ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of Aloe vera on Submandibular Salivary Gland of Streptozotocin-induced Diabetic Rats.

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Manuscript Info

Manuscript History:

Received: 15 November 2015
Final Accepted: 16 December 2015
Published Online: January 2016

Key words:
Lyophilized Aloe vera, streptozotocin, glibanclamide, inducible nitric oxide synthase, and aquaporin-5.

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Abstract

Salivary gland hypofunction has a negative impact on the oral tissues. Diabetes mellitus is one of different causes for salivary gland hypofunction. There are many structures in the salivary gland that may have a relation to its hypofunction such as aquaporin-5, which important for transcellular water transport in the salivary glands. It is suggested that the disturbance in aquaporin-5 causes reduction in the salivary secretion. Salivary gland hypofunction also has been associated with inflammation and nitric oxide over production. However glibenclamide is the most common and more safe hypoglycemic drug, it cause unneeded side effects like any other synthetic drugs. For that reason, there is another alternative therapies using herbs and plants. One of the plants that have antidiabetic action is Aloe vera. This study aimed mainly to evaluate the biological effect of Aloe vera on the submandibular salivary gland in streptozotocin induced diabetic white albino rats. sixty-two albino rats were divided into four groups: I) control group, II) diabetic group without any treatment, III) diabetic group + Aloe vera, IV) diabetic group + glibanclamide. Induction of diabetes was done by single intrapritoneal injection of streptozotocin. Then blood glucose levels and body weight were measured, submandibular salivary glands were isolated, and immunohistochemical for inducible nitric oxide synthase and aquaporin-5 were done. The Aloe vera treated group showed, at the end of experiment, decrease in the blood glucose level, and nearly normal body weight, and immunohistochemically, inducible nitric oxide synthase expression and aquaporin-5 expression were not significantly changed compared to control group.

Introduction:-

Salivary glands importance is related to their secretion (saliva). Saliva has a pivotal role in maintenance of oral homeostasis. **(Proctor GB & Carpenter GH, 2007)** Water secretion is regulated by water channel proteins on the secretory cells, called aquaporin-5 (AQP5). It is supposed that any change in AQP5 may cause change in the salivary secretion. (**Matsuzaki et al., 1999)** Normally, nitric oxide is produced in the salivary gland for benefit action through a reaction catalyzed by inducible nitric oxide synthase (i-NOS). (**Lomniczi et al., 1998**) If nitric oxide is overproduced, it leads to destruction in salivary gland's cells. **(Konttinen et al., 1997)**

Diabetes mellitus is a metabolic disorder with multiple etiology, characterized by chronic hyperglycemia resulting from abnormal insulin secretion and/or action. **(American Diabetes Association, 2010)** It has many complications

that affect several tissues and organs. These complications usually are long-term complications. **(Nathan DM, 1993)** There are several oral manifestations associated with diabetes, such as salivary dysfunction, periodontal destruction, and oral mucosal lesions. These manifestations may be related to reduction in the salivary secretion. (**Shrimali et al., 2011; Chandna et al., 2010)**

In the last years, There are more concern toward herbal treatment to avoid the side effects caused by medical treatment. (**Dzeufiet PD et al., 2007**) There are many plants are found to have antidiabetic action, such as Aloe vera**. (Okyar et al., 2001)** Aloe vera is a one of the safe herbal treatment that has different therapeutic uses. It contain a lot of constituents, such as enzymes, minerals, vitamins, and sugars. **(Rajasekaran et al., 2005)** This study was designed mainly to find whether the diabetes mellitus affect on inducible nitric oxide synthase (i-NOS) and aquaporin-5 (AQP5) levels in submandibular salivary gland and if the Aloe vera has a protective effect on the same structure or not.

Materials and methods:-

- **Animals:-**

In this study, 62 white albino rats (200-250 gm body weight) were housed in Mansoura experimental research center, faculty of medicine, Mansoura university, according to the ethical and animal care guidelines of Mansoura university. They were divided into four groups:

- **Group I (Control group):**
  This group consisted of 12 rats not received any treatment and not diabetic.
- **Group II (diabetic group):**
  The animals of this group consisted of twenty rats, that received streptozotocin (Sigma) for induction of diabetes and received distilled water by oral gastric tube.
- **Group III (Aloe vera treated group):**
  The animals of this group consisted of twenty rats, that received streptozotocin (STZ) for induction of diabetes as group II, moreover were treated daily by lyophilized powder of Aloe vera gel (Swanson®) 300 mg/kg mixed in 1 ml distilled water using oral gastric tube. (**Noor et al., 2008)**
- **Group IV (Glibenclamide treated group):**
  The animals of this group consisted of twenty rats, that received streptozotocin for induction of diabetes and received glibenclamide (***DAONIL***®) 600 µg/kg in distilled water by oral gastric tube.

- **Induction of diabetes:-**

After fasting the rats for 18 hours, induction of diabetes was done by intraperitoneal injection of 50 mg/kg STZ freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. **(Szkudelski T, 2001)** After three days of induction, Diabetes induction was confirmed by measuring blood glucose levels. Animals with blood glucose level above 300 mg/dl were defined as diabetic and were used in the study.

Measuring the body weight and blood glucose level:-

Blood glucose level was measured by bioneme blood glucose tester and body weight also was measured.

- **Biopsy collection:**

The rats of each group were euthanized by sodium thiopental (Ravonal®, Tanaba Seiyaku, Osaka, Japan) overdose 40 mg/kg by intrapritoneal injection at 1, 2, 4, 6, and 8 weeks and then the submandibular salivary glands were excised. (**Hara et al., 2012)**

- **Sections of specimens were prepared for:**
  1. Haematoxylin & Eosin stain.
  2. Immunohistochemical stains with rabbit anti-iNOS polyclonal antibody and rabbit anti-AQP5 polyclonal antibody by avidin-biotin complex method.

Computer Assisted digital image analysis:-

5 slides from each case were prepared, 5 random fields from each slides were analyzed.
Slides were photographed by Olympus® digital camera that was installed on Olympus® microscope with 1/2 X photo adaptor, using 400X objective lens. The result images were analyzed on Intel® Core I3® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for stain quantification and area measurement.

Statistical analysis:-

Data were tabulated, coded and analyzed by using the computer program Statistical package for social science (SPSS) (version 17). Data were expressed as Mean and Standard deviation (±SD).The statistical comparison between the different groups, the significance of difference was tested using one of the following tests:-

1- **Analysis of variance** (**ANOVA**):- was used to make comparison between more than two groups of parametric (numerical) data followed by post hoc tukey for multiple comparisons.
2- **Repeated ANOVA:** Used to compare between more than two groups with time series of numerical (parametric) data followed by post hoc tukey for multiple comparisons.

Pearson correlation coefficient test was used correlating different parameters.

A (*P* value) was considered statistically significant, if it was <0.05.

Results:-

❖ **General observations:**

1- Body weight:- (Table 1, & 2) (Fig. 1)

There was reduction in body weight after 1 week from induction of diabetes in group II (**171.67±3.56)**, III (**191.5±6.09)**, and IV (**199.17±6.97)** compared to group I (**251.33±18.13)**. In group II, there was more reduction in weight till the end of the experiment (**156.3±2.12)**. While in group III, and IV, there were reduction after induction of diabetes and recover latter on till the end of experiment to be after 8 weeks from induction of diabetes (**236.5±4.6), &** (**250.33±9.95)** respectively.

2- Blood glucose level:- (Table 1, & 2) (Fig. 1)

After 1 week from induction of diabetes, there was increase in the blood glucose level on group II (**602.0±20.36),** group III (**336.17±23.77)**, & group IV **(315.5±34.77).** The blood glucose level was nearly normalized in group III (**158.33±29.98)**, and IV (**126.33±7.76)** at the end of experiment.

❖ **Histological results:**

1- Haematoxylin and eosin stain:- (Fig. 2)

After 1 week, there were cytoplasmic vacuolations and pyknotic nucleus in the groups II, III, and IV. In addition to that, sections in group II showed focal loss of salivary architecture, and few lining cells loss from excretory duct. While in group IV, focal loss of salivary architecture was also seen.

After 2 weeks, in group II, there was more cytoplasmic vacuolation and pyknotic nucleus with focal areas of degeneration and widening in interacinar and periductal spaces, while group III showed less amount of cytoplasmic vacuolation, and group IV showed a lot of cytoplasmic vacuolation and pyknotic nucleus with vacuolar degeneration of salivary structures.

After 4 weeks, the slides show the same changes similar to changes at 2 weeks.

After 6 weeks, both group II and IV showed a lot of cytoplasmic vacuolations and pyknotic nucleus with degenerated salivary structure. While group III showed nearly normal salivary architecture.

After 8 weeks, both group II and IV showed a lot of cytoplasmic vacuolations and pyknotic nucleus with degenerated salivary structure and fat globules in connective tissue septa. While group IV had normal acinar outline.

2- Immunohistochemical stains:- (Fig. 1, & 3)

a) Anti- Inducible nitric oxide synthase:- (Table 1, &4)

The positive immune-reaction for iNOS appeared mainly in the cytoplasm of ductal cells and also in the cytoplasm of acinar cells. iNOS expression in the submandibular salivary gland of group II (**3.061±1.07)** is marked and increase along the experiment to be after 8 weeks (**6.99±1.81)** compared to that of group I (**1.16±0.2)**, III (**1.08±0.18)**, & IV (**1.87±0.65)**.

b) **Anti-Aquporin-5:** (Table 2,& 4)

AQP5 was expressed on the cell membrane of acinar cells and cytoplasm of ductal cells. After 1 week, group II showed reduction in AQP5 expression (**7.04±2.15)** compared to groups I (**8.92±4.68)**, III (**8.78±3.09)** and IV **(7.58±2.15)**. The expression of AQP5 decreased in group II more after 8 weeks (**1.97±0.3)** than that of groups III (**7.04±1.83)** and IV (**6.22±0.96).**

Discussion:-

Saliva have a pivotal role in maintaining oral tissues healthy. Hypofunction in the salivary glands will have a bad impact on the oral tissues. **(Proctor GB & Carpenter GH, 2007)** This hypofunction may be occurred due to drugs, or systemic disease such as diabetes. Submandibular salivary glands secrete a large amount of saliva, so that this study was done on SMG. (**Fedirko et al., 2006)**

Diabetes cause many complications including cardiovascular disease, kidney disease, neuropathy, blindness, and lower-extremity amputation. **(Deshpande et al., 2008)** There are also oral complications related to diabetes such as periodontitis, hyposalivation, taste alteration and halitosis. **(Shrimali L et al., 2011; Chandna S et al., 2010)** The three most significant risk factors are hyperglycemia, high blood pressure, and hypercholesterolemia, so adequate control of blood glucose levels, blood pressure, and blood lipid levels can prevent or even delay the onset of diabetes-related complications. (**Assmann et al., 1988)**

According to **Ravi et al.** (**2004)**, glibenclamide is the standard hypoglycemic drug and have been used for many years to treat diabetes, so the effect of A. vera was compared with glibenclamide.

In this study, there was a detectable weight loss in all rats of group II (171.67±3.56), III (191.50±6.09) and VI (199.17±6.97) after 1 week as compared to that of group I (351.33±18.13). The body weight in group II had more reduction in weight till the end of experiment (156.3±2.12).

This result is in accordance with **Tanaka et al. (2006)** who revealed weight loss in diabetic mice to losing more calories due to glucose loss in the urine. While **Rajkumar L and Govindarajulu P.** (1991) explained the decrease in body weight in diabetic rats to loss or degradation of structural proteins, which contribute to body weight.

While group III (236.5±4.6) and group IV (250.33±9.95) had increase in body weight starting from 4 weeks after induction.

For group III (A. vera treated group) the result of this experiment is in harmony with **Noor et al. (2008),** and **Helal et al. (2003)** While group IV weight gain result is in agreement with **Pari L and Satheesh M.A. (2004).** These results may be due to hypoglycemic action of A. vera and glibenclamide.
In this study, A. vera treated group (III) had increase in blood glucose level after induction of diabetes by 1 week (336.17±23.27) than normal, while the blood glucose level decreased later on to be (160.00±22.60) after 6 weeks and (158.33±29.98) after 8 weeks compared to control group that had the blood glucose level (102.50±14.43) and glibenclamide treated group (126.33±7.76) after 8 weeks, so glibenclamide is more effective in lowering the blood glucose level than A. vera.

In contrast to our results, **Rajasekaran et al. (2006)** who used A. vera gel extract for one diabetic group 300 mg/kg and other diabetic group use 600 µg/kg glibenclamide but after 21 days found that A. vera decreased the blood glucose level and increased the insulin level more than glibanclamide treated group. **Rajasekaran et al. (2006)** explained the antihyperglycaemic activity of A. vera could be due to an insulinogenic activity of the gel extract by stimulating insulin secretion from the remenant β-cells and/or from regenerated β-cells. **Mohamed EK (2011)** added to this explanation that A. vera could increase the total antioxidant capacity that found to be decreased in diabetic rats.

As A. vera have many components, **Tanaka et al. (2006)** used 1 µg of phytosterols (4-monomethyl and 4-dimethyl sterols) derived from A. vera gel, that had antioxidant action, and found that it lowered the blood glucose levels in diabetic mice**. (Law MR, 2000; Wang T et al., 2002)**

In the current study, glibenclamide treated group (IV) had a reduction in blood glucose level after 1 week from diabetes induction (315.50±34.77) compared to untreated group (602±20.36). Previous studies explained this hypoglycemic effect of glibenclamide is by inhibition of the ATP-sensitive K+ channels, which leads to depolarization of the β-cells and subsequently insulin secretion. (**Proks et al., 2002)**

Histological section in SMG with ordinary staining for group II after 1 week showed cytoplasmic vacuolation, pyknotic nucleus, focal loss of salivary architecture that increased latter on with widening in interacinar and periductal spaces and degeneration of salivary structures.

In the same direction **Hidayat et al.** (2014) documented these results after 6 weeks found lipid infiltration of the parotid gland of diabetic rats in the form of lipid vacuoles with degeneration of the serous acini and they revealed the changes in parotid gland to oxidative stress (liberation of nitric oxide, lipid peroxidation, generation of free radicals, decreased levels of catalase and glutathione peroxidase, protein glycosylation, as well as DNA single-strand breaks) that increased in diabetes.

In the present study, Aloe vera treated group had less vacuolation in the cytoplasm of acini cells and less degeneration in salivary structures that appeared after 1 week and decreased latter on with regeneration of the normal salivary architecture starting from 4 weeks till the end of experiment. In agreement with these results, **Nejaim et al**. (2014), found that A. vera has a protective action on salivary gland in rats exposed to ionizing radiation which cause free radical formation and revealed this radioprotective action to the antioxidant effect of A. vera, so The beneficial effect of Aloe vera on SMG may be related to antioxidant effects that can protect cells against inflammatory processes and oxidative damage caused by diabetes. **(Donath MY and Shoelson SE, 2011)**

The immunohistochemical reaction in the current study revealed that mild immunoreactivity to **iNOS** in normal salivary gland cells in harmony with **Correia et al.** (2010)
While the expression of iNOS was marked in group II compared to control group I throughout the experiment.

This is in accordance with **Astaneie et al**., (2005) who found that there was increase in the amount of nitric oxide in the saliva of diabetic patient indicating the existence of oxidative stress. The mechanism of this hypofunction is by increase in the production of NO that produced by a reaction catalyzed by iNOS. NO at high concentration inhibits various iron-containing DNA synthetases and mitochondria1 enzymes, and thus inhibits cell growth and division, finally leading to cell death. **(Kleinert et al., 2004; Konttinen et al., 1997)**

While in the group III, iNOS expression is more or less approach control group's values. Also **Kim et al. (2009)** found that complex containing Aloe vera cause decrease iNOS in liver in hepatotoxicity. This in harmony with **Sarkar et al. (2005),** who found that Aloe vera cause reduction in NO production in macrophages, and so inhibit the release of prostaglandin, causing suppression of inflammation.

In group IV from this study, there was a little increase in the iNOS expression than group I and III. As glibenclamide can reduce iNOS this in accordance with **Wu et al. (1995)** who make induction for iNOS by lipopolysaccharide (as endotoxin) and found that glibenclamide inhibits the induction of iNOS which is supposed to be the mechanism glibenclamide's beneficial hemodynamic effect with septic shock.

In this study immunohistochemically, AQP5 expression in salivary gland was found in control group in cell membrane of acini cells and a ductal cells' cytoplasm.

This in accordance with **Matsuzaki et al. (1999), Gresz et al. (2001),** and **Nielsen et al**. **(1997)** but the AQP5 was localized SMG in the apical plasma membrane of the acinar cells and in the secretory canaliculi between the cells only. While **Ishikawa et al**. **(2005)**, and **Cotroneo et al. (2008),** observed some apical staining in interlobular duct cells in addition to apical plasma membrane of the acinar cells and in the secretory canaliculi.

This expression for AQP-5 was decreased in diabetic group compared to control group. This result in accordance with **Wang et al. (2011)** The explanation may be due to diabetes induced hyperlipidemia and this in accordance with **Fangqin et al**. **(2012),** who found a reduction in the expression of AQP5 in the submandibular glands of hyperlipidemic rats.

In the other hand, the AQP5 expression in SMG of A. vera treated rat was near the normal level. This result may be related to antihyperglycemia and antihypercholesteremia effect of Aloe vera in accordance to
Rajasekaran et al. (2006), and Huseini et al (2012).

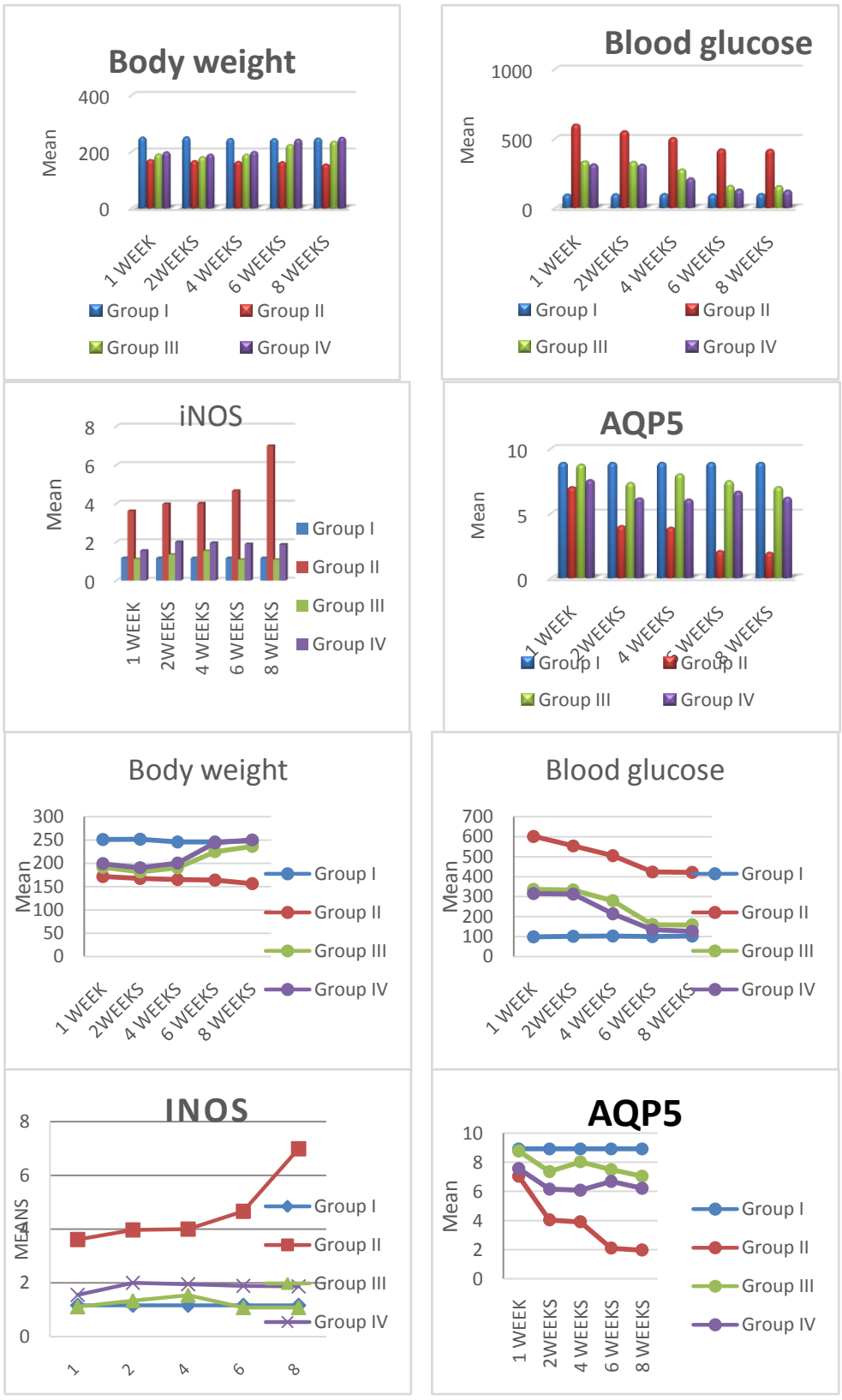

Fig. 1: histograms showing the mean at the periods of sacrifaction for the studied groups. And line charts showing the mean for the studied groups at different periods of sacrifaction.

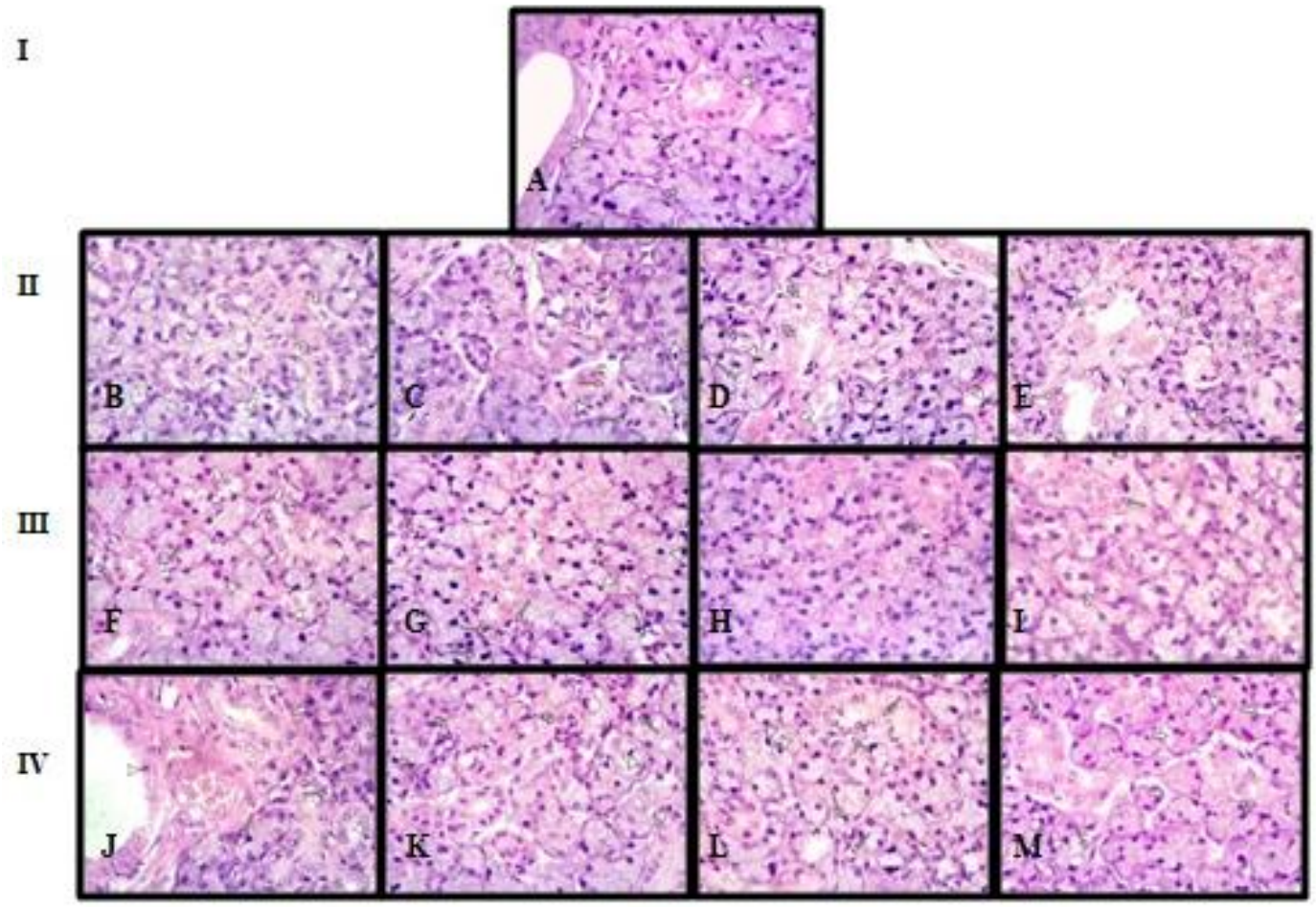

Fig. 2: photomicrographs of SMG showing: A) group I. B, F, J) group II, III, and IV respectively after 1 week. C, G, K) group II, III, and IV respectively after 2 weeks. D, H, L) group II, III, and IV respectively after 6 weeks. E, I, M) group II, III, and IV respectively after 8 weeks. (H&Ex400)

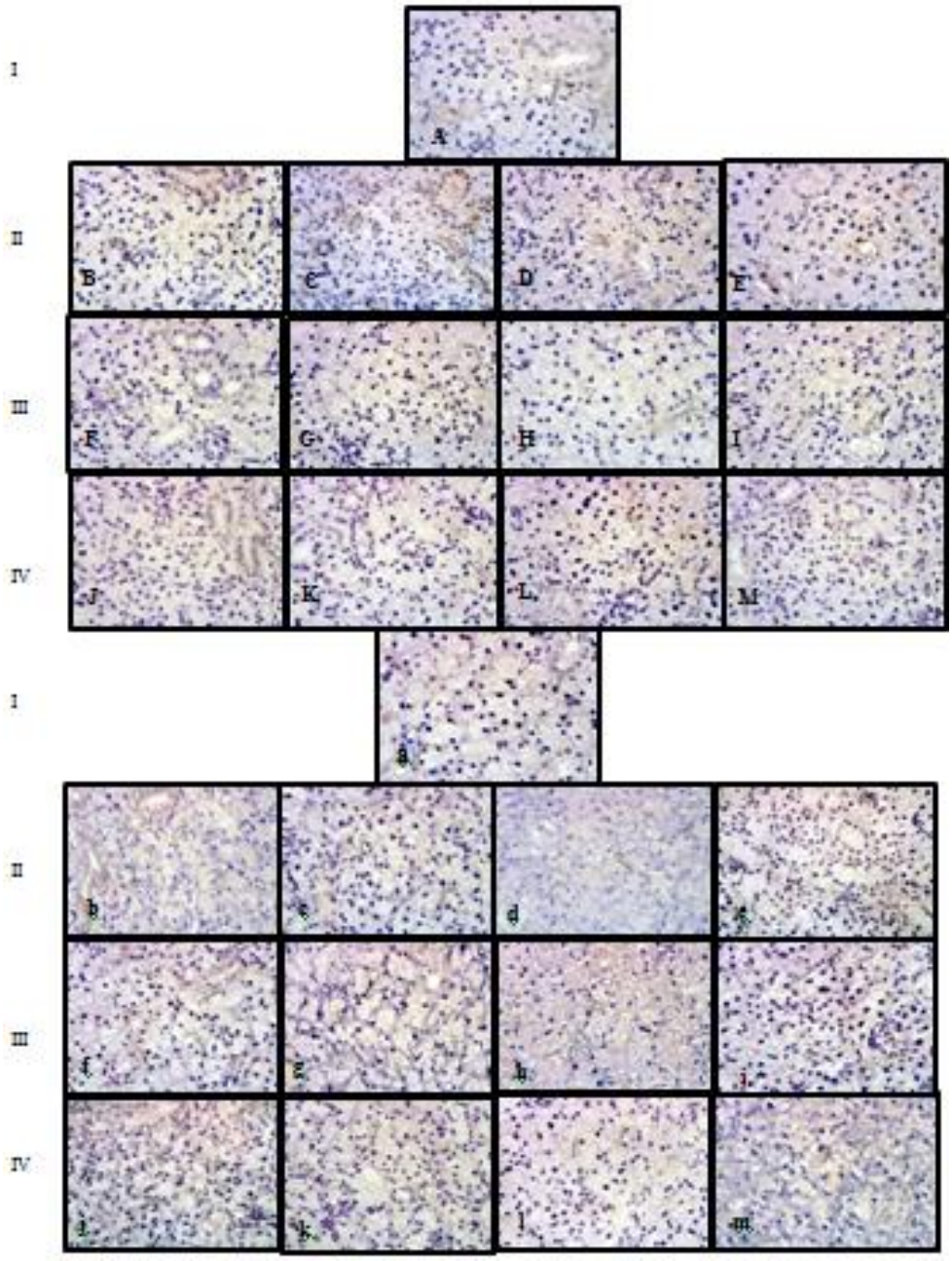

Fig. 3: photomicrographs of SMG immunolabelled with iNOS in photos with capital letters photos, and with AQP5 in photos with small letters. A: for group I showing the expression of iNOS in cytoplasm of ductal (arrow) and acinar cells (arrow head), a: for group I showing the expression of AQP5 in the acinar cell membrane (arrow) and cytoplasm of ductal cells (arrow head), B/b, F/f, J/j: groups II, III,& IV respectively after 1 week, C/c, G/g, K/k: groups II, III, & IV respectively after 2 weeks, D/d, H/h, L/l: groups II, III, & IV respectively after 6 weeks, and E/e, I/I, M/m: groups II, III, & IV respectively after 8 weeks. (IHCx400).

Table 1: showing the mean ±SD of the rat's body weight, and blood glucose level at different periods of sacrifaction

for the studied groups.

		Body Weight				P	Blood glucose				P
		Groups					Groups				
		I	II	III	IV		I	II	III	IV	
1W.	Mean	251.33	171.67[a]	191.50[ab]	199.17[ab]	<0.0001	98.17	602.0 [a]	336.17 [ab]	315.5 [ab]	<0.0001
	±SD	18.13	3.56	6.09	6.97		10.76	20.36	23.27	34.77	
2W.	Mean	252.00	167.67[a]	181.50 [a]	190.83[ab]	<0.0001	100.5	554 [a]	333.33 [ab]	312.83 [ab]	<0.0001
	±SD	15.10	11.36	5.58	15.30		16.48	50.47	23.68	24.33	
4W.	Mean	246.00	165.33[a]	190.33 [ab]	200.50 [ab]	<0.0001	102.0	505.0[a]	279.17 [ab]	214.17 [ab]	<0.0001
	±SD	14.38	6.38	2.88	5.54		18.57	114.67	28.97	21.94	
6W.	Mean	245.83	163.83[a]	224.83 [b]	244.00 [b]	<0.0001	99.17	423.5[a]	160.00 [b]	134.00 [b]	<0.0001
	±SD	9.37	10.09	19.83	11.26		11.41	96.31	22.60	13.34	
8W.	Mean	248.00	156.3 [a]	236.5 [b]	250.33 [b]	<0.0001	102.5	421.0 [a]	158.33 [b]	126.33 [b]	<0.0001
	±SD	14.55	2.12	4.6	9.95		14.43	86.45	29.98	7.76	

P: Probability Test used: ANOVA followed by post-hoc tukey
a: significance relative to Group I (with gps II,III,IV)
b: significance relative to Group II (with gps III,IV)
c: significance relative to Group III (with gp IV)

Table 2: showing the mean ± SD of the rat's body weight and blood glucose level of the studied groups at different periods of sacrifaction.

		Body weight					P	Blood glucose					P
GROUPS		1 WEEK	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS		1 W	2 W	4 W	6 W	8 W	
I	Mean	251.33	252.00	246.00	245.83	248.00	0.7	98.17	100.50	102.00	99.17	102.50	1.00
	±SD	18.13	15.10	14.38	9.37	14.55		10.76	16.48	18.57	11.41	14.43	
II	Mean	171.67	167.67	165.33	163.83	156.3 [a]	0.02	602.00	554.00	505.00	423.50 [a]	421.00 [a]	0.014
	±SD	3.56	11.36	6.38	10.09	2.12		20.36	50.47	114.67	96.31	86.45	
III	Mean	191.50	181.50	190.33	224.83 [b]	236.5[abc]	<0.0001	336.17	333.33	279.17	160.00 [ab]	158.33[ab]	<0.0001
	±SD	6.09	5.58	2.88	19.83	4.6		23.27	23.68	28.97	22.60	29.98	
IV	Mean	199.17	190.83	200.50	244.00[abc]	250.33[abc]	<0.0001	315.50	312.83	214.17	134.00 [ab]	126.33[ab]	<0.0001
	±SD	6.97	15.30	5.54	11.26	9.95		34.77	24.33	21.94	13.34	7.76	

P: Probability Test used: repeated ANOVA followed by post-hoc LSD
a: significance relative to Week1 (with other weeks 2,4,6,8) in each group.
b: significance relative to Week2 (with other weeks 4,6,8) in each group.
c: significance relative to Week4 (with other weeks 6,8) in each group.
d: ***significance relative to Week6 (with weeks 8) in each group.***

Table 3: showing the mean ± SD of iNOS and AQP5 of the studied groups at different periods of sacrifaction.

		iNOS					P	AQP5					P
GROUPS		1 W	2 W	4 W	6 W	8 W		1 W	2 W	4 W	6 W	8 W	
I	Mean	1.16	1.16	1.16	1.16	1.16	-	8.92	8.92	8.92	8.92	8.92	-
	±SD	.20	.20	.20	.20	.20		4.68	4.68	4.68	4.68	4.68	
II	Mean	3.61	3.97	4.00	4.66	6.99[abc]	0.04	7.04	4.04	3.91[a]	2.10[abc]	1.97[abc]	0.018
	±SD	1.07	1.10	1.12	.46	1.81		2.15	1.18	.90	0.71	.3	
III	Mean	1.11	1.34	1.54	1.08	1.08	0.8	8.78	7.36	8.03	7.50	7.04	0.8
	±SD	.31	.46	.56	.49	.18		3.09	2.39	1.93	1.98	1.83	
IV	Mean	1.55	2.00	1.95	1.89	1.87	0.9	7.58	6.17	6.09	6.69	6.22	0.7
	±SD	.55	.54	.26	.50	.65		2.15	2.86	.65	1.84	.96	

P: Probability Test used: repeated ANOVA followed by post-hoc LSD
a: significance relative to Week1 (with other weeks 2,4,6,8) in each group.
b: significance relative to Week2 (with other weeks 4,6,8) in each group.
c: significance relative to Week4 (with other weeks 6,8) in each group.
d: ***significance relative to Week6 (with weeks 8) in each group.***

Table 4: showing the mean ±SD of AQP5 and iNOS expression at different periods of sacrifaction for the studied groups.

		AQP5					iNOS				P
		Groups				P	Groups				
		I	II	III	IV		I	II	III	IV	
1W.	Mean	8.92	7.04	8.78	7.58	0.4	1.16	3.61[a]	1.11[b]	1.55[b]	<0.0001
	±SD	4.68	2.15	3.09	2.15		0.20	1.07	0.31	0.55	
2W.	Mean	8.92	4.04	7.36	6.17	0.07	1.16	3.97[a]	1.34[b]	2.00[b]	<0.0001
	±SD	4.68	1.18	2.39	2.86		0.20	1.10	0.46	0.54	
4W.	Mean	8.92	3.91[a]	8.03	6.09	0.015	1.16	4.00[a]	1.54[b]	1.95[b]	<0.0001
	±SD	4.68	.90	1.93	.65		0.20	1.12	0.56	0.26	
6W.	Mean	8.92	2.10[a]	7.50[b]	6.69[b]	0.002	1.16	4.66[a]	1.08[b]	1.89[bc]	<0.0001
	±SD	4.68	.71	1.98	1.84		0.20	0.46	0.49	0.50	
8W.	Mean	8.92	1.97[a]	7.04[b]	6.22[b]	0.001	1.16	6.99[a]	1.08[b]	1.87[b]	<0.0001
	±SD	4.68	.30	1.83	.96		0.20	1.81	0.18	0.65	

P: Probability Test used: ANOVA followed by post-hoc tukey
a: significance relative to Group I (with gps II,III,IV)
b: significance relative to Group II (with gps III,IV)
c: significance relative to Group III (with gp IV)

Conclusion:-

In the light of findings of this study, we can conclude that the lyophilized powder of A. vera gel can lower the blood glucose level and preserve the submandibular salivary gland in diabetic rats, so it can be used as adjunctive therapy with antidiabetic drugs.

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