The Effect of Different Levels from Ashwagandha Roots Powder *(Withania somnifera)* on Rats Suffering from Diabetes

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مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2021.64310.1270

المجلد السابع العدد 35 .يوليو 2021

الترقيم الدولي

P-ISSN: 1687-3424 E- ISSN: 2735-3346

موقع المجلة عبر بنك المعرفة المصري https://jedu.journals.ekb.eg/

موقع المجلة http://jrfse.minia.edu.eg/Hom

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

The effect of different levels from Ashwagandha roots powder *(Withania somnifera)* on rats suffering from diabetes

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Abstract

The present study aims to investigate the effect of different levels from Ashwagandha roots powder (ARP) on rats suffering from diabetes. Thirty male albino rats weighing (150±10g) were divided into two main groups. The first main group (n=6) was a negative control group fed on basal diet. The second main group (n=24) was injected subcutaneously with alloxan (150mg/kg BW) to induce diabetes; which classified into four subgroups as follow; subgroup 1 was a positive control group fed on a basal diet, subgroups (2,3 and 4) were fed on a basal diet containing 2.5, 5 and 7.5% ARP. The obtained results revealed that the chemical composition of ARP contained 6.34, 3.92, 0.72, 5.38, 31.48 and 52.16% for moisture, protein, fat, ash, crude fiber and total carbohydrates respectively, while caloric value recorded 230.80 kcal/100g. Injected rats with alloxan exhibited hyperglycemia, elevation in lipid profile (total cholesterol, triglycerides, low and very low density lipoprotein-cholesterol), liver enzymes, and kidney functions and reduction in antioxidant enzymes activity. Treatment with different levels of ARP revealed gradual improvement in all parameters, when compared to the untreated diabetic group. The most pronounced improvement was in the treated group that received the highest level of ARP 7.5%. The histological examination of pancreas confirmed a gradual improvement in all treated groups. Sensory evaluation results showed that all samples obtained a higher than 75% in overall

acceptability. Conclusion, the present study indicates that ARP has potent hypoglycemic activity and can be used as a supplement in the diet of diabetic patients.

key words: Ashwagandha roots - pan bread - hyperglycemia - lipid profile - antioxidant enzymes.

INTRODUCTION:

Diabetes is getting higher as a public health concern in Egypt. Its high incidence tends to rise as a result of increased prevalence of central obesity, sedentary lifestyle, increased prevalence of hepatitis C, and likely increased use of uncontrolled pesticides **(Hegazi *et al.*, 2015).** Besides that, smoking among adults, illiteracy in health and inadequate attention to care raised the rate of diabetes complications **(Nelson *et al.*, 2010).** Egyptian officials are trying to improve diabetes treatment with a small health care budget, but a range of quality of care interventions and recommendations are also required to expand this initiative **(Hegazi *et al.*, 2015)**. **The International Diabetes Federation (2019)** ranked Egypt in the top 10 countries in the world in terms of the number of diabetes patients. It is concerning that the prevalence of diabetes in Egypt has risen steadily within a comparatively short time from approximately 4.4 million in 2007 to 7.5 million in 2013, this figure is projected to jump to 13.1 million by 2035. Medicinal plants contain a variety of active compounds having a therapeutic properties **(Abouzekry *et al.*, 2021).**

For the last few decades, there is increasing interest in information about the drugs derived from plants which help to control diseases. Also the herbal products are safer than synthetic products which may be harmful and unsafe to the human body and environment **(Jamshidi-Kia *et al.*, 2018).** There is no ultimate method possible to prevent and treat diabetes mellitus but techniques are required to reduce the complications of the disease; thus, many herbs and

plants have been described as possessing hypoglycaemic activity and beneficial for reducing oxidative stress and its harmful effects. One of these plants is *Withania somnifera (L.)* which is commonly known as Ashwagandha; it is a perennial plant belonging to the family *Solanaceae* and extensively used in Ayurvedic system of medicine in India **(Anwer *et al.*, 2017; Ahmad and Dar, 2018).**

Roots of ashwagandha have a several bioactive chemical compounds such as amino acids, reducing sugars, volatile oil, starch, flavonoids, tannin, sitoindosides, glycosides, withanicil, withaniol, steroidal lactones, and alkaloids **(Kalra and Kaushik, 2017; Mukherjee *et al.*, 2020)**. The major chemical constituents of ashwagandha roots are alkaloids and steroidal lactones. Withanine is the primary constituent of the numerous alkaloids. Other alkaloids include somniferine, msomnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropine, choline, cuscohygrine, isopelletierine, anaferine, and anahydrine **(Bharti *et al.*, 2016; Chen *et al.*, 2011).** Numerous studies have shown that the root extracts of Ashwagandha have been used for their therapeutic properties, such as anticancer, anti-inflammatory, antimicrobial, anti-tumor, anti-stress and antioxidant **(Singh, *et al.*, 2021; Krutika *et al.*, 2016).** It also has beneficial effects on on boosting the immune system **(Mishra and Mishra, 2020),** endocrine, cardiopulmonary, hepatoprotective, and central nervous system **(Chaturvedi *et al.*, 2018).**

Recently, Ashwagandha in the form of tea is usually used to improve immunity, cardiovascular system and facilitate detoxification, which helps preserve better health. Ashwagandha roots were found to be devoid of any toxic effect in acute and sub-acute toxicity studies **(Durg *et al.*, 2020).** Extracts didn't cause any mortality, nor showed any changes in normal behavior in rats **(Mukherjee *et al.*, 2020).** Additionally, fairly high daily *W. somnifera* extracts

doses are well tolerated without any severe adverse effects and that they could be effective and safe herbal therapeutic alternatives against diabetes associated hyperlipidemia and other comorbidities **(Usharani *et al.*, 2015).**

Considering the above, the present study investigated the effect of different levels from Ashwagandha roots powder on type 2 diabetic rats, besides evaluating the sensory characteristics of pan bread supplemented with ARP.

MATERIALS AND METHODS

Materials

- Casein, vitamins, minerals, cellulose, choline chloride and alloxan were purchased from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

- Bread manufacturing components: Whole-wheat flour, dry yeast, salt, sugar, skim milk powder, corn oil obtained from the local market of Damietta; Damietta Governorate, Egypt.

- Ashwagandha roots were purchased from local Egyptian market.

Methods

Determination of gross chemical composition

Ashwagandha roots were grinded and analyzed for moisture, ash, total protein, crude fiber and fat contents, while total carbohydrates were calculated by difference according to **A.O.A.C (2000).** Caloric value was calculated according the following equation:

Caloric value = 4 (protein%+ carbohydrates%) + 9 (fat%).

The biological assay

Thirty healthy adult male albino rats Sprague Dawley Strain weighing 150±10 g per each were obtained from Nile Center for Experimental Researches, Mansoura City. Rats were individually housed in wire cages under the normal laboratory condition and were fed on basal diet for one week as an adaptation period. The basal diet prepared according to the following formula as mentioned by **Reeves *et al.* (1993)** as follow: casein (14%), corn oil (4%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.25%), cellulose (5%), and the remained is corn starch. The used vitamins mixture component was that recommended by **Campbell, (1963)** while the salts mixture used was formulated according to **Hegested *et al.* (1941).** Thereafter, rats were randomly divided into two main groups, the first group [group 1: negative control group (C-), 6 rats] fed on basal diet and the second group (24 rats) was injected subcutaneous with alloxan (150 mg/kg BW) to induce diabetes in rats after fasting overnight **(Buko *et al.*, 1996),** which classified into four subgroups of 6 rats each as follow; subgroup 1: positive control (C+) fed on basal diet, subgroup 2: fed on basal diet containing ARP 2.5%, subgroup 3: fed on basal diet containing ARP 5% and subgroup 4: fed on basal diet containing ARP 7.5%. Rats were maintained under standard conditions (23±2°C temperature, 55±5% relative humidity, 12h light/12h dark cycle). The biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council **(NRC, 2011).** The animals were fed diet and water ad-libitum for a period of 4 weeks. The diets consumed and body weights were recorded twice weekly.

At the end of experiments, all rats were fasted up to 12 hours and then sacrificed. Blood samples were collected from the aorta. The blood samples were centrifuged, separated and stored frozen at -20°C until further analysis. Internal organs: liver, kidneys and spleen of each rat were

removed, washed in saline solution, dried by filter paper and weighed separately according to method mentioned by **Drury and Wallington, (1980).**

Biochemical analysis

Enzymatic determination of serum glucose was carried out colorimetrically according to **Yound, (1975).** Serum total cholesterol, triglyceride and high density lipoprotein were determined using the methods described by **Allain *et al.* (1974); Fassati and Prencip (1982); and Lopez-virella (1977)** respectively. The determination of low density lipoprotein cholesterol and very low density lipoprotein cholesterol were carried out according to the methods described by **Lee and Nieman, (1996).** Serum levels of urea and uric acid were determined according to **Pattn and Crouch (1977); and Schultz (1984),** respectively. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in serum according to **Tietz, (1976).** Antioxidant enzymes; glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) activities were measured in RBC's by the method as described by **Flohé and Gunzler 1984; Aebi, 1984; and Mc Cord and Fridovich, 1969,** respectively.

Histological Examination

Tissues from liver and pancreas of the sacrificed rats were examined as described by **Bancroft and Cook, (1998).**

Preparation of Pan Bread

The straight dough method for pan bread production was carried out according to the method described by **A.A.C.C. (2002)** as follows: The ingredients consisted of whole-wheat flour (1000g), water (550g), dry yeast (20g), salt (10g), sugar (40g), and corn oil (20g). The ingredients were mixed for 5 minutes. The dough was left to rest for 20

min at 28 – 30°C (first proofing) then divided, rolled and molded. Each piece was placed in metal pan and left to ferment for 60 min (final proofing), then the baking process was carried out in electrically oven at 210-220°C for 15-20 min. After baking, bread allowed to cool at room temperature before sensory evaluation. Control pan bread was made from 100% whole wheat flour. Different formulas of pan bread were made from different levels of Ashwagandha Roots Powder (2.5%, 5% and 7.5%).

Sensory evaluation

Sensory evaluation of Pan bread was done as described by **A.A.C.C. (2002).** using 10 panelists of staff members from Home Economic Department, Faculty of Specific Education, Damietta University, Damietta, Egypt. Samples of the pan bread were prepared one day earlier before the evaluation, cooled for 1-2h at room temperature (25±3ºC). Sensory attributes for color (20), taste (20), odor (20), texture (20), general appearance (20) and overall acceptability (100) were evaluated.

Statistical analysis

Data obtained were statistically analyzed by SPSS computer software SPSS 2000. The results were expressed as mean ± standard deviation (SD) and tested for significance using one-way analysis of variance ANOVA test, according to **Armitage and Berry, (1987).**

RESULTS AND DISCUSSION

Proximate chemical composition of Ashwaganda Roots Powder

Data in Table (1) presented proximate chemical composition of Ashwagandha roots powder (ARP). Moisture, total protein, fat, ash, crude fiber and

carbohydrate were receded 6.34, 3.92, 0.72, 5.38, 31.48 and 52.16%, respectively. whereas, caloric value recorded 230.80 K.cal. From these data it was revealed that Ashwagandha root powder is rich source of carbohydrates followed by crude fiber. **Mishra and Mishra (2020)** found that Ashwagandha roots powder contained 7.40% Moisture, 4.44% Ash, 0.4% Fat, 33.4% Crude Fiber, and 48.9% Carbohydrate while energy value recorded 245k.cal. Meanwhile, **Veer *et al.* (2019)** indicated that Ashwagandha root powder had a nutrient composition; 7.2% moisture, 4.2% ash, 0.9% fat, 3.3% protein, 33% crude fiber, and 51% carbohydrates.

Table (1): Proximate chemical composition of Ashwaganda (% on dry weight basis)

Components (g)	Ingredients
Moisture	6.34±0.09
Total Protein	3.92±0.46
Fat	0.72±0.08
Ash	5.38±0.18
Crude fiber	31.48±0.25
Carbohydrate*	52.16±0.23
Caloric Value (Kcal)	230.80±2.18

Each value represents the mean ± SD.
* Total carbohydrates were calculated by differences

Food Intake, Body weight gain BWG% and relative weight of internal organs

Data presented in Table (2) showed an effect of feeding ARP diet on feed intake FI, body weight gain BWG% and relative weight for internal organs. From these data, it could be noticed that mean values of feed intake (g/28 day) and body weight gain % in diabetic rats (C+)

were significantly ($p \leq 0.05$) lower when compared with the normal group (C-). In this respect, **Diabetes Control and Complications Trial Research (1993)** reported that the decrease of BWG that observed in diabetic rats could be accounted by the body's inability to utilize glucose, so it tries to overcome this by lipolysis and muscle degradation for obtaining energy, consequently the weight loss occurs.

Basal diets containing ARP with different levels had a tendency to increase the corresponding values of FI (g/28 day) and BWG%, whereas, basal diet containing 7.5% of ARP showed extremely significant decrease ($p \leq 0.05$) in mean value of BWG%, as compared to control positive group. On the other hand, results in the same Table revealed that the relative weight of some internal organs - spleen and liver- were increased significantly ($p \leq 0.05$) in diabetic rats (C+) as compared to the normal group (C-). All supplemented diets with different ratios of ARP resulted in a significant decrease in the relative weight of spleen and liver, as compared to control positive group.

Results are in line with **Udayakumar *et al.* (2009)** who found that body weight loss was observed in diabetic rats; whereas oral treatment with Ashwagandha roots extracts protected diabetic rats from massive body weight loss when administered everyday for eight weeks.

Table (2): Effect of ARP on feed intake, BWG % and some organs relative weight of diabetic rats

Parameters / Groups	FI g/28 day	BWG %	Organs relative weight	
			Spleen	Liver
Normal (C^-)	380.00	39.83 ^{a} ± 3.65	0.21 ^{d} ± 0.03	2.06 ^{d} ± 0.08
Diabetic (C^+)	345.27	13.23 ^{d} ± 2.44	0.32 ^{a} ± 0.04	3.72 ^{a} ± 0.16
2.5% ARP	391.56	28.31 ^{b} ± 3.90	0.27 ^{b} ± 0.02	3.11 ^{b} ± 0.10
5% ARP	387.40	17.54 ^{c} ± 2.84	0.25 ^{bc} ± 0.06	3.00 ^{bc} ± 0.15
7.5% ARP	392.11	7.97 ^{e} ± 2.18	0.23 ^{c} ± 0.01	2.85 ^{c} ± 0.19

ARP, Ashwagandha Roots Powder. Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Serum glucose

A primary priority in the treatment of individuals with diabetes is to achieve optimal glycemic control since chronic rise in blood glucose levels are associated with organ and nerve harm and an elevated risk of cardiovascular disease **(Ramdath *et al.*, 2016).** Data presented in Table (3) illustrated the effect of ARP on serum glucose levels of rats suffering from diabetes. Results indicated that, alloxan-induced diabetes resulted in significant increase $P \leq 0.05$ in serum glucose level relative to (C-) group (normal rats) in the initial experimental period (1st day), the mean values ± SD were (243.38 ± 4.00 vs. 81.23 ± 2.10, respectively). This was due to the injection of alloxan which developed hyperglycemia, induced insulin deficiency and a decrease in β-cells. Data also observed a non-significant changes in serum glucose levels between all diabetic groups for the same period, this

hyperglycaemia status may be was due to abnormalities in hormones secretion.

After the middle (14th day) and the end (28th day) of the experimental period, the mean values ± SD of serum glucose level for C+ group showed a highly significant increase P<0.05, as compared to C- group. On the other hand, diabetic rats fed on a basal diet containing different ratios of ARP 2.5%, 5%, and 7.5% showed a significant decrease P<0.05 in serum glucose levels as compared to the C+ group for the same periods. From all current results, it could be noticed that the values of serum glucose for all treated groups were decreased gradually with increasing the percentage of ARP, which means the lowest serum glucose concentration in all treated groups was exist in the groups fed on a basal diet containing 7.5%.

Results were agree with **Udayakumar *et al.* (2009)** who found that *W. somnifera* root extract showed hypoglycaemic activity on alloxan-induced diabetic rats similar to the standard drug glibenclamide. **Safhi and Anwer, (2011)** found that anti-diabetic activity of repetitive daily treatments with ashwagandha in animals. **Sarangi *et al.* (2013)** revealed that *W. somnifera* root extract when administered to diabetic rats; restored the biochemical parameters to normal and decreased blood glucose level. Also, **Anwer *et al.* (2017)** established that oral administration of *W. somnifera* for 5 weeks resulted in a significant reduction in glucose.

Table (3): Effect of ARP on serum glucose of diabetic rats

Parameters / Groups	Glucose (mg/dl)		
	1st day	14th day	28th day
Normal (C^-)	81.23 [b] ± 2.10	80.15 [e] ± 2.92	83.35 [e] ± 4.58
Diabetic (C^+)	243.38 [a] ± 4.00	245.19 [a] ± 6.31	248.50 [a] ± 4.12
2.5% ARP	240.38 [a] ± 2.06	216.11 [b] ± 3.71	182.72 [b] ± 5.04
5% ARP	241.38 [a] ± 2.09	192.40 [c] ± 7.68	160.20 [c] ±8.15
7.5% ARP	245.38 [a] ± 3.16	179.75 [cd] ± 6.21	143.85 [cd] ±10.30

ARP, Ashwagandha Roots Powder. Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Serum lipid fractions

In addition to higher blood sugar, other causes like dyslipidemia or hyperlipidemia, also play an important role in the progression of vascular complications of type 2 diabetes which eventually contribute to coronary heart disease and other co-morbidities and death **(Tiwari *et al.*, 2014).** The finding in a Table (4) presented the effect of ARP on lipid fractions of rats suffering from diabetes. The mean values (mg/dl) of serum TC (125.26± 4.20), TG (97.47± 4.40), VLDL-c (19.48± 0.98) and LDL-c (83.93± 3.15) increased significantly ($p \leq 0.05$), while HDL-c (21.85± 2.99) decreased significantly ($p \leq 0.05$) for the C+ group, as compared to the C- group. From the same table, it could be observed that rats injection by Alloxan and received basal diets with different concentrations of ARP had lower mean values (mg/dl) of TC (97.71± 4.07, 88.40± 3.12, and 81.35± 4.65), TG (84.26± 4.88, 74.10± 3.12, and 64.56± 4.89), VLDL-c (16.85±0.92, 14.82± 0.92, and 12.91± 0.78) and LDL-c (54.38± 0.94, 39.62± 1.62, and 30.14± 1.25) than the C+ group. On the contrary, HDL-c had higher mean values (26.49±2.81, 33.96±2.30, and

38.31± 2.73) than the C+ group. The rate of improvement in lipid fractions was increased with the increase of the ARP.

The same trend was observed in a study published by **Andallu and Radhika (2000)** who mentioned that *W. somnifera* root powder can cause a reduction in serum cholesterol and triglycerides in hyperlipidemic patients. **Singh *et al.* (2014)** indicated that *W. somnifera* root showed a significant decrease in total lipids cholesterol and triglycerides in plasma and also regulate blood pressure. Also, **Anwer *et al.* (2017)** indicated that oral administration of Ashwagandha for 5 weeks caused in a significant reduction in TC, TG, LDL-C, VLDL-C levels with significant rise of HDL-C levels. **Adi *et al.* (2019)** results showed that *W. somnifera* significantly improved LDL-c and HDL-c compared to the control group.

Table (4): Effect of ARP on serum lipid fractions of diabetic rats

Parameters / Groups	TC	TG	VLDL-c	HDL-c	LDL-c
	mg/dl				
Normal (C^-)	76.44 ^{d} ± 5.05	58.68 ^{d} ± 4.11	11.74 ^{d} ± 1.17	47.79 ^{a} ± 3.19	16.91 ^{e} ± 1.12
Diabetic (C^+)	125.26 ^{a} ± 4.20	97.47 ^{a} ± 4.40	19.48 ^{a} ± 0.98	21.85 ^{e} ± 2.99	83.93 ^{a} ± 3.15
2.5% ARP	97.71 ^{b} ± 4.07	84.26 ^{b} ± 4.88	16.85 ^{b} ±0.92	26.49 ^{d} ± 2.81	54.38 ^{b} ± 0.94
5% ARP	88.40 ^{c} ± 3.12	74.10 ^{c} ± 3.12	14.82 ^{c} ± 0.92	33.96 ^{c} ± 2.30	39.62 ^{c} ± 1.62
7.5% ARP	81.35 ^{d} ± 4.65	64.56 ^{d} ± 4.89	12.91 ^{d} ± 0.78	38.31 ^{b} ± 2.73	30.14 ^{d} ± 1.25

ARP, Ashwagandha Roots Powder. Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

liver enzymes and kidney functions

Over time, A high blood glucose level can cause complications in many parts of the body. **John (2016)** reported that diabetic nephropathy is a common complication of type 2 diabetes. uncontrolled diabetes can cause damage to the clusters of the blood vessel in the kidneys that filter blood waste. This can lead to kidney damage and cause high blood pressure. Furthermore, Type 2 diabetes is related to abnormally elevated hepatic enzymes. Besides, excessive fat accumulation in the liver can exacerbate insulin resistance and lead to serious metabolic dysfunction. Fatty liver and hyperglycemia can damage hepatocytes and cause higher morbidity and mortality for diabetic patients **(Mohamed *et al.*, 2016).** In addition, the liver is among the primary organs susceptible to the effects of hyperglycemia-induced oxidative stress, which can contribute to liver tissue damage **(Palsamy *et al.*, 2010).**

The effect of ARP on liver enzymes activity and kidney functions in diabetic rats were shown in Table (5). Concerning liver enzymes, results observed that serum AST and ALT (u/l) levels were significantly ($p \leq 0.05$) increased in the diabetic group (C+) compared to the normal group (C-) (149.15 & 80.59 vs 59.79 & 19.75, respectively). This was due to the injection of alloxan which increased serum levels of AST and ALT in diabetic rats. Results observed that the mean values (u/l) of serum AST and ALT in all treated groups decreased gradually with increasing the ratios of ARP, compared with the C+ group. The higher effects in the manipulation of the liver enzymes disorders induced by diabetes in rats were recorded for the group of rats treated with 7.5% of ARP. The study by **Jeyanthi and Subramanian (2010)** had shown the support hepatoprotective and

nephroprotective functions of *W. somnifera* root extract against various chemical agents by inhibition of oxidative stress and cytoprotective mechanisms. Moreover, **Adi *et al.* (2019)** reported that *W. somnifera* showed an inhibitory effect on hepatic enzymes and possible liver toxicity. Therefore, *W. somnifera* could be considered as a supplement for the treatment of dyslipidemia.

As shown in the same table, the mean values of uric acid and urea nitrogen (mg/dl) of the C+ group were significantly ($P \leq 0.05$) increased as compared with C- group (3.25±0.18 & 67.12±3.12 vs. 1.82±0.54 & 25.35±1.48, respectively). This was due to the injection of alloxan which increased serum levels of uric acid and urea nitrogen in diabetic rats. Feeding diabetic rats on a basal diet containing 2.5%, 5%, and 7.5% ARP resulted in a significant decrease in uric acid and urea nitrogen, as compared to C+. The higher amelioration effects in kidney disorders induced by diabetes in rats were recorded for the group which treated with 7.5% of ARP. These results agree with **Harikrishnan *et al.* (2008)** who indicated that ashwagandha can improve kidney functions by increasing glomerular filtration rate that may be due to some of its active components like phenolic compounds and flavonoids. **Das *et al.* (2010)** who showed that *W. somnifera* roots lower the dehydration-induced increase in the level of serum urea and creatinine that indicates the protective effect of this plant against renal injury. In addition, the study by **Shimmi *et al.* (2011)** who found that the mean values of serum urea nitrogen and uric acid were significantly lower in experimental groups treated with ashwagandha roots as compared to the positive control. **Vasavan *et al.* (2020)** indicated that treatments with ashwagandha ameliorate liver

enzymes, total protein, creatinine, urea levels, and pathological damage caused to liver and renal tissue.

Table (5): Effect of ARP on liver enzymes and kidney functions of diabetic rats

Parameters / Groups	AST	ALT	Uric Acid	Urea Nitrogen
	u/l		mg/dl	
Normal (C^-)	59.79 ^{e} ± 3.01	19.75 ^{e} ± 2.03	1.82 ^{e} ± 0.54	25.35 ^{e} ± 1.48
Diabetic (C^+)	149.15 ^{a} ± 5.36	80.59 ^{a} ± 3.47	3.25 ^{a} ± 0.18	67.12 ^{a} ± 3.12
2.5% ARP	132.81 ^{b} ± 3.49	61.38 ^{b} ± 3.25	2.35 ^{b} ± 0.91	57.83 ^{b} ± 4.71
5% ARP	108.37 ^{c} ± 4.27	46.38 ^{c} ± 3.19	2.05 ^{c} ± 0.14	42.27 ^{c} ± 2.26
7.5% ARP	75.16 ^{d} ± 4.75	29.25 ^{d} ± 3.42	1.63 ^{d} ± 0.07	35.45 ^{d} ± 2.58

ARP, Ashwagandha Roots Powder. Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Antioxidant enzyme activities

Diabetes is a metabolic condition that contributes to a rise in oxidative stress that tends to be the main source of all diabetic disorders such as neuropathy, nephropathy, myocardial injury and retinopathy **(Ansley and Wang, 2013; and Mukherjee *et al.*, 2015).** On the other hand, Oxidative stress is a condition of imbalance due to excess formation of free radicals and decreased activity of antioxidant defense systems. The levels of reactive oxygen species (ROS) are regulated by a variety of cellular defense mechanisms consisting of enzymic and non-enzymic systems. Antioxidant levels in the blood and tissues are an important factor of sensitivity of individual tissues to oxidative stress **(El-Abhar and Schaalan, 2014).**

Table (5) showed impact of ARP on antioxidant parameters in RBC's. From such data it could be noticed that diabetic rats (C+) had a significant decrease ($p \leq 0.05$) in GPx, CAT and SOD activities in RBC's by 50.9, 23.8, and 56.6% as compared to normal controls (C-), respectively. Supplementation of the rat diets with 2.5, 5 and 7.5% of ARP induced significant increasing on these parameters concentration by the ratio of 14.9, 68.8 and 84.7%; 6.7, 14.8 and 20.1%; and 26.8, 63.8 and 91.1% as compared to C+ group, respectively. The higher amelioration effect in GPx, CAT and SOD activities in RBC's rising induced by diabetes in rats was recorded for 7.5% ARP treatment followed by 5% respectively.

In patients suffering from diabetes mellitus, oxidative stress induced by dysregulation of glucose homeostasis accompanies chronic inflammation, which eventually leads to tissue damages **(Rochette *et al.*, 2014; Domingueti *et al.*, 2016; and Hasnain *et al.*, 2016).** On the other hand, brain and nervous tissues are rich in lipid and iron content that facilitates the synthesis or generation of free oxygen species, rendering them more vulnerable to free radical damage compared to other physiological systems **(Singh and Singh, 2019).** Numerous studies indicated that *W. somnifera* root extracts is rich in bioactive compounds such as Withanolides which exhibited antioxidant activities in different biological systems, and regulate the oxidative stress and cellular stress responses **(Heyninck *et al.*, 2014; Cui *et al.*, 2014; Mohan and Bargagna-Mohan 2016)**.

Results are in line with **Anwer *et al.* (2012)** who had shown the protective role of Withania somnifera against oxidative stress and pancreatic β-cell damage in type 2 diabetic rats; where, *W. Somnifera* has demonstrated good free radical scavenging ability and has helped to improve non-enzymatic and enzymatic antioxidants in diabetic

type2 rats. Another study by **Anwer *et al.* (2017)** indicated that oral administration of *W. somnifera* for 5 weeks resulted in a significant increased levels of antioxidant enzymes like GSH, GPx, GR, GST, SOD and CAT. They also reported that *W. somnifera* is responsible to increase the amount of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) **(Singh and Singh, 2019).**

Table (5): Effect of ARP on antioxidant enzyme activities in RBC's of diabetic rats

Parameters / Groups	GPx	CAT	SOD
	U/g Hb		
Normal (C^-)	20.14 [a] ± 0.89	169.47 [a] ± 3.01	49.75 [a] ± 1.03
Diabetic (C^+)	9.87 [e] ± 0.49	129.15 [e] ± 3.16	21.59 [e] ± 1.47
2.5% ARP	11.35 [d] ± 0.61	137.81 [d] ± 2.19	27.38 [d] ± 1.25
5% ARP	16.67 [c] ± 0.55	148.37 [c] ±3.27	35.38 [c] ± 2.19
7.5% ARP	18.23 [b] ± 0.27	155.16 [b] ± 2.75	41.25 [b] ± 1.42

Mean values in the same column which is not followed by the same letter are significantly different ($p \leq 0.05$).ARP: Ashwagandha Roots Powder.

Histological examination of pancreas

Fig. (1) showed the histopathological examination of pancreas. Microscopically, pancreas of rat from C- group which fed on basal diet revealed the normal histological structure for islets of Langerhan's (Photo1).While, pancreas of rats from diabetic C+ group showed vacuolations of the islet cells of Langerhan's nucleus pyknosis (photo 2).

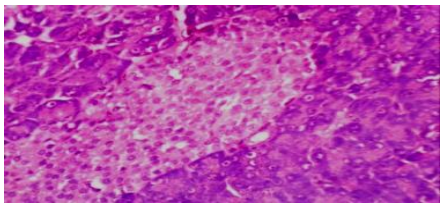
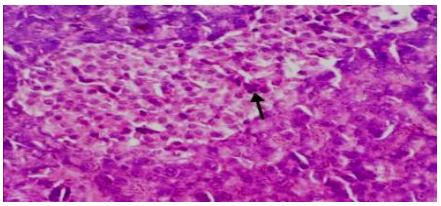
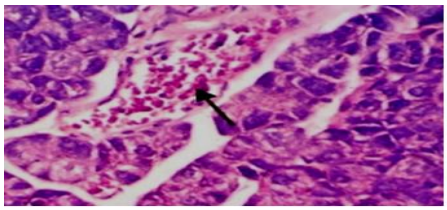
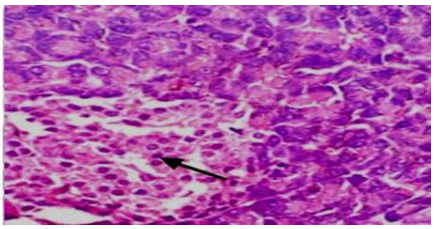
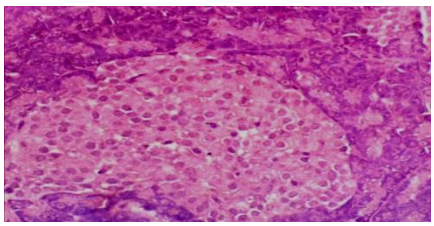
(Photo1) Normal histological structure for islets of Langerhan's (H & E X 400).	
(Photo 2) Vacuolations of the islet cells of Langerhan's nucleus pyknosis (H & E X 400).	
(Photo 3) Vacuolation and necrosis of some cells of Langerhan's islets (H & E X 400).	
(Photo 4) A necrosis in some sporadic cells of Langerhan's islets and some sections showed a normal pancreatic tissue (H & E X 400).	
(Photo 5) No histopathological changes and normal pancreatic tissue (H & E X 400).	

Fig (1): Histological examination of pancreas

In this respect, (Photos 3, 4 & 5) showed a gradual improvement of pancreas tissue and islet cells of Langerhan's in diabetic rats that received a basal diet containing different ratios from ARP. Results were agreed with by **Ram and Krishna (2021)** who found that *W.*

somnifera root extract ameliorates the histology of the pancreas.

Sensory evaluation

Bakery products are widely consumed prepared food products, bread is one of the bakery products that is consumed daily. Since, nutrition plays an important role in diabetes management, the consumption of whole grains like Whole-wheat bread diet lead to a postprandial blood glucose improvement when compared to a refined grains diet like white bread. On the other hand, making bread with Ashwagandha roots powder is a good step in upgrading the nutritive value of the bread.

Table (6) presented the sensory characteristics of prepared pan bread supplemented with Ashwagandha roots powder. The results observed that the color and texture of all pan bread samples supplemented with different levels from ARP 2.5, 5, and 7.5% didn't differ significantly ($p \leq 0.05$), as compared to the control sample. On the other hand, whole-wheat flour replaced by ARP from 5% and 7.5% caused a significant ($p \leq 0.05$) reduction in pan bread taste and odor as compared to the control sample. While pan bread sample that containing 2.5% from ARP showed non-significant changes in the same sensory evaluation attributes. Adding 2.5% from ARP to pan bread showed no significant differences ($p \leq 0.05$) in general appearance as compared to the control. In general, results from overall acceptability occurred that all samples obtained higher than 75%, which means that Ashwagandha roots powder is an acceptable herb and can be used in the preparation of pan bread which is appropriate for diabetic patients.

Table (6): Sensory evaluation of pan bread supplemented with ARP

Sensory Characteristics	Control 0%	Pan bread with ARP %		
		2.5%	5%	7.5%
Color (20)	19.36 [a] ±.337	19.08 [a] ±.407	18.97 [a] ±.496	18.91 [a] ±.574
Taste (20)	19.33 [a] ±.286	18.92 [a] ±.289	18.02 [b] ±.736	16.52 [c] ±.407
Odor (20)	19.26 [a] ±.206	18.95 [a] ±.481	17.44 [b] ±.427	16.34 [c] ±.547
Texture (20)	19.48 [a] ±.391	19.22 [a] ±.393	19.24 [a] ±.327	19.18 [a] ±.308
General Appearance (20)	19.76 [a] ±.291	19.61 [a] ±.375	18.19 [b] ±.572	17.14 [c] ±.632
Overall Acceptability (100)	97.19[a] ±.869	95.78[b] ±.784	91.86[c] ±1.407	88.09[d] ±1.023

Mean values in the same row which is not followed by the same letter are significantly different ($p \leq 0.05$). ARP: Ashwagandha Roots Powder.

CONCLUSION

The present study has shown that Ashwagandha roots powder serve as an important source of many bioactive compounds. Consumption of Ashwagandha roots powder improves glucose homeostasis and Lipid Profile. Besides, maintains the integrity of the kidney and liver functions and Lowering oxidative stress in the body. Ultimately, Ashwagandha roots powder can be incorporated in the diet of diabetic patients**.**

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تأثير نسب مختلفة من مسحوق جذور الأشوجاندا علي الفئران المصابة بالسكري

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استهدفت الدراسة التعرف علي تأثير النسب المختلفة من مسحوق جذور الاشواجاندا على الفئران المصابة بمرض السكري. تم استخدام 30 فأر من نوع الالبينو (150 ± 10جم)، قسمت الفئران إلي مجموعتين رئيسيتين، المجموعة الاولي (6 فئران) أصحاء كمجموعة ضابطة سالبة، المجموعة الثانية (24 فأر) تم تغذيتهم علي الوجبة الاساسية، ثم حقنهم تحت الجلد بمادة الالوكسان (150ملجم / كجم من وزن الجسم) للحث على ارتفاع سكر الدم، ثم تقسيمها الي 4 مجموعات فرعية كالتالي: المجموعة (2) تم تغذيتها علي غذاء اساسي كمجموعة ضابطة موجبة، المجموعات (3 و4 و5) أضيف لغذائهم الاساسي نسبة 2.5 و 5 و 7.5% من مسحوق جذور الاشواجاندا. أظهرت النتائج أن التركيب الكيميائي لمسحوق جذور الاشوجندا يحتوي على 6.34، 3.92، 0.72، 5.38، 31.48، 52.16٪ للرطوبة والبروتين والدهون والرماد والألياف والكربوهيدرات علي التوالي، بينما سجلت السعرات الحرارية 230 كيلو كالوري/100جم. اظهرت نتائج الدراسة البيولوجية أن المجموعة الضابطة المصابة قد أظهرت ارتفاعاً في مستوي سكر الدم و مستوى دهنيات الدم (الكوليسترول، الجلسريدات الثلاثية وكوليستيرول البروتينات الدهنية منخفضة الكثافة جدًا) ومستوي انزيمات الكبد ووظائف الكلى بينما انخفض مستوي مضادات الاكسدة الانزيمية. أظهرت المعالجة بالمستويات المختلفة من مسحوق جذور الاشواجاندا تحسنًا تدريجيًا في مستويات التقديرات السابقة عند مقارنتها بالمجموعة الضابطة المصابة

بالسكر، سُجل التحسن الأكثر وضوحاً في المجموعة المعالجة بنسبة 7.5٪. كما اظهرت نتائج الفحص الهستولوجي تحسناً تدريجياً في أنسجة البنكرياس لدي كافة المجموعة المعالجة. كما أظهرت نتائج التقييم الحسي أن جميع عينات الخبز حصلت على أعلى من 75٪ في القبول العام. استنتجت الدراسة ان مسحوق جذور الاشواجاندا ذو تأثير فعال في خفض سكر الدم ويمكن استخدامه كمكمل في النظام الغذائي لمرضى السكري.

الكلمات المفتاحية: جذور الاشواجاندا – الخبز – ارتفاع سكر الدم – دهنيات الدم – مضادات الأكسدة الانزيمية .