Abstracts:

Diabetes prevalence was 20.0% in urban Egypt. There are new trend to use traditional natural medicines mainly originated in herbs, which is showing a bright future in the therapy of diabetes mellitus (DM) and its complications. Accordingly, this study aimed to study the effect of different doses of *Stevia rebaudiana* plants on blood glucose, lipid profile and kidney functions level in induced diabetic rats. The results indicated that, after six weeks of injection with alloxan diabetic rats treated with high dose of stevia tended to have blood sugar close to healthy group. Using the three levels from stevea 2.5%, 5% and 7.5% separately in treated diabetic rats led to significant decreases in serum triglycerides, as compared to the positive control group.

The high level from stevia induced significant decrease in serum triglycerides and cholesterol in diabetic groups, as compared to the treated groups with low and medium levels from those materials. high-density lipoprotein of hyperglycemic groups which treated with high level from stevea increased significantly at $p<0.05$, as compared to hyperglycemic groups which treated with medium levels. Three levels from stevea -decreased serum LDL-c in rats suffering from hyperglycemia, as compared to positive control group. All treated groups with different tested diets produced an improvement in serum urea

nitrogen and creatinine levels, as compared to positive control group. Dose of 7.5% of *stevia* have therapeutic potential. They possess hypolipidemic and hypoglycemic properties.

Introduction:

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels(WHO, 2013).

In 2014, 9% of adults 18 years and older had diabetes. In 2012 diabetes was the direct cause of 1.5 million deaths. More than 80% of diabetes deaths occur in low- and middle-income countries. Unfortunately, Egypt is classified as one of middle-income countries(WHO, 2013).

Some region of Northern Africa including Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, South Sudan and Western Sahara are the most countries that dispersed this disease (Bos and Agyemang 2013). It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes (Naglaa and Ghada, 2010).

Diabetes prevalence was 20.0% in urban Egypt. Undiagnosed diabetes is common in Northern Africa with a prevalence ranging from 18% to 75%. (Bos and Agyemang, 2013).

The control and treatment of diabetes and its complications mainly depend on the chemical or biochemical agents. There are new trend to use traditional natural medicines mainly originated in herbs, that is showing a bright future in the therapy of diabetes mellitus (DM) and its complications (Li et al, 2004).

Stevia rebaudiana (Bertoni) is an herb of the 950 genera of Asteraceae family. It is also known as sweet herb, sweet leaf, honey leaf, candy leaf and honey yerba (Carakostas et al., 2008).

Stevia rebaudiana(Bertoni) has been used for the treatment of diabetes in many countries. Although, a positive effect on antidiabetic and its complications has not been unequivocally demonstrated.This herb has been proven safe and effective over hundreds of years.

Accordingly, this study aimed to study the effect of different doses of Stevia rebaudianaplants on blood glucose, lipid profile, kidney and liver functions level in induced diabetic rats.

Materials and Methods

Biological Experiment:

Male albino rats Sprague Dawley strainweighing 110-120g were maintained under standard conditions for one week before starting the experimental for acclimatization.

After acclimatization period for one week, rats will feeding balance diet and inject by aloxan150 mg/Kg body

weight of recrystallized alloxanexpect the negative group (non-diabetic rats) to induce hyperglycemia Buko et al., (1996).

Rats (n= 30) were divided into five main groups: each group contains 5 rats as following:

Group (1): Fed on the basal diet only and kept as negative control group (healthy group).

Group (2): Injected with alloxan and Fed on the basal diet without any treatment and kept as positive control group.

Group (3): Injected with alloxan and fed on the basal diet containing stevia at dose of 2.5g/100g of basal diet.

Group (4): Injected with alloxan and fed on the basal diet containingstevia at dose of 5g/100g of basal diet.

Group (5): Injected with alloxan and fed on the basal diet containing stevia at dose of 7.5g/100g of basal diet.

At the end of the experimental period (6weeks), rats were fasted for 12 hours then sacrificed. Blood samples were collected from eye.

Biochemical analysis:-

Determination of serum glucose:

Fasting plasma glucose was analysis according to (Tietz 1986)

Determination of serum lipids:

Serum cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were determined by kits according to(Knight et al.,1972) methods.

Determination of serum transaminases:

Serum aspartate transferase (AST) and serum alanine transferase (ALT) and lipid peroxide (Malonaldehyde) activities were measured colorimetrically according to (Reitman and Frankel., 1957).

Results and Discussion:

Stevia possess the ability to increase the insulin effect on cell membranes, increase insulin production, stabilize glucagon secretion and blood sugar levels, and improve glucose tolerance to ingested carbohydrates and lower post-prandial blood sugar levels in both animals and humans(Dyrskog, et al., 2005).

Table (1): The effect of different doses of stevia on serum blood glucose of hyperglycemic rats.

Parameters / Groups		mg/dl.	
		Initial blood glucose	Final blood Glucose
Healthy rats. Control (-)		$92.75^{a} \pm 3.31$	$86.73^{a} \pm 3.30$
Hyperglycemic rats. Control (+)		$132.13^{b} \pm 18.2$	$154.80^{c} \pm 2.35$
Hyperglycemic rats fed on diet and treated with stevia	2.5g/100g	$128.02^{b} \pm 12.35$	$123.90^{b} \pm 3.56$
	5g/100g	$133.18^{b} \pm 10.18$	$120.23^{b} \pm 5.12$
	7.5g /100g	130.10b±11.92	83.43 a ± 2.40

Values are expressed as mean ± SD.Significance at p<0.05Values, which don't share the same letter in each column, are significantly different.

From the data illustrated in table (1), it could be concluded that, there was no significant statistical has been observed on initial blood glucose between injected groups with alloxan. On contrast, healthy group (control negative group) tended to have the lowerinitial blood glucosethan groups injected with alloxan. It may be due to Alloxan is a specific toxin that causes massive destruction of the pancreatic beta cells**(Singh, et al., 2013).**

After six weeks of injection with alloxan and treating with different doses of stevia, there were significant change has been observed between groups. Diabetic rats treated with high dose of stevia tended to have blood sugar close to healthy group, even no significant difference has been observed between them. On the other hand all treated groups with stevia had blood sugar lower than control positive group. Groups treated with low and medium doses of stevia had blood sugar lower than control positive group but still higher than control negative group, even significant statistical was observed between them. The current results were within with the openion that Stevia is shown to provide a comprehensive set of mechanisms that counter the mechanics of type II diabetes and its eventual complications **(Dyrskog, et al., 2005).**

The plasma sugar data illustrated in this study was agree with early study showed that 10 g % of powdered Stevia leaves in high-carbohydrate diets given to rats caused

a significant reduction in blood glucose level following 4weeks of treatment **(Takahashi et al., 2001and Geuns, 2003**).treatment of diabetic micerats with dried steavia leaves for 6 months considerably lowered the blood sugar level. This could be due to the presence of some biomolecules present in the plant extract that may have stimulate the beta cells of langerhans to release insulin, leading to the improvement in the carbohydrate metabolizing enzymes and thus establishing normal blood glucose level. The previous openion was agree with Hossain, et al. openion **(Hossain, et al, 2011).**Thus, sugars can be replaced with steviol glycosides or stevioside of Stevia leaf to support healthy gluco-regulation. Fortunately, all treated group with steiva with different concentrations tended to have blood glucose lower than control positive group. Accordingly, the oxidative stress will be reducing. The previous opinion is within with opinion who reported that, oxidative stress along with hyperglycemia, plays a major role in the parthenogenesis of DM **(Ceriello et al., 1997 and Kasiviswanath. et al., 2005).**

With regard to the effect of different levels from stevea on serum lipoprotein cholesterol and triglyceride of diabetic rats are presented in table (2).Data in this table (2) revealed that, the mean value of total serum cholesterol in rats suffering from hyperglycemia decreased gradually with increasing the level of stevea.

Treatments of diabetic rats with the lowest level from stevea recorded the lowest decrease in serum cholesterol, in comparison with other two treated groups. While, the highest decrease in the mean value of serum cholesterol was observed in diabetic groups treated with the highest levels from stevia. The results indicated that, significant difference in serum cholesterol was observed between diabetic groups fed on basal diet (control positive) and treated with different stevia doses.

Table (2): The effect of different doses of stevia on cholesterol and triglycerides of hyperglycemic rats.

Groups \ Parameters		mg/dl.	
		Cholesterol	**Triglycerides**
Healthy rats. Control (-)		107.97d±9.19	103.62d ± 2.47
Hyperglycemic rats. Control (+)		150.90a±0.91	150.90a ± 0.91
Hyperglycemic rats fed on diet and treated with stevia	2.5g/100g	143.42b±3.00	175.98b ± 2.91
	5g/100g	141.48b±2.40	163.40c ± 2.69
	7.5g /100g	135.75c±2.93	106.20d ± 2.66

Values are expressed as mean ± SD. Significance at p<0.05 Values, which don't share the same letter in each column, are significantly different.

Treatments of diabetic rats with the lowest level from stevea recorded the lowest decrease in serum cholesterol, in comparison with other two treated groups. While, the highest decrease in the mean value of serum cholesterol was observed in diabetic groups treated with the highest levels from stevia. The results indicated that, significant difference in serum cholesterol was observed between diabetic groups fed on basal diet (control positive) and treated with different stevia doses.

On the other hand, the best results in serum cholesterol observed for hyperglycemic group fed on basal diet and treated with the high levels from stevea. Unfortunately, this group still have total cholesterol higher than control negative group (healthy group), even showed significant differences in serum cholesterol between them.

Table (2) illustrated the effect of treating diabetic rats with different levels from stevia on serum triglycerides.The data in this table showed that, triglycerides in serum diabetic rats (control positive) increased significantly at $p < 0.05$, as compared to non-diabetic rats (healthy group).

Using the three levels from stevea 2.5%,5% and 7.5% separately in treated diabetic rats led to significant decreases in serum triglycerides, as compared to the positive control group. The high level from stevia induced significant decrease in serum triglycerides in diabetic groups, as

compared to the treated groups with low and medium levels from those materials.

Results in this table indicated that, significant differences in serum triglycerides was observed between healthy group (control negative) and groups suffering from hyperglycemia and treated with (2.5% and 5%). Treating diabetic groups with 7.5% levels from stevea achieved the best results, because these groups did not differ significantly in serum triglycerides, as compared to the negative control group. The data in this table showed that injected rats with 150 mg alloxan/kg body weight to induce hyperglycemia (control –ve group) led to significant increase ($p<0.05$) in the serum, as compared to the negative control group. It should be noted that, control positive group tented to have total cholesterol and triglyceride much higher than control negative group. It may be due to the main cause of the lipid changes associated with diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells (Chahil and Ginssberg, 2006).

With regard to the effect of different levels from stevea on serum lipoprotein (LDL-c, HDL-c and VLDL-c) of diabetic rats are presented in table (3). Results showed that, treating hyperglycemic groups with the three levels from stevea resulted in significant increase at $p<0.05$ in serum HDL-c, as compared to the positive control group.

Table (3): Effect of different doses from stevia on serum lipoproteins of hyperglycemic rats.

Parameters / Groups		mg/dl. HDL-C	LDL-c	VLDL-c
Healthy rats. Control (-)		$56.52^{ab} \pm 0.9$	$30.73^{c} \pm 8.52$	$20.72^{d} \pm 0.49$
Hyperglycemic rats. Control (+)		$32.53^{d} \pm 8.6$	$83.35^{a} \pm 11.5$	$37.08^{a} \pm 0.45$
Hyperglycemic rats fed on diet and treated with stevia	2.5g/100g	$46.40^{c} \pm 1.90$	$61.92^{b} \pm 4.2$	$35.10^{b} \pm 0.69$
	5 g /100g	$61.58^{a} \pm 3.1$	$52.93^{b} \pm 5.2$	$21.24^{d} \pm 0.53$
	7.5g/100g.	$50.54^{b} \pm 11.24$	$56.41^{b} \pm 18.6$	$29.36^{c} \pm 7.12$

Values are expressed as mean ± SD. Significance at p<0.05Values, which don't share the same letter in each column, are significantly different.

The results indicated that, high-density lipoprotein of hyperglycemic groups which treated with high level from stevea decreased significantly at $p<0.05$, as compared to hyperglycemic groups which treated with medium levels. Rats suffering from hyperglycemic and treated with medium level of stevea tended to have the highest level of HDL-c among of all treated groups.

Results in table (3) showed that the three levels from stevea -decreased serum LDL-c in rats suffering from hyperglycemia, as compared to positive control group. The

data in this table showed that, serum LDL-c is approximately the same in all treated groups, even no significant difference has been observed between them.

The highest decrease in serum LDL-c recorded for hyperglycemic group, which treated with level 5% 52.93b ± 5.27 mg/dl, followed by the treated group 7.5 % 56.41 ± 18.62 mg/dl, and the group treated with 2.5% level from (stevea) 56.41 ± 18.62 mg/dl.

The same table illustrated the effect of treating diabetic rats with different levels from stevea on serum VLDL-c. The data showed compared to non-diabetic rats (healthy group).

Using the three levels from stevea in treated diabetic rats led to significant decrease in serum VLDL-c, as compared to the positive control groupthat, VLDL-c in serum diabetic rats increased significantly at $p < 0.05$, as. Statistical analysis in this table showed that, significant change in serum VLDL-c was observed between the groups which treated with different stevia doses.

The 5% level from stevea induced significant decrease in serum VLDL-c in diabetic groups, as compared to the other treated groups.

Treating diabetic groups with 5% levels from stevia achieved the best results, because these groups did not differ significantly in serum VLDL-c, as compared to the negative control group. From the data presented in the tables (2 and 3) appeared that, Alloxan administration at the dose of

150mg/kg body weight resulted in significant increase in the level of serum Total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and Very low density lipoprotein (VLDL). The previous results were agreed with earlier study (Harrison, 2001).

From the data present in the current study appeared that all treated groups have lipid profile lower than untreated groups. It may be due to S. rebaudiana possess significant anti-hyperglycemic, anti-hyperlipidemic and anti oxidative properties. This opinion is completely agreed with the previous Singh and Grag opinion (Singh and Grag, 2014).

From the above mentioned data, it be concluded that stevia leaves has anti-lipedemic affect. It may be due to leaves have bioactive component(s) such as phytochemical which responsible for the anti-lipidemic activities. It has also been reported earlier that the leaves possess high phenols and flavanoid contents, which in turn are responsible for its antioxidant activity(Harrison, 2001).

Effect of some levels from stevia on kidney functions of hyperglycemic rats:

Table (4) illustrated the changes in serum levels of urea nitrogen and creatinine (mg/dl) as a result of the treatments with three levels from (stevia) for rats suffering from hyperglycemia.

From the presented data it could be observed that alloxan injection lead to abnormal changes in kidney

functions. Serum creatinine and urea nitrogen has been increased significantly at $p< 0.05$ in diabetic group fed on basal diet, as compared to healthy groupe fed on the same diet.

Table (4): Effect of some levels from stevia on kidney functions of hyperglycemic rats.

Groups \ Parameters		mg/dl.	
		Creatinine	Urea nitrogen
Healthy rats. Control (-)		$4.42^{b} \pm 0.33$	$6.03^{c} \pm 0.71$
Hyperglycemic rats. Control (+)		$6.35^{a} \pm 0.19$	$8.72^{a} \pm 0.60$
Hyperglycemic rats fed on diet and treated with stevia	2.5g/100g	$7.45^{b} \pm 0.40$	$7.45^{b} \pm 0.40$
	5 g /100g	$6.50^{c} \pm 0.28$	$6.50^{c} \pm 0.28$
	7.5g/100g.	$4.45^{d} \pm 0.30$	$4.45^{d} \pm 0.30$

Values are expressed as mean ± SD.Significance at p<0.05Values which don't share the same letter in each column are significantly different.

Data in table (4) showed the changes in serum urea nitrogen levels of diabetic rats as a result of administration of stevia in experimental diets. From the above mentioned data, it could be observed that all treated groups with different doses of stevia produced an improvement in serum urea nitrogen and creatinine levels, as compared to positive

control group. Even there are significant differences have been observed between them.

The findings showed that, the increasing levels of stevia in the diets which prepared for treating rats led to gradual decrease in serum urea nitrogen.

The highest decrease in serum urea nitrogen in all tested groups recorded for the group which treated with the highest levels from stevia 7.5% followed by the treated groups with (5% and 2.5%). The group fed in highest dose of stevia decreased serum urea nitrogen by about 48% than that of the positive control group.

The mean value of serum creatininefor treated group has been decreased, as compared to positive control group. It should be noted that, all treated group with different doses of stevia tended to have similar serum creatinine, even no statistical difference has been observed between them. From the above mentioned data, it could be concluded that, stevia improve the kidney functions. It may be due tointravenous infusion of crude extract of Stevia leaves increase both sodium and potassium excretion (El-Gengaihi etal.,2011).

Moreover the previous author suggested that the extract may have a direct effect on salt and water transport in renal tubules. The fact that water and sodium excretion increased following sweet glycoside infusion in spite of an unchanged glomerular filtration rate (Melis ,1992a). Accordingly, stevia can improve the kidney functions**.**

Conclusion:

Oral administration of stevia caused significant declines in the blood levels of triglycerides, total cholesterols, and LDL-cholesterol, but increased HDL-cholesterol. Moreover, it seemed that stevia had hypolipidemic potential. This may be an indication of progressive metabolic control of stevia on mechanisms involved in elimination of the lipids from the body, this hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use.

Dose of 30 g/day of stevia have therapeutic potential. They possess hypolipidemic and hypoglycemic properties, but the hypoglycemic effect was more pronounced. Also, they did not show any negative impact on kidney function.

This was only a preliminary study to prove the hypoglycemic and hypolipidemic effect of stevia. Further investigation should target determination the active constituents of stevia and the mechanism of stevia and many foods that have the hepatoprotective.

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