sodium acetate for gallic tannins. All assays were adapted from the work of Koudoro et al., (2014) and Sangaré et al, (2017).

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was performed as per described previously by Su et al (2009). Briefly, 2 ml of the following reaction components were mixed in assay tube: the 6 mM aqueous FeSO₄ solution, the crude extract and the 6 mM H₂O₂ solution then incubated at room temperature (24°C) for 10 minutes. Then, the 6 mM aqueous solution of salicylic acid was added to the tube and incubated for 30 min. After complete change in color, the optical density was read at 510 nm. A test with three replicates was performed and the radical scavenging activity was determined.

Body weight, samples harvesting and biochemistry test

Animals were weighed at for time points during the study as presented in the Table 1 experimental days (ED) 1, 7, 14, 21 and 28. Blood was collected in appropriate tubes on ED28 then centrifuged. Plasma or serum was used for blood biochemistry assays for glucose, total cholesterol, high-density lipoprotein (HDL), alanine aminotransferase, and aspartate aminotransferase. All assays were performed as per the manufacturer's instruction. Blood glucose level was monitored on ED 1, 7, 14, 21 and 28 by cutting the tail-tip of the rats and using glucometer strips (ONE TOUCH).

Data Analysis

Data were analyzed using one way analysis of variance (one-way ANOVA) followed by Turkey multiple comparison test in Minitab 16 program. Values were considered significant at P < 0.05.

Results:-

Phytochemical content and antioxidant power of extract

The table 2 presents the principal secondary metabolites identified in the crude aqueous extract of Hibiscus S. as follows:

Table 2:- Secondary metabolites in Hibiscus S.

Hibiscus S.		
Alcaloides		+
Flavonoides		+
Tanins	Catechic type	+
	Gallic type	+
Anthocyan		-
Mucilages		+
Saponosides		-
Leucoanthocyan		+
Anthraquinones		+
Terpens et sterols		-

Coumarines	-
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As presented the table 2, crude aqueous extract of Hibiscus S. contains alkaloids, flavonoids gallic and catechic types of tannins, leucoanthocyanins, and anthocyanins. Other types of secondary metabolites such as terpenes and sterols anthocyanins, saponoside and coumarines were not found in our extract. In addition, our extraction yielded in 6.20 ± 0.48 % of crude extract.

Table 3:- The IC_{50} of Hibiscus S.

IC_{50} ($\mu\text{g/mL}$) of Hydroxyl radical	
Extract of H.S	18 ± 2.80
Gallic acid	15 ± 1.6
Quercetin	125 ± 7.75

The crude extract of Hibiscus S. presented an IC_{50} of 18 ± 2.80 similar to that gallic acid, a reference phenolic compound and significantly higher than that of quercetin, another reference compound (Table 3). These data support the in vitro antioxidant effects of the extract of Hibiscus S.

Effect of crude extract of body and blood glucose

Increased blood glucose known as hyperglycemia characterizes diabetes. As previously reviewed (Skovsø, 2014), type 2 diabetes is preceded by an increased in body weight. In this experiment, we recorded body weight change during the experiment. As presented by the figure 1, HFD significantly increased animals' body weight from ED21 and so until the end of our experiment (Figure 1). This ensures us that all rat developing hyperglycemia upon low dose of STZ would be type 2 diabetes models. Hence, injection of STZ from ED14 to HFD fed animals significantly increased their blood glucose after one week, i.e. on ED21 (Figure 2) and the blood glucose level was higher until ED28 in T2D and T2D + LHS groups but not in T2D + HS, compared to N group. This supports the anti-hyperglycemic effect of HS. More importantly, when the treatment of rats with HS begins earlier (from ED1 for T2D + HS) not late (ED14 for T2D + LHS), the effect of HS becomes pronounced and HS significantly reduced and normalized the blood glucose level (Figures 2 & 3).

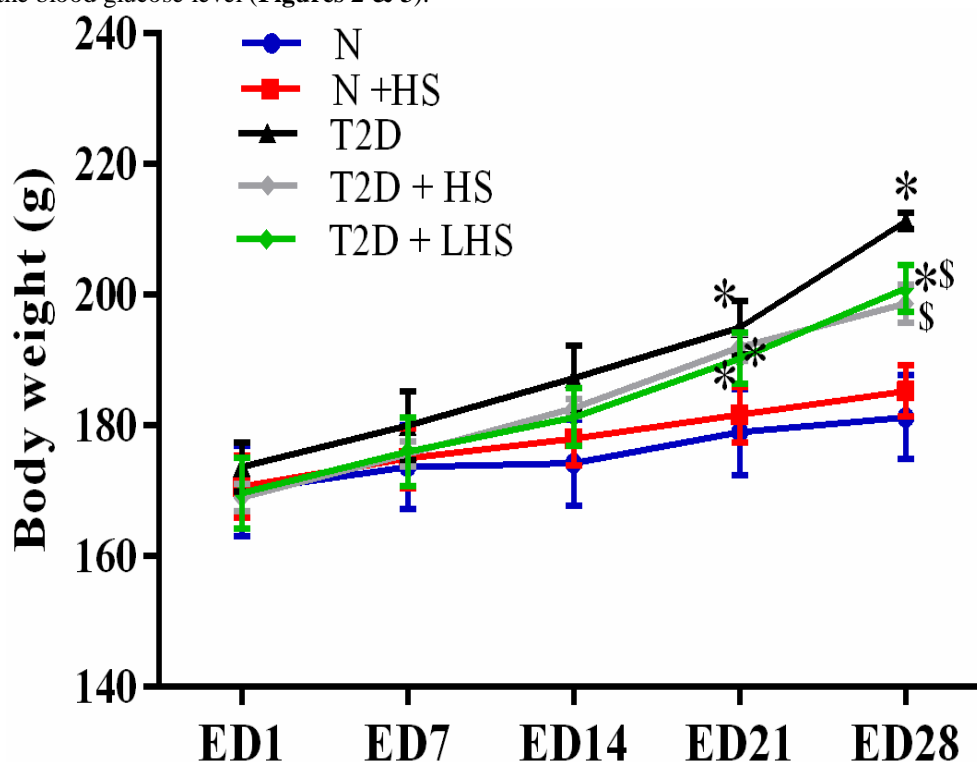


Figure 1:- Change in body weight of rats. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. *: $P < 0.05$ vs N on the same date; \$ $P < 0.05$ vs T2D on the same date.

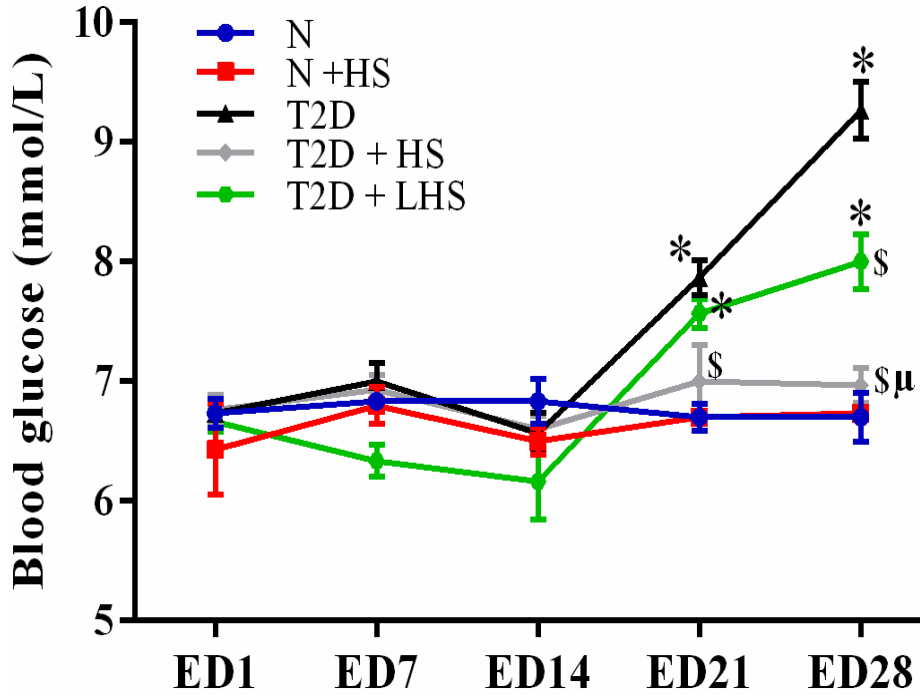


Figure 2:- Weekly change in blood glucose of rats using glucometer. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. *: $P < 0.05$ vs N on the same date; \$ $P < 0.05$ vs T2D on the same date; μ $P < 0.05$ vs. T2D + LHS.

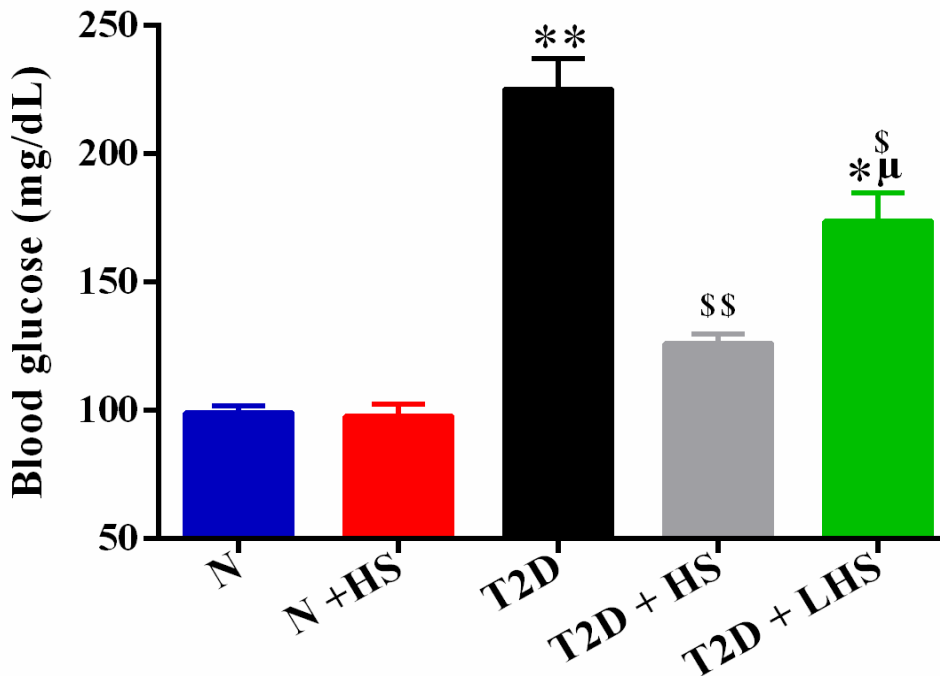


Figure 3:- Blood glucose of rats on ED28. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. ** $P < 0.01$ vs N on the same date; \$ $P < 0.05$ vs T2D on the same date \$ $P < 0.01$ vs T2D on the same date; μ $P < 0.05$ vs. T2D + LHS.

Effect of crude extract of lipid profile

Dyslipidemia, defined as abnormal circulating lipid profile, is one of the main characteristics of type 2 diabetes patients. To ensure the lipid profile of our animals in study and assess the antilipidemia effect of HS, we evaluated the circulating level of triglycerides, total cholesterol, HDL-cholesterol and LDL-Cholesterol. Our blood biochemistry analysis revealed that HFD followed by STZ injection significantly increased the circulating level of triglycerides (Figure 4), that of total cholesterol (Figure 5) and that of LDL-cholesterol (Figure 7) and reduced the circulating level of HDL-cholesterol (Figure 6) in T2D group. Interestingly, the administration of crude extract of HS significantly reduced the circulating level of triglycerides (Figure 4), that of total cholesterol (Figure 5) and that of LDL-cholesterol (Figure 7) and increased the circulating level of HDL-cholesterol (Figure 6) in the two treated groups (T2D + HS and T2D + LHS). Noticeably, although significant changes were observed in treated groups, the variation of parameters from the T2D group level was more pronounced in T2D + HS, which received treatment from the beginning, than in T2D + LHS. This remark is in line with the level of blood glucose measured by two different methods.

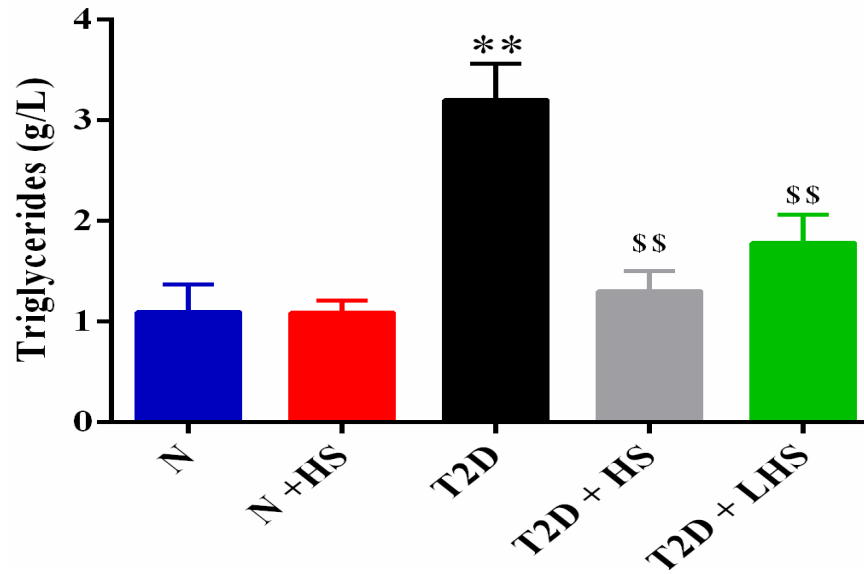


Figure 4:- Blood triglycerides of rats on ED28. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. **P < 0.01 vs N on the same date; \$P < 0.05 vs T2D on the same date \$\$ P < 0.01 vs T2D on the same date.

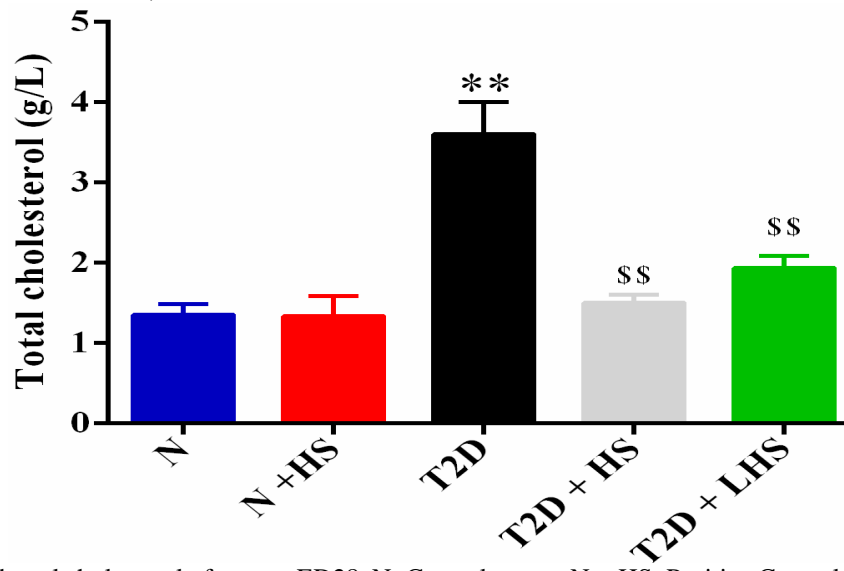


Figure 5:- Blood total cholesterol of rats on ED28. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1;

T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. **P < 0.01 vs N on the same date; \$\$ P < 0.01 vs T2D on the same date.

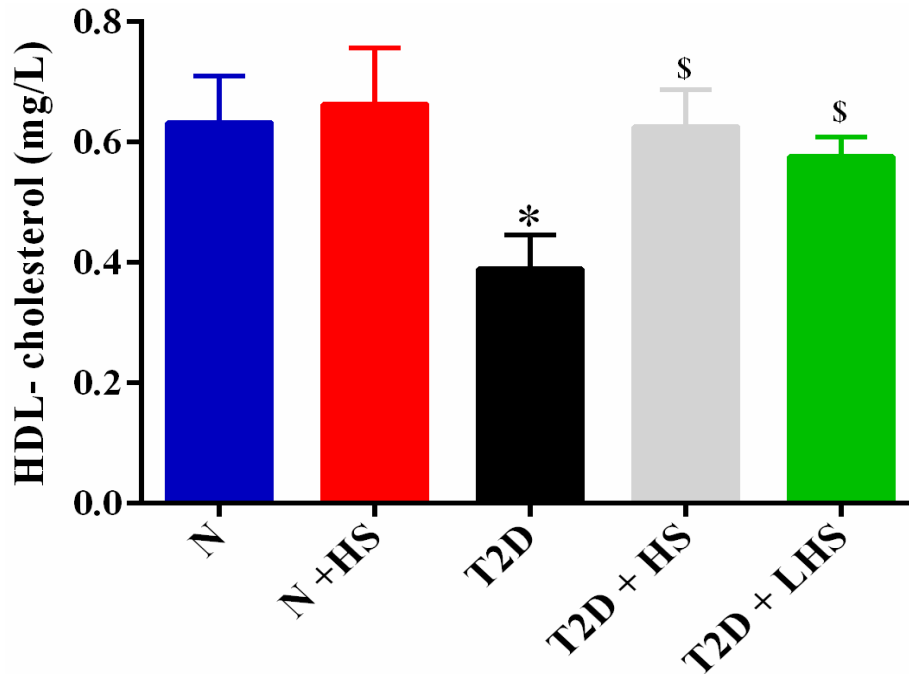


Figure 6:- Blood HDL-cholesterol of rats on ED28. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. ** < 0.05 vs N on the same date; \$P < 0.05 vs T2D on the same date.

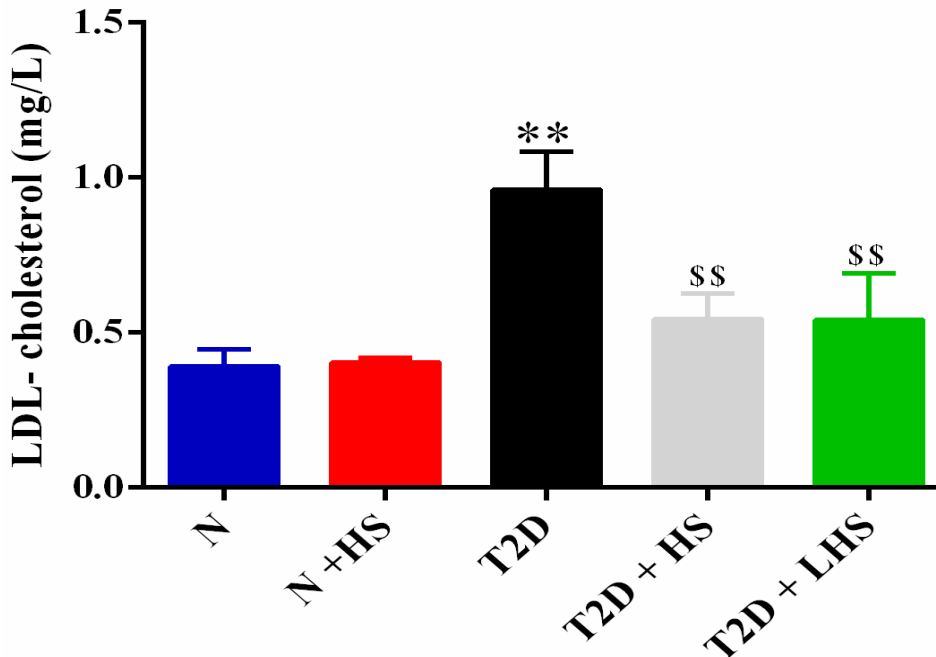


Figure 7:- Blood total cholesterol of rats on ED28. N: Control group; N + HS: Positive Control administered with Hibiscus S.; T2D: Type 2 diabetes group; T2D + HS: Treated Type 2 diabetes group with Hibiscus S. from ED1; T2D + LHS: Lately Treated Type 2 diabetes group with Hibiscus S. from ED14. ED: Experimental day. **P < 0.01 vs N on the same date; \$\$ P < 0.01 vs T2D on the same date.

Discussion:-

To date, the use of medicinal plants remains common and popular in many developing countries as part of health care system. There are still thousands of those plants under use in many African countries to manage a variety of illnesses including metabolic diseases among which we have the diabetes. In this study, we investigated the antidiabetic effect of the crude aqueous extract of Hibiscus S. in a type 2 diabetes model. The main findings of this study are 1) two weeks administration of HS attenuated HFD + STZ induced increased body weight and blood glucose and 2) early intake of HS prevented HFD + STZ induced increased body weight and blood glucose more than late intake.

The development of diabetic rat model has been carried out to study the antidiabetic potential of many substances. Beside genetically manipulated models, the STZ or alloxan induced diabetes phenotypes represent the main models to study the antidiabetic drugs (Khan et al., 2014). High dose of STZ (3 x 30mg/Kg of body weight) has been used to induce type 1 diabetes. As for the type2 diabetes, a variety of doses and treatment timing has been reported to induce type 2 diabetes features including progressive increase in body weight, blood glucose and lipid profile. In this study, we chose two weeks HFD followed by single dose of STZ at 30mg/kg to develop the model as described before (Zhang et al, 2008). The features of our model are similar to those described earlier for type 2 diabetes (Zhang et al, 2008, Skovsø, 2014). This supports and validates our model.

The phytochemical study of HS revealed the presence of many phenolic compounds such as tannins and flavonoids. Our findings regarding the phytochemical components agree with previous studies for most secondary metabolites (Hopkins, 2013). In addition, our extraction yielded in 6.20% of crude extract. This yield is overall similar to those found by other authors using water as extraction solvent. These compounds were shown in a variety of plant extract to own several beneficial biological effects for human and animals. In addition to their antioxidants effects, as confirmed in this study, phenolic compounds from HS and many other medicinal plants or green tea where demonstrated to possess antidiabetic effect. Similar to our finding, previous works on HS aqueous, ethanolic or methanolic crude extracts showed that these extracts reduced blood sugar, blood lipidemia and reduced blood pressure in hypertensive animals (Hopkins et al, 2013, Joven et al, 2014). This suggests the potential of HS as anti-metabolic syndrome medication, which is characterized by increased body weight, increased blood glucose, dyslipidemia (Riaz et al, 2018) and hypertension. In this study, we found that HS, when administered before the injection of STZ, significantly prevent the onset of hyperglycemia and dyslipidemia compared to late administration. Such finding not only agrees with previous reports on the anti-hyperglycemic effect HS crude extract (Riaz et al, 2018) but also bring new evidence that regular intake of HS may offer preventive effect for hyperglycemia. Moreover, our current findings, regarding the lipid profile in treated rats, are supported by previous studies on the effects of different types of HS in rat diabetic rat, hypertensive rats and metabolic syndrome models of rat (Riaz et al, 2018, Hopkins et al, 2013, McKay et al, 2010). Taken together, our data support the popular use of HS as tea or other type of beverages in different region of the globe.

Conclusion:-

In conclusion, this work showed the anti-hyperglycemic effect crude extract of HS in T2D rat model induced by HFD and STZ. Early intake of crude extract provided better effect than late administration. This confirms and supports the use of HS as tea and may offer protective effect to consumers by regulating blood sugar and lipid profile which need further investigation

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