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Comparative Study between the Effect of Marjoram (*Origanum marjoram*) and Ginger (*Zingiber officinale*) Extract on the Fertility of Diabetic Male Albino Rats

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ABSTRACT

This research was applied to assess the impacts of marjoram (*Origanum marjoram*) and ginger (*Zingiber officinale*) water extract on diabetic male rats' fertility. A total of forty eight Sprague Dawley albino rats allocated into eight groups were utilized in this research. Varying doses of the extract 100, 200 and 400 mg/kg b.w (Body weight) were utilized. Weight of testis, histological study, hormonal assay and sperm analysis were studied. Findings of marjoram and ginger demonstrated no significant change in weight of testis except at the high level, inhibition of testicular tissue peroxidation, improve testosterone (T) levels, parameters of sperm analysis such as sperm concentration and motility than the controls. Elevated serum leptin, and prolactin (PRL) with declined serum T and rise in aromatase action in testis, of the positive controls were observed than negative controls. While, diabetic rats received tested extract showed significantly decreased in leptin, prolactin and aromatase with increasing serum T. Histopathological changes, involving seminiferous tubules (ST) degeneration, with vacuolization, sloughing and decline of spermatogenic cells were also observed in controls. Orally marjoram (ME) or ginger extract (GE), with diabetic rats appeared to avoid these changes by decreased accumulated testicular lipid, increased sperm count and androgens, as well as improve testicular structure. So, this research recommends that intake of marjoram and ginger as a drink or add it to any other food product (Such as) had a positive impact on the fertility potentials of the diabetic male rats.

Keywords: Marjoram and Zingiber -Diabetic male- Antioxidant - Fertility- Histopathology.

INTRODUCTION

Infertility is considered as one of the most significant health issues, and about 30% of this issue is caused by male factors (Isidori *et al*., 2006). Many conditions may inhibit spermatogenesis and diminish the quality and quantity of sperm. Several disorders and conditions, including coronary heart disease, diabetes mellitus (DM), chronic liver disease, smoking, pesticide toxins, air pollutants, and vitamin deficiency, have been shown to negatively impact spermatogenesis (Mosher and Pratt, 1991). On the contrary, according to prior research, the use of vitamins A, C, and E and antioxidants may strengthen the testicular blood barrier's integrity and preserve sperm DNA from oxidative stress induced by active free radicals (Jedlinska *et al*., 2006). Primary or idiopathic DM is a chronic condition of carbohydrate, lipid, and protein metabolism characterized by insulin dysfunction, hyperglycemia, and glycosuria. This syndrome might have a function in developing atherosclerosis, microangiopathy, nephropathy, and neuropathy (Butler *et al*., 2017). DM has been linked to both male and female sexual dysfunction. In addition to diminished vaginal lubrication and orgasm dysfunctions, it is considered that neuropathy, vascular insufficiency, and psychological issues may be implicated in the pathophysiology of some phenomena, such as impotence, ejaculation abnormalities, and decreased libido (Berardis *et al*., 2019). In several regions of the globe, traditional plants are employed as a source of therapy for a variety of maladies. Currently, the utilization of plant extract as an adjuvant method of medical therapy is immensely popular. Herbal treatment of sickness is almost widespread in non-industrialized countries (Anquez, 2011).

Numerous plants have traditionally been utilized to promote fertility, and at least some of the herbs studied by contemporary science have been shown to have fertility-enhancing properties (Kneri *et al*., 2016). One of the most well-known fragrant herbs is marjoram (*Origanum marjoram*, *Lamiaceae*), that is indigenous to the Mediterranean area (Ramadan *et al*., 2012). In folk medicine, marjoram extract (ME) is used to treat coughs, cramps, melancholy, vertigo, digestive issues, migraine, and nervous headaches. Marjoram showed some pharmacological influences including hepatoprotective, cardio protective, and immunostimulating activities. Recently, it explored some protective effects of marjoram against collagen -induced liver injury, bactericidal, dyslipidemia, antiseptic, analgesic, antiviral, and laxative agent. Marjoram or its extract found likewise to be useful in preserving healthy weight and improving metabolism. Furthermore, natural antioxidants of marjoram leaves and their extract are generally documented as safe and effective in preventing lipid peroxidation/cellular injury (Ahmed *et al*., 2009 and Stephan *et al*., 2017). Ginger

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is the Zingiber officinale plant's rhizome, which is used as a delicacy, medicinal, or spice. It is the namesake of its genus and family (Zingiberaceae). Turmeric, cardamom, and galangal are more important members of this family. Because of their same flavor, the dissimilar dicots of the genus Asarum are collectively mentioned as wild ginger (An *et al*., 2016). Ginger has up to three percent of a fragrant essential oil whose primary ingredients are sesquiterpenoids, with (-)-zingiberene being the most abundant. A tiny monoterpenoid portion (β-phelladrene, cineol, and citral) and smaller quantities of additional sesquiterpenoids (β-sesquiphellandrene, farnesene and bisabolene) have also been found. Ginger's strong flavor is attributed to nonvolatile phenylpropanoid-derived chemicals, namely gingerols and shogaols that arise when gingerols are dried or heated.

During this process, gingerols are also converted into Zinger one, a less pungent molecule with a spicy-sweet fragrance. Ginger has a sialagogue effect, boosting saliva production, which facilitates swallowing (Khaki *et al*., 2016 and He *et al*., 2018). As well as, ginger rhizome contains several antioxidant components, including vitamin E, ascorbic acid, pyridoxine, beta-carotene, quercetin, lutein, lycopene, tannin, and genistein. In addition, ginger rhizome includes a number of vital minerals, including selenium, phosphorus, manganese, iron, zinc, and copper (De Lima *et al*., 2018). So, this research was applied to evaluate the impacts of ME and GE on diabetic male fertility.

MATERIALS AND METHODS

Materials

Marjoram (*Origanum marjoram*) and ginger (*Zingiber officinale*) were purchased from the Aromatic Research Department Horticulture Research Institute Center, Giza, Egypt.

Animals: Forty eight adult male albino rats of Sprague Dawley strain weighed 150±5g b.w were obtained from Giza Memorial Institute for Ophthalmic Research, Animal House, Ministry of Health, Giza, Egypt.

Alloxan was acquired from El-Gomhoryia Company, Cairo, Egypt all kits and basal diet were prepared according to AIN (1993); Materials of applied biscuits were acquired from local market from Cairo, Egypt.

Preparation of freeze dried herb extracts

The ginger rhizome and marjoram petals are slightly cut, then the ginger and marjoram are washed with distilled water. It is dried by air oven at 40 C for 48h. The dried petals and rhizome were turned into powder using an electric machine then extracted by soxhlet for 12 hours with distilled water (1:10 w: v) after extraction the extract was freeze dried and kept at - 18 ºC (Arslan *et al*., 2010).

Chemical analysis of freeze dried herb extracts and fortified biscuits

Chemical analysis to assess moisture, fat, protein, ash and crude fiber contents regarding AOAC (2000).Total carbohydrates was determined by difference.

Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber)

Determination of Ascorbic acid (vitamin C) and vitamin A

Vitamin C was assessed by 2, 6- dichloro-phenol-indophenol dye (Lu *et al.*, 2017). And vitamin A determined by Colorimetric procedures with Antimony trichloride (Cl_3Sb) according to the official method (AOAC, 1984).

Determination of minerals content

Minerals content (Na, Ca and K) were assessed in the diluted solution of ash specimens by emission flam photometer (Model Corning 410). The other minerals (Cu, Zn, Fe, P and Mg) were assessed by Atomic absorption spectrophotometer (PerKin – Elmer Instrument Model 2380, Germany), regarding Nzikou *et al.*, (2009).

Determination of total flavonoid and total phenols

Total flavonoid was assessed regarding Ordonez *et al., (*2006). Total phenolic compounds was assessed by Folin Ciocalteu method regarding Wolfe *et al*., (2003) and the findings were presented as mg gallic acid equivalent (GAE).

Identification of phenols and flavonoid compounds

Extraction, separation and quantification of phenolic and flavonoid compounds using Perkin Elmer PE200 HPLC were assessed regarding Goupy *et al.*, (1999).

Biological experiment.

Forty-eight adult male albino rats of Sprague Dawley strain weighed 150±5g were obtained from Giza Memorial Institute for Ophthalmic Research Giza, Egypt. All rats were adapted and ate a standard diet for one week. After adaptation period, forty-eight rats were divided randomly into 8 groups of rats (each group was included 6 rats). One of these groups was kept as a negative control (G1), and the other groups were injected by 150 mg/kg body weight of Alloxan monohydrate which dissolved in 0.9 % w/v of NaCl. The previous dose was divided to three doses for three days (50mg/kg/d) until the level of fasting blood glucose was more than >250 mg/dl (Porchezian *et al.*, 2000). Rats were taken glucose solution (20% w/v) to avoid fatal post-Alloxan hypoglycemia (Ozougwu *et al*., 2009) . The diabetic groups were divided to as following:

Group 2 fed on the basic diet as a positive group. Groups 3, 4, and 5 fed on the basic diet and were administered 100, 200, and 400 mg/kg/day of marjoram extract respectively. Groups 6,7, and 8 were received the basal diet and were administered 100, 200, and 400 mg/kg/day of ginger extract respectively. (Rao, 1987 and Sinha, 1990). Basal diet was prepared according to AIN(1993) It consisted of diet comprised of 10 %, 5 %t cellulose, 4 % salt combination, 1 % vitamin mixture, 10 % oil, and 70 % corn starch .

At the conclusion of the experiment, blood specimens were obtained from the orbital plexus veins of rats that had fasted overnight and centrifuged at 3000 rpm for 10 minutes. The serum was then separated and kept at -20 °C. (Kondeti *et al*., 2011). The testis, prostate gland and seminal vesicle were removed and weighed after being dissected. The testis was kept in a 10% neutral formalin solution until the histological investigation was performed.

Assessment of biochemical parameters

The concentrations of serum glucose, Total lipids (TLs), Total cholesterol (TC), and triglycerides (TGs) were measured regarding Kaplan (1984); Zolliner & Kisch (1962); Allain *et al*. (1974) and Fossati & Prencipe, (1982) respectively.

Serum testosterone (T) and prolactin (PRL) levels were assessed, regarding Tietz (1995), was assessed by Enzyme linked Fluorescent Assay (ELFA) utilizing kits of BioMeriuex, regarding Sapin and Simon (2001). Estradiol

was determined by the method of Santen *et al.* (2010). Leptin was estimated by enzyme-linked immunosorbentassay (ELISA) technique by commercially available kit (DRG instruments, Gmbh, Germany), regarding Considine and Siha (1996).The quantitative assessment of aromatase action was accomplished using a solid phase (ELISA) sandwich assay, regarding Roselli (1998). In microtiter plate wells covered with biotin-conjugated aromatase-specific antibody, samples were treated. Following incubation, a sandwich complex was created, and the unbound material was washed off Next. Avidin peroxidase enzyme complex was introduced to detect bound aromatase at 450 nm using "streptavidin not avid in" (Hendrickson, 1985). Malondialdehyde (MDA) was assessed in testicular homogenates supernatant regarding Buege and Aust (1978).

Semen analysis

Semen analysis Sperm specimens were obtained from the cauda epididymis. and separated according to Hamilton (1975), then put in a petri dish, grist, and maintained in 2 ml of preheated sterile physiological saline (37°C) to discharge the sperms from the epididymis (incubating media) (Filler, 1993).

Sperm motility%

The percent of motile spermatozoa was microscopically (Olympus BX41, Japan) evaluated by using one drop of semen between a prewarmed cover slide and a glass slide placed on a hot stage microscope Taking the average reading of 5 fields, the percent of motile to non-motile spermatozoa was estimated (Mohamed *et al*., 2011).

Sperm Viability (Live/Dead %)

At 37°C, Eosin-Nigrosin smears were produced, and the viability of at least 100 sperms was determined utilizing a bright-field microscope (x1000). Briefly, a 10-l aliquot of the material was combined with a drop of (1 percent Eosin and 10 percent Nigrosin) stain on a clean, pre-warmed glass slide that was then spread over another slide and left to air-dry. Live spermatozoa don't take the stain (still white) while dead spermatozoa membrane allows the stain to pass throw (stain pink). Two slides were made from each specimen. The viability % was estimated by counting the live /dead ratio (Morakinyo *et al*., 2009).

Sperm concentration measurements

The counting chambers of the hemocytometer were manufactured in accordance with WHO (1999) guidelines for sperm analysis. Under a light microscope, spermatozoa are examined and counted; the Hemocytometer is split into nine fields; however, spermatozoa are counted and recorded for just five random fields (Tomlinson *et al*., 2001).

Method of applied biscuits

Fortified biscuits with the best dose from tested freeze dried herb extracts were prepared using the following:

Ingredients	Amount
Margarine	200gm
Fortified wheat flour 72% extraction	1000gm
Milk	400gm
Sugar	300gm

Preparation of fortified biscuits was carried out using replacing 8 gm from wheat flour by adding 8 gm of tested freeze dried herb extracts at the level 8gm (400mg/kg bw) /100gm biscuit dough ,fat and sugar are creamed, followed by the addition of milk and flour. The dough is softly kneaded till smooth, smoothed out, poked, and formed. Preparation of the control product required 10-15 minutes of baking at 180°C regarding (Panel and Sonthgate, 1978) .

Histopathological examination

Rat testicles were removed and treated in a neutral formalin solution 10%. Next, the fixed samples were cut, cleaned, and dehydrated in escalating alcohol concentrations. These samples were xylene-cleaned, paraffin-embedded, cutted to 4-6 microns, stained with Hematoxylin and Eosin (H & E), and then inspected microscopically regarding Luna (1968).

Statistical analysis

Data were recorded as means and analyzed by (SPSS) (Ver.10.1). One-way analysis of variance (ANOVA) and Duncan comparisons were tested to signify differences among different treatments of tested herbs SAS (1988).

RESULTS AND DISCUSSION

The chemical composition of freeze dried herb extracts

Data presented in table (1) show the chemical structure of ginger and marjoram freeze dried extract. It is clear to notice that the percentage of moisture, fat, protein, ash, carbohydrates, fiber, and energy of ginger freeze-dried extract were 15.89,5.86,4.51,3.93,9.70, 60.11%and 304.15 kcal/100g, respectively.

On the other hand, the percentage of the above mentioned composition of marjoram freeze-dried extract were16.34%, 10.71%, 7.54%, 8.68%, 18.07%, 38.66% and 267.74 kcal/100g, respectively.

From the obtained results, it was noticed that marjoram was significantly higher than ginger freeze-dried extract in all chemical composition except carbohydrates and energy value. These findings are in line with Koch *et al*.(2017), they revealed the GE had the fat content between 4.11 to 9.65%, protein was between 4.65 to 5.11% and fiber content ranged from 8.99 - to11.44%. However, El-Ashmawy *et al.*, (2007) discovered the total fat content of marjoram in the range of 7.06 , 15.76% the protein 9.04-13.55% and fiber was between 15.64 to 18.99%.The fiber content contributes to the preservation of human health by lowering the body's cholesterol level and decreasing the risk of certain malignancies, as well as improving overall health.

Table 1. Chemical composition of freeze dried **tested herb extracts**

Constant (%)	Ginger freeze-dried extract	Marjoram freeze-dried extract
Moisture	15.89±1.03 [b]	16.34±2.34 [a]
Protein	5.86±0.93 [b]	10.71±0.57 [a]
Fat	4.51±0.56 [b]	7.54±1.22[a]
Ash	3.93±0.44 [b]	8.68±1.44[a]
Fiber	9.70±0.23 [b]	18.07±2.31 [a]
Carbohydrates	60.11±3.56 [a]	38.66 ±5.22 [b]
Energy value(Kcal /100 g)	304.15±10.34 [a]	267.74±11.32 [b]

Values are means ± SD (n = 3).Values superscripted are significantly different at $p \leq 0.05$.

Minerals content of freeze dried herb extracts (mg/100gm).

Data given in table (2) showed the minerals content of tested freeze dried herb extracts. It is clear to mention that the highest minerals content of GE was recorded for potassium, phosphorus and magnesium. 412.54, 46.76 and 45.22,% respectively. On the contrary, the lowest minerals

content of the same extract was recorded for copper, zinc, and iron. The values were 0.34, 1.02 and 1.32%, respectively.

In case of the marjoram freeze dried herb extracts. The main minerals were potassium and calcium while the lowest levels were recorded in copper and zinc. From the data, marjoram freeze-dried extract had higher levels of tested minerals than ginger freeze dried herb extracts.

These findings are in line with (Kukula *et al*., 2018), they demonstrated the ginger is a good source of mineral elements for humans especially in aqueous extract. While Ahmed *et al*., (2009) showed aqueous extract of marjoram low in zinc and high in potassium, calcium and iron. Thus, consuming aqueous of tested herbs could supply more mineral elements, while consuming raw tested herbs could supply the lowest comprehensive amounts of nutritional substances.

Table 2. Minerals content of freeze dried **tested herb extracts (mg/100gm)**.

Minerals	Concentration%	
	Ginger freeze-dried extract	Marjoram freeze-dried extract
Calcium (Ca)	18.33	202.98
Iron (Fe)	1.32	32.43
Magnesium (Mg)	45.22	75.65
Phosphorus (P)	46.76	124.87
Potassium (K)	412.54	632.0
Sodium (Na)	15.07	78.22
Zinc (Zn)	1.02	4.08
Copper (Cu)	0.34	1.08

Vitamins A and C contents in freeze dried **tested herb extracts**

Data tabulated in table (3) showed the vitamins A and C contents in dried extract. It is clear to mention that the values of ascorbic acid (mg/100g) dried extract and vitamin A (IU) in ginger dried extract were 10. 31and 24.66 respectively while it amounted in marjoram dried extract 51.4 and2068 respectively which was significantly higher than the ginger dried extract.

These findings are in line with Patel and Srinivasan (2004), who revealed ginger had vitamin C and vitamin A as a β-carotene which had a good effect as natural antioxidants and effective in preventing lipid peroxidation/cellular injury including hepatoprotective, cardio protective, and immunostimulating activities .Tajkarimi and Cliver (2017) found that vitamins A and C found in abundance in marjoram have anti-inflammatory, antibacterial, and antioxidant activities. Marjoram may benefit hormonal health, particularly in women.

Table 3. Vitamins A and C contents in freeze dried **tested herb extracts.**

Vitamins	Ginger extract	Marjoram extract
Vitamin A (IU)	24.66±5.63 [b]	2068 ±12.65 [a]
Vitamin C (mg)/100g	10.31±2.66 [b]	51.4±6.22 [a]

Values are means ± SD (n = 3).Values superscripted are significantly different at $p \leq 0.05$.

Total phenolic and flavonoid in freeze dried **tested herb extracts**

Total phenolic and flavonoid of the freeze dried GE and ME were presented in Table (4). Total phenolic and total flavonoid of GE were significantly higher than ME. On the other hand, Flavonoids are extensively dispersed in many plant sections and serve a variety of functions. They are one of the most prevalent classes of phenolic found in plants. Because of their significance to human health, it would be advantageous to have a greater knowledge of flavonoid and biological activities that might suggest their potential in treatment, as well as for forecasting and managing the quality of food and medicinal plants (Chan *et al*., 2008). GE and ME had total flavonoid concentrations of 25.9 and 13.9 g/mg quercetin equivalents, respectively. They are the most essential plant pigments for flower coloring, creating yellow, red, or blue pigmentation in petals to attract pollinating animals. 50 percent aqueous extraction of ginger was shown to provide the maximum overall phenolic concentration. According to authors, this is due to the 50% aqueous extract's higher dielectric constant, which results in a greater release of the compound's total phenolic content. It has been reported that reported that total phenolic content is higher in ginger than in marjoram of different ginger species (Çakmakçı *et al*., 2015).

Table 4. Total phenolic and flavonoid contents in freeze dried tested herb extracts (mg/1000gm)

Content	Ginger extract	Marjoram extract
Total phenolic	137.5±11.32 [a]	25.8±4.28 [b]
Total flavonoid	25.9±2.63 [a]	13.9±2.14 [b]

Values are means ± SD (n = 3).Values superscripted are significantly different at $p \leq 0.05$

Identification of phenolic compounds in freeze dried **tested herb extracts using HPLC:**

Data given in Table (5) showed the identified phenolic compounds of GE and ME. The obtained results indicated that the highest phenolic compounds of GE were for Catechol and Catechein. The values were 406.52 and 411.24 mg/kg, respectively while in ME recorded that p-Hydroxybenzoic acid and Catechein, while amounted in values were 321.1 and 300.98 mg/kg, respectively.

Table 5. Identification of phenolic compounds in freeze dried tested herb extract using HPLC.

Phenolic compounds	Concentration(mg / 100gm)	
	Ginger extract	Marjoram Extract
Pyrogallol	264.3	142.4
Gallic acid	39.6	29.8
Catechol	406.52	102.23
Catechein	411.24	300.98
Chlorogenic acid	243.10	197.87
P-OH-benzoic	125.52	ND
p-Hydroxybenzoic acid	29.4	321.1
Caffeic acid	91.2	9.8
Vanillic acid	89.4	101.2
Caffeine	75.08	ND
Ferulic acid	224.7	88.8
Iso-Ferulic acid	10.71	ND
p-Coumaric acid	170.2	291.4
Coumarin	7.66	1.65
3,4,5- methoxycinnamic	31.04	23.56

On the other hand, the lowest phenolic compounds of GE recorded for Coumarin, and Iso-Ferulic acid. The values were 7.66 and 10.71 mg/kg, respectively. While MG, the lowest levels were1.65 and 9.8 as Coumarin and Caffeic acid. These findings are in line with Çakmakçı *et al*., (2015), who revealed the various *marjoram* species have been extensively analyzed for their antioxidant chemical profiles. As well as vitamins C and A, the other antioxidants include

the Catechein, p-Hydroxybenzoic acid, Pyrogallol, Iso-Ferulic acid and Coumarin in a few amount. Ginger (Zingiber officinale Roscoe) is a widely used and familiar spice. It involves a variety of chemical elements, including as phenolic chemicals, terpenes, lipids, polysaccharides, organic acids, and raw fibres. Ginger's health advantages are mostly attributable to its phenolic chemicals, which consist primarily of gingerols, shogaols, and paradols. Ginger has many biological effects, involving anti-inflammatory, antioxidant, antibacterial, neuroprotective,anticancer, cardiovascular protective, antiobesity, antidiabetic, respiratory protective, antinausea, and antiemetic actions, according to accumulated research (Prasad *et al.,* 2015).

Identification of flavonoid compounds in freeze dried **tested herb extracts using HPLC**.

Data given in table (6) showed the Identified flavonoid compounds of extracts. The obtained findings revealed the highest flavonoid compounds of GE recorded for apignin 6-arabinose, hespirdine and narengin and in ME, the major flavonoid compounds were apignin 6-arabinose, narengin and hespirdine.

Table 6. Identification of flavonoid compounds in freeze dried tested herb extracts using HPLC .

Flavonoid compounds	Concentration (mg/100g)	
	Ginger extract	Marjoram extract
Apignin 6-arabinose	1762.42	457.87
Rosmaric	14.81	33.25
Luteolin 7-glucose	36.43	--
Hespirdine	444.43	106.43
Rutin	14.41	10.32
Apignin 7- glucose	4.36	1.32
Apig.7-O-neohespiroside	35.66	25.65
Quercetrin	18.13	12.54
Narengenin	20.94	34.65
Quercetin	15.42	32.87
Hespirtin	5.24	3.65
Campferol 3-2-P-coumaryl	33.57	50.54
Acacetin 7neo-rutinoside	18.83	37.84
Campferol	1.54	2.87

On the other hand, the lowest flavonoid compounds of GE and ME recorded forcampferol, and apignin whereas Luteolin 7-glucose didn't found in ME. These findings are in line with (Skrovankova *et al*., 2017) they revealed the subclass flavonol was the most prevalent group of flavonoids found in all ginger and MEs examined. Quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside, quercetin-3-O-arabinoglucoside, catechin, epigallocatechingallate, epigallocatechin, ferulic, chlorogenic, isoferulic, and caffeic acid were among the most abundant phenolics discovered in both the extract.

Effect of oral administration of GE and ME for 12 weeks on TLs, TC and TGs in serum and testes of diabetic adult male rats.

The results of serum lipids profile demonstrated significant decline in epididymal sperm count, with significant increase in serum & testicular TLs, TC, and TGs of diabetic rats when compared with rats got basal diet as negative control (Table 7).Treating with levels of both GE and ME led to decreased in the values of TLs, TC and TGs than positive controls but it were still increasing when compared with negative control group. With the increasing the levels of tested extract demonstrated significant decline in the tested lipid profile in serum and testis of tested diabetic groups. The groups 5 and 8 are non significat with negative control group in TC and TGs in testis was significant. In DM, an increase in lipid peroxidation is caused by an excessive production of free radicals. Other sources of oxidative stress include autooxidation, glycosylated protein, decreased ascorbic acid and superoxide dismutase enzyme, and a shortage of declined glutathione. Elevated amounts of lipid peroxide in DM may result from altered erythrocyte membrane function. This inhibits the function of the superoxide dismutase enzyme, resulting in the buildup of superoxide radicals, which are responsible for tissue damage in DM and the greatest lipid peroxidation (Velazquez *et al.,* 2018).

Table 7. Effect of oral administration of ginger and marjoram extract for 12 weeks on total lipids in serum and testis of diabetic adult male rats.

Groups	Lipid profile in serum (mg/dl)			Lipid profile in testis (mg /dl)		
	TL	TC	TG	TL	TC	TG
G1	401.16 ± 6.35^{e}	81.05 ± 5.48^{e}	108.98 ± 1.48^{e}	209.98 ± 2.74^{e}	21.40 ± 0.56^{d}	34.07 ± 1.75^{c}
G2	762.52 ± 9.49^{a}	125.49 ± 2.39^{a}	154.11 ± 4.75^{a}	331.48 ± 13.8^{a}	40.61 ± 1.57^{a}	65.79 ± 1.29^{a}
G3	629.22 ± 9.87^{b}	107.89 ± 1.50^{b}	139.45 ± 3.36^{b}	312.59 ± 4.36^{b}	35.51 ± 0.68^{b}	54.08 ± 1.52^{b}
G4	534.16 ± 6.35^{c}	97.48 ± 1.88^{c}	129.40 ± 1.87^{c}	280.33 ± 1.11^{c}	29.03 ± 0.87^{c}	48.31 ± 1.40^{b}
G5	473.13 ± 8.67^{d}	88.19 ± 0.96^{d}	119.35 ± 1.51^{d}	267.08 ± 1.76^{d}	22.16 ± 0.81^{d}	37.22 ± 1.87^{c}
G6	622.22 ± 7.45^{b}	103.75 ± 1.48^{b}	133.98 ± 1.48^{b}	308.87 ± 2.22^{b}	35.48 ± 0.84^{b}	53.09 ± 1.45^{b}
G7	527.85 ± 4.30^{c}	96.46 ± 1.17^{c}	128.45 ± 2.68^{c}	276.33 ± 1.11^{c}	28.03 ± 0.87^{c}	43.31 ± 1.40^{b}
G8	477.74 ± 9.19^{d}	86.19 ± 3.11^{d}	118.35 ± 1.51^{d}	262.08 ± 1.76^{d}	23.16 ± 0.81^{d}	36.09 ± 1.07^{c}

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day) , Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w. Values are means ± SD (n = 6).Values superscripted are significantly different at $p \leq 0.05$.

Effect of oral administration of GE and ME for 12 weeks on blood glucose of diabetic adult male rats

The results of serum glucose levels were elevated by feeding the tested dried extract during the end of period of diabetic rats. From table (8) , the positive control group had the increased values while rats got basal diet as negative control had the lowest values. Treating with levels of both GE and ME led to descend decreasing in the values of blood glucose than positive controls but it were still increasing than negative controls. The groups 5 and 8 values than negative controls were significant. Marjoram improved blood glucose levels also improved insulin secretion, exerted antioxidant effects, enhanced renal function, and also treated diabetic retinopathy and neuropathy. It involves phenolic terpenoids, flavonoids, tannins and phenolic glycosides .The extract of marjoram showed very high antioxidant effect (Skrovankova *et al*., 2017). Ginger pretreatment inhibited the induced hypoinsulinemia and

hyperglycemia. They found dosage concentration influences the reaction to ginger components. Ginger has been demonstrated to influence the release of insulin. Ginger enhances glucose clearances in peripheral tissues that respond to insulin, which is essential for maintaining blood glucose homeostasis (Prasad *et al.*, 2015).

Table 8. Effect of oral administration of ginger and marjoram extracts for 12 weeks on blood glucose of diabetic adult male rats.

Groups	Blood glucose in serum (mg/dl)			
	The beginning of the experiment	first month	second month	End of the experiment (the third month)
G1	98.05± 1.06 [c]	98.28± 1.22 [d]	100.01± 2.99 [f]	100.76± 6.76 [f]
G2	258.30 ± 4.23 [a]	275.87± 3.34 [a]	296.97 ± 10.65 [a]	318.50 ± 9.16 [a]
G3	255.50 ± 8.22 [a]	242.22 ± 4.22 [b]	229.44 ± 4.98 [b]	210.50 ± 2.06 [b]
G4	253.54 ± 8.99 [b]	239.07 ± 6.24[b]	219.64 ± 9.67 [c]	198.90 ± 4.06 [c]
G5	250.65 ± 10.32 [b]	228.04 ± 10.06 [c]	196.07 ± 4.52[d]	160.59 ± 11.76 [d]
G6	258.32 ± 0.98[a]	240.01 ± 8.22 [b]	228.90 ± 6.76 [b]	218.80 ± 8.22 [b]
G7	255.05 ± 9.99 [b]	237.99 ± 5.96 [b]	217.97 ± 4.97[c]	198.45 ± 9.99 [c]
G8	250.07 ± 2.33[b]	220.11 ± 9.54 [c]	185.88 ± 11.06 [e]	150.97 ± 4.67 [e]

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day) , Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w. Values are means ± SD (n = 6).Values superscripted are significantly different at p ≤ 0.05.

Effect of oral administration of GE and ME for 12 weeks on some sexual hormones of diabetic adult male rats.

Diabetic rats revealed significant elevation in serum leptin, PRL and Estradiol with significant decline in serum T in opposite of the negative control group which recorded significant decrease serum leptin, PRL and estradiol and high serum T. Administration of ME or GE at different levels to diabetic animals showed significantly declined serum leptin, PRL, and estradiol, but elevated serum T with elevated epididymal sperm count than positive control rats (Table 9).levels of GE were more effective on these hormones than the ME levels but the differences are no significant except estradiol.

Table 9. Effect of oral administration of GE and ME for 12 weeks on some sexual hormones of diabetic adult male rats.

Groups	Sexual hormones			
	Serum Testosterone (ng/ml)	Estradiol (ng/ml)	Leptin (ng/ml)	Prolactin (ng/ml)
G1	5.94 ± 0.90 [a]	22.65 ± 2.44[c]	14.88 ± 3.83 [d]	11.09 ± 1.22 [e]
G2	2.49 ± 0.44 [d]	29.42 ± 0.83[a]	31.42 ± 0.73[a]	26.53 ± 1.96[a]
G3	3.63 ± 0.23 [c]	26.03 ± 0.75[b]	26.16 ± 1.59[b]	22.63 ± 0.61[b]
G4	4.94 ± 0.20 [b]	25.65 ± 0.44[b]	22.88 ± 0.83 [c]	17.04 ± 1.22 [c]
G5	5.09 ± 0.27 [a]	24.84 ± 0.38[b]	16.95 ± 0.69 [d]	14.14 ± 0.35 [d]
G6	3.17 ± 0.38[c]	26.24 ± 0.83[b]	25.06 ± 1.09[b]	23.03 ± 1.22[b]
G7	4.47 ± 0.18 [b]	25.05 ± 2.4 [b]	19.96 ± 2.61[c]	18.54 ± 0.99[c]
G8	5.04 ± 0.18 [a]	23.14 ± 0.38[c]	15.25 ± 1.55 [d]	14.74 ± 1.05 [d]

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day) , Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w.

Values are means ± SD (n = 6).Values superscripted are significantly different at p ≤ 0.05.

In rats fed copper ox chloride, ginger has been shown to boost T, follicle-stimulating hormone (FSH), and testicular total antioxidant. It has been shown that marjoram boosts sperm count and motility in rats while decreasing the number of defective sperm. In the same research, phenolic acids were shown to enhance the levels of T hormones, decline MDA and PRL in the testicle, reduce the generation of reactive oxygen species (ROS), and lower testicular cholesterol. The drop in T levels may have been caused by damage to the Leydig cells (LC), where the hormone is released. DM has been observed to reduce the number of LC. Similar to prior research, the impact of ginger on T, estradiol, and PRL was shown in this study. This may be related to the length of follow-up or the dose administered (Hatzimouratidis *et al.*, 2017 and Kumar *et al.*, 2019).

Effect of oral administration of GE and ME for 12 weeks on aromatase (ng/g), ACP (U/g) and MDA concentration of diabetic adult male rats.

Results revealed significantly reduced activities of ACP (acid phosphatase) with increase of aromatase action in testis of the diabetic rats as positive control group than negative control. Administration of ME or GE to diabetic rats substantially enhanced all indicated enzymatic alterations than diabetic animals who did not receive the extracts (Table 10). Overall, the data suggest that both ME and GE may have potential activity against diabetic and associated biochemical changes; however, ginger appeared to be more effective than marjoram, although the differences among the two extracts were not significant but ACP, the highest level of both extracts exhibited non-significant alternations with the negative control group except ACP of ginger. MDA (Malondialdehyde) concentrations were nearly stable in GE and ME treated groups.

Groups which were treated with different levels of both extract especially at the high level revealed lower levels than the controls. This proved the rate -control of oxidation is significantly lower in the treated groups than diabetic rats without treated.

It has been shown that ACP activities increase as testicular steroidogenesis increases. A reduction in ACP activity would thus indicate a decrease in testicular steroidogenesis, which may be connected with a drop in gonadotrophin production found in diabetic rats. A reduction in ALP activity may suggest a condition of retarded steroidogenesis in which intracellular and intercellular transport are diminished due to slower steroidogenesis-related metabolic processes.

The ACP gene is up regulated by androgens and down regulated by estrogens in the testis. It has been shown that ACP activities increase as testicular steroidogenesis increases, a reduction in ACP activity would thus indicate a decrease in testicular steroidogenesis, which may be connected with a drop in gonadotrophin production. And production found in diabetic rat, due to the presence of vitamin C and phenols in their extract, aromatic herbs such

as marjoram and ginger have received considerable interest in this regard. the treatment of ME or GE to diabetic rats resulted in an enhanced lipid profile in both blood and testis, along with lowered serum, leptin, and PRL levels, suggesting increased fertility.

Given the above information, it is plausible to establish a connection among lowered blood glucose and hyperlipidemic condition by ME and GE and increased fertility. Elevated blood glucose in these conditions is related with low levels of T and sperm count, increased serum concentration of leptin, and decreased activity of aromatase, a critical regulator of E2 synthesis that is widely expressed in adipose tissue. Thus, it is possible that the decreased adiposity induced by ME and GE may impact adipose tissue-specific hormones that have a significant function in regulation of T production and male fertility.(Ahmed *et al.,* 2009; Ramadan *et al.,* 2012; Li *et al.,* 2013; .Long *et al*., 2015 and Dupont *et al.,* 2019).

Table 10. Effect of oral administration of ginger and marjoram extract for 12 weeks on aromatase (ng/g), ACP (U/g) and Malondialdehyde concentration of diabetic adult male rats.

Groups	Aromatase (ng/g)	ACP (U/g)	Malondialdehyde concentration(umol mg-1)
G1	5.70 ± 0.86 [a]	3.59 ± 0.61 [a]	0.44±0.16 [d]
G2	2.49 ± 0.44[d]	1.59 ± 0.33 [e]	1.53±0.013 [a]
G3	3.99 ± 0.23 [c]	2.10 ± 0.08 [d]	0.93±0.06 [b]
G4	4.94 ± 0.20 [b]	2.40 ± 0.10[c]	0.70±0.01 [c]
G5	5.09 ± 0.27 [a]	2.79 ± 0.19 [b]	0.52±0.12 [d]
G6	4.17 ± 0.38[c]	2.42 ± 0.04 [c]	0.92±0.17 [b]
G7	4.47 ± 0.18 [b]	2.83 ± 0.39 [b]	0.76±0.13 [c]
G8	4.99 ± 0.18[a]	3.28 ± 0.21 [a]	0.55±0.11 [d]

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day) , Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w.

Values are means ± SD (n = 6).Values superscripted are significantly different at $p \leq 0.05$

Effect of oral administration of GE and ME for 12 weeks on the weight of sexual organs of diabetic adult male rats.

Orally GE and ME at 100, 200 and 400 mg/kg b.w. to adult diabetic rats for 12 weeks significantly elevated the testis weight, Vas deferens and seminal vesicle and decreased the prostate glands weight than positive control group while than the negative control group, the weight of testis, Vas deferens and seminal vesicle were decreased and increased the weight of prostate glands (Table 11). Treated with GE and ME at 400 mg/kg b.w. /day for 12weeks significantly improve the tests, vas deferens and seminal vesicles weights than the other group and positive control group. Whereas in case prostate glands, all treated groups with different extract showed significantly improved the prostate weight than positive controls and the treated group with the highest level of both extract showed more improvement in its weight than the other treated groups and it was statically no significant with the negative control group. DM is a significant and growing global health concern. Generally, DM arises due to a mix of genetic, cultural, environmental, and behavioural variables (Although the complex aetiology of high blood glucose, dietary factors notably, increasing intake of high fat diet (HFD) represents a key risk for its development. Similar trend was seen in the current investigation, where diabetic rats had a much lower adiposity index, accompanied by a decline in the weight of their sexual organs than normal rats (Ferris and Crowther, 2011).

Table 11. Effect of oral administration of GE and ME for 12 weeks on the weight of sexual organs of diabetic adult male rats.

Groups and Treatment	Sexual organs weight (g) Mean ± SE			
	Testis	Seminal vesicles	Vas deferens weight	Prostate glands
G1	2.81 ± 0.04 [a]	1.96 ± 0.042 [a]	0.243 ± 0.02 [a]	0.72± 0.01 [c]
G2	2.56 ±0.53 [c]	1.70 ± 0.22 [c]	0.206± 0.01 [b]	0.99± 0.01 [a]
G3	2.58 ±0.02 [c]	1.79 ± 0.24 [b]	0.212± 0.02 [b]	0.83± 0.01 [b]
G4	2.61±0.43 [b]	1.83± 0.16 [b]	0.239 ± 0.03 [a]	0.80± 0.03 [b]
G5	2.79 ±0.27 [a]	1.95± 0.18 [a]	0.24 ± 0.03 [a]	0.74 ± 0.12 [c]
G6	2.58 ±0.03 [c]	1.72± 0.12 [c]	0.21± 0.03 [b]	0.83± 0.01 [b]
G7	2.63±0.02 [b]	1.92± 0.12 [a]	0.237 ± 0.03 [a]	0.80± 0.01 [b]
G8	2.80±0.92 [a]	1.95 ± 0.15 [a]	0.241± 0.03 [a]	0.73 ± 0.04 [c]

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day), Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w.

Values are means ± SD (n = 6).Values superscripted are significantly different at $p \leq 0.05$.

Effect of oral administration of GE and ME for 12 weeks on the parameters of sperm analysis of diabetic adult male rats.

Table (12) showed the positive control group results in a significant decrease in both sperm concentration, progressive motility and viability while it recorded significant increase in the percentage of no progressive motility and immotile sperm than the negative control group (Table 12). Administration of GE and ME avoided a decline in the sperm analysis parameters to a reasonable degree

These parameters were improved by adding the high levels of herbs extract of GE and ME when compared with diabetic group whereas, the sperm concentration and its motility levels of ME extract were increased as compared to GE and the differences statically with positive control group were significant while the statically differences between the two herbs were no significant. From data obtained in the same table (Table 12), it was found that there is no significant between G5 , G8 and negative control group in Sperm concentration (total count) and immotile sperm percent and significant changes in the other parameters. DM has a direct effect on human fertility.

The likelihood of infertility is greater in men. It was found that there is no significant between G5, G8 and negative control group in Sperm concentration (total count) and immotile sperm percent and significant changes in the other parameters. DM has a direct effect on Semen parameters like sperm motility tends to be poorer in diabetics and abnormal sperm forms tend to be higher. DM is usually associated with obesity; this contributes to lower T levels and loss of libido (sex drive), thus, reducing the frequency of intercourse and chances of conception. DM is associated with nerve damage and also damage to blood vessels. This results in a host of sexual issues encompassing erectile dysfunction and ejaculation issues further hampering fertility. Traditional light microscopic analysis of the ejaculate suggests that the effect of DM on sperm quality

is negligible, and Ginger and marjoram are potent anti-oxidant compounds that may attenuate or prevent free radical production (Elberti and Zimmet, 2019). Oxidants, It is now evident that the ongoing production of pro-oxidants, including oxygen free radicals, is an important characteristic of aerobic life, Oxidative stress has been characterized as a disruption in the pro. Reactive oxidant/antioxidant system oxygen species (ROS) are highly reactive molecules classified as free radicals due to the presence of an unpaired electron, such as a superoxide ion organism; they are primarily confined to cell compartments and are counterbalanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers.

Both of GE and ME have been extensively studied for a broad range of biological activities, especially antioxidant activities found that they significantly lowered lipid per oxidation by maintaining the activities of the antioxidant enzymes –super oxide dismutase, catalase and glutathione peroxides in rats.

A lack of equilibrium between ROS production and scavenging activities causes cellular damage in the sperm. Extreme ROS generation that surpasses threshold levels may overcome the antioxidant defence mechanisms of spermatozoa and seminal plasma, resulting in oxidative stress in human fertility. The likelihood of infertility is greater in men. The scientific explanation for this is that the oxidative stress generated by high glucose levels destroys sperm DNA (Maresch *et al*., 2018). (Ramadan *et al*., 2012 and He *et al*., 2018).

Table 12. Effect of oral administration of ginger and marjoram extract for 12 weeks on the parameters of sperm analysis of diabetic adult male rats.

Groups and Treatment	Sperm /concentration (total count) no of sperm rat×10^6	Motility (%)			Viability (%)
		Immotile Sperm Percent	No progressive Motility.	Progressive Motility	
G1	194.11±4.55 [a]	34.21±1.72 [d]	30.08± 0.61 [c]	35.71± 6.33 [a]	65.65± 4.91 [a]
G2	107.34±0.13 [e]	50.9± 0.087 [a]	40.32± 0.91 [a]	8.78± 0.001 [e]	48.74± 3.99 [d]
G3	140.28±0.21 [d]	46±0.000 [b]	40.09± 1.51 [a]	13.91± 4.91 [d]	53.75± 6.88 [c]
G4	168.76±0.10 [b]	41.05±2.76 [c]	37.95± 0.78 [b]	21± 0.000 [c]	58.03± 0.08 [b]
G5	189.92±0.09 [a]	36.01±0.92 [d]	35.6± 5.31 [b]	28.39± 0.07 [b]	63.76± 0.08 [a]
G6	155.14±0.37 [c]	46.4± 0.000 [b]	40.05± 0.66 [a]	13.55± 0.63 [d]	53.04± 3.99 [c]
G7	172.32±0.17 [b]	40±0.000 [c]	37.6± 0.39 [b]	22.4± 2.01 [c]	59.75± 6.88 [b]
G8	191.24±0.23 [a]	36.05±0.96 [d]	35.3± 0.89 [b]	28.65± 0.08 [b]	63.73± 0.58 [a]

Negative control (G1), Positive control (G2), Group 3: Treated with GE (100 mg/kg b.w. /day), Group 4: Treated with GE (200 mg/kg b.w. /day), Group 5: Treated with GE (400 mg/kg b.w. /day) , Group 6: Treated with ME (100 mg/kg b.w. /day) , Group8: Treated with ME(400 mg/kg b.w. Values are means ± SD (n = 6).Values superscripted are significantly different at $p \leq 0.05$.

The sensory evaluation of applied biscuits:

A committee of faculty members of (Nutrition and food science - Home economics Department-Faculty of Specific Education, Mansoura University) evaluated the colour, odor, flavor, texture, and general acceptability of biscuits made with 100 percent wheat flour and biscuits with the most ginger and marjoram as shown in table (13).

Table 13. Sensory properties of control biscuit and fortified biscuits

Organoleptic Properties	Color 10	Odor 10	Flavor 10	Texture 10	Overall acceptability 10
Control biscuits	9.50[a]	9.53[a]	9.57[a]	9.59[a]	9.61[a]
Biscuits with ginger	8.8[a]	9[a]	8.7[a]	9[a]	9[a]
Biscuits with marjoram	7.5[b]	9[a]	8[b]	8.89[a]	9[a]

Values superscripted are significantly different at $p \leq 0.05$. When compared different groups with control group

Data showed that, all organoleptic properties for fortified biscuits with ginger and marjoram didn't differ as compared to control sample except color and flavor of marjoram biscuits were significantly lower than the control and ginger biscuits. The decrease in attractiveness evaluations with increasing replacement levels was mostly attributable to Egyptian panelists, who often favor lighter grades over darker ones (Akubor *et al*., 2003).

Chemical composition of control biscuits and fortified biscuits.

Control biscuits and fortified biscuits with best level of tested level were evaluated for their chemical structure (protein, fat, ash, fiber and carbohydrates) table (14). From the obtained results, it could be noticed that there is no significant between fortified and control sample in protein, fat and carbohydrates while ash and fiber in marjoram sample significantly were increased than the other samples. This conclusion corresponded with that stated by (FAO, 2002). In addition, Mari *et al*., (2008) discovered that the addition of herbs to a school lunch induced positive changes in children's development, morbidity, and cognitive functions than when no herbs were included.

Table 14. Chemical composition of control biscuits and fortified biscuits on dry weight basis.

Samples	Protein	Fat	Ash	Fiber	Carbohydrates
Control biscuits	9.53±0.23 [a]	17.12± 1.05 [a]	1.52±0.02 [b]	1.43±0.01 [c]	70.40± 3.98 [a]
Biscuits of ginger level 400mg	9.06±.0.45 [a]	17.25± 0.25 [a]	1.51±1.11 [b]	2.17±0.42 [b]	70.01± 4.98 [a]
Biscuits marjoram of level 400mg	9.45 ±0.13 [a]	17.46± 1.33 [a]	2.09±0.08 [a]	2.84±0.23 [a]	68.16±3.89 [a]

Values superscripted are significantly different at $p \leq 0.05$.

Histopathological examination

Testis from positive controls (photo 2) revealed seminiferous tubules (ST) of this group revealed degeneration in the form of thickening of the basement membranes of the tubules and marked hypo-spermatogenesis. In addition, necrosis of spermatogenic epithelium with pyknotic nuclei in some tubules was also seen. Meanwhile, their lumens showed desquamated necrotic spermatogenic cells and tissue debris. While testis of normal rats (G1) as negative control exhibited normal

structural organization of ST. Normal sperm (SP) number and LC appearance were detected (photo 1). GE and ME at the high level (G5and G8)(photos 5 and 8) marked improved testicular and epididymal lesions with restored normal spermatogenic series more effective in GE but diabetic rats received ginger (photo 3) or marjoram (photo 6)extract at the level 100mg exhibited marked recovery, where the tubes of the testis of this group showed marked improvement with the restoration of the normal spermatogenic series (regular distributed spermatogenic cells and increased number of sperms) in the photos (4 and 7) for groups received 200mg from extract. Chronic hyperglycemia leads to an elevation in forming free radicals, particularly ROS, in all diabetic tissues as a result of glucose autoxidation and protein glycosylation. High amounts of free radicals and a reduction in the efficacy of antioxidant enzyme defence may result in damage to cellular organelles and enzymes and an increase in lipid peroxidation. Mammalian sperm cells have an abundance of particular lipid composition, including plasmologen, polyunsaturated fatty acid, and sphingomyelin, but lack an antioxidant action. Mammalian spermatozoa are more vulnerable to perooxidative damage due to their high levels of polyunsaturated fatty acids and limited antioxidant capability. According to a recent study, high levels of ROS and free radicals reduce sperm motility and fertility in DM individuals. High antioxidant qualities in ginger and marjoram have scavenging abilities against free radicals. Antioxidant action is mediated by polyphenols and phenols found in key protein structures. Anti-inflammatory properties and a reduction in blood glucose levels were among the protective benefits of the studied extract on various tissues, as determined by experiments (Ahmed *et al*., 2009; Long *et al*., 2015 and Dupont *et al*., 2019).

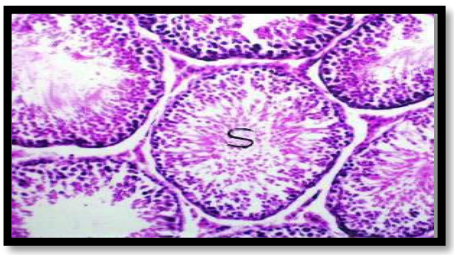

Photo 1. Histopathology of testes of the normal rat's group showing structural organization of seminiferous tubules (ST) and normal sperm (SP) (H&E,X10)

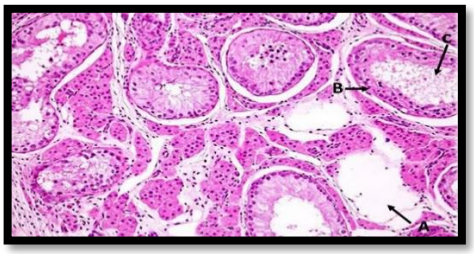

Photo 2. Histopathology of testes of the diabetic rat's group showing (A) degeneration of some seminiferous tubules (ST). (b) Pyknotic nuclei in the epithelium of some ST tubules. (c) some tubules show necrotic cells in their lumen (H&E,X10).

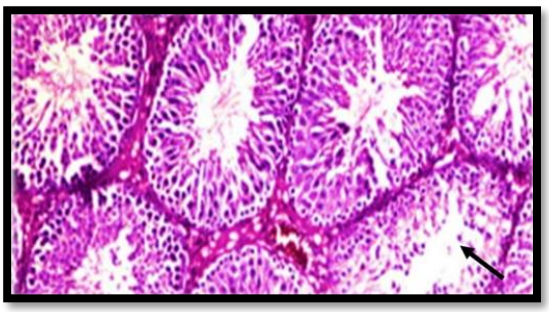

Photo 3. Histopathology of testes of the diabetic rat's group treated with 100 kg b.w. /day GE showing mild degeneration of the seminiferous tubules (ST). (H&E,X10).

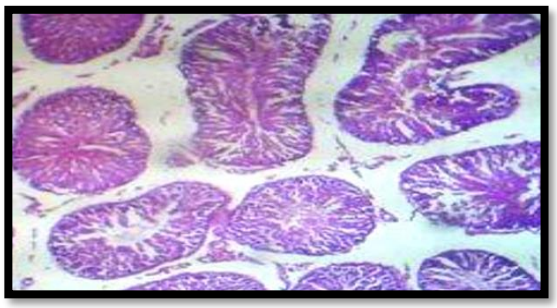

Photo 4. Histopathology of testes of the diabetic rat's group treated with 200 kg b.w. /day GE regular distribution of spermatogenic cells (H&E,X10).

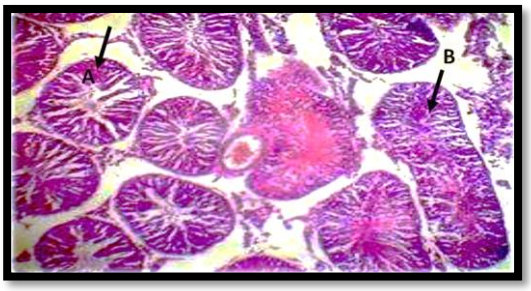

Photo 5. Histopathology of testes of the diabetic rat's group treated with 400 kg b.w. /day GE showing (A) normal spherical seminiferous tubules (ST) with regular spermatogonia distribution. (B) the lumen full of sperm cells (H&E,X10).

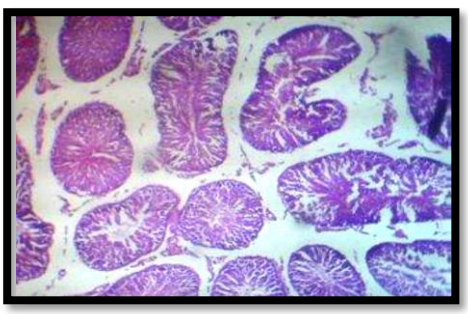

Photo 6. Histopathology of testes of the diabetic rat's group treated with 100 kg b.w. /day ME showing marked recovery (H&E,X10).

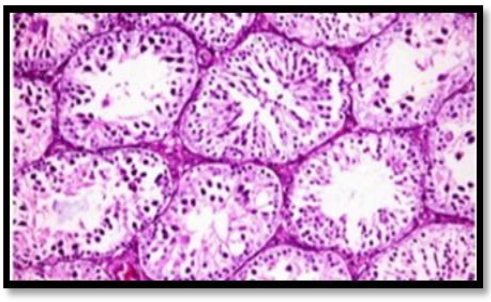

Photo 7. Histopathology of testes of the diabetic rat's group treated with 200 kg b.w. /day ME showing regular distribution of spermatogenic cells and increased number of sperms (H&E,X10).

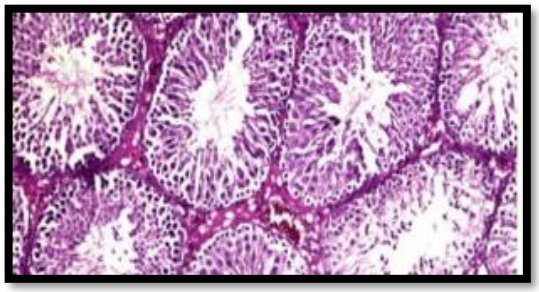

Photo 8. Histopathology of testes of the diabetic rat's group treated with 400 kg b.w. /day ME showing regular spermatogonia distribution and the lumen full of sperm cells (H&E,X10).

CONCLUSION

From the histopathology analysis, it was determined that the current data convincingly demonstrated a correlation between DM and the development of testicular function and structural disruptions. A change in the hormonal profile, particularly leptin and PRL, may explain this association. The administration of ME and GE had a significant preventative effect against changed hormones, along with an improvement in testicular health. Thus, revealing the strongest potential of extract's natural components to prevent DM -related male infertility.

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دراسة مقارنة بين تأثيرمستخلص البردقوش والزنجبيل على خصوبة ذكور الجرذان البيضاء المصابة بداء السكري

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الملخص

أجريت هذه الدراسة لمعرفة تأثير المستخلص المائى لكل من البردقوش والزنجبيل على خصوبة ذكور الفئران المصابة بداء السكرى, وقد تم استخدام عدد 48 فأرا مقسمة إلى ثماني مجموعات كل مجموعة 6 فئران،المجموعة الأولى (الكنترول السالبة) والتى تناولت الغذاء القياسى – المجموعة الثانية (الكنترول الموجبة المصابة) والستة مجموعات الباقية أعطيت جرعات مختلفة من كلا من ممستخلص البردقوش والزنجبيل هى (100 و 200 و 400 مجم / كجم من وزن الجسم) على التوالى ،و تم عمل تحاليل الحيوانات المنوية وقياس الأنسجة التشريحية . و أظهرت النتائج عدم وجود تغير معنوى فى الأوزان المختلفة سواء للخصية أو الحيوانات المنوية أو غدد البروستاتا الا بنسب طفيفة عند بعض التركيزات المختلفة ،وعلى الجانب الآخر تم تحسين مستويات هرمون التستوستيرون وزيادة عدد الحيوانات المنوية وحركتها مقارنة بالمجموعة الضابطة . كما لوحظ أيضا زيادة فى مستويات هرمون اللبتين والبرولاكتين مع انخفاض هرمون التستوستيرون وارتفاع فى نشاط الاروماتيز فى الخصية مقارنة بالمجموعة السالبة ، بينما أظهرت الفئران المصابة بداء السكري بعد تناولها المستخلص بالجرعات المختلفة اظهرت انخفاضًا معنويًا في اللبتين والبرولاكتين والأروماتيز مع زيادة هرمون التستوستيرون في الدم. لوحظ أيضا عدم حدوث تغيرات معنوية فى الأنسجة التشريحية وعدد الحيوانات المنوية مقارنة بالمجموعة الضابطة .كما أن تناول كلا من مستخلص البردقوش و الزنجبيل للفئران المصابة بداء السكري يمنع التغيرات المختلفة مثل تراكم دهون الخصية ، وارتفاع الأندروجينات وعدد الحيوانات المنوية ، بالإضافة إلى تحسين شكل وعدد الحيوانات المنوية وبنية الخصية .لذلك توصي هذه الدراسة بأن تناول البردقوش والزنجبيل كمشروب أو اضافته لأى منتج غذائى آخر (مثل البسكويت) يكون له تأثير إيجابي على زيادة الخصوبة لدى ذكور الجرذان المصابة بداء السكري.

الكلمات الدالة: البردقوش والزنجبيل - ذكور فئران مصابة بالسكرى - مضادات الأكسدة - الخصوبة - الحيوانات المنوية - التشريح المرضي