Effect of Extracts of Some Herbs on Fertility of Male Diabetic Rats

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Abstract: Diabetics have been recognized as a leading cause for male infertility. This study was carried out to investigate reproductive disorders caused by diabetics and the possible prevention through the use of lemongrass, marjoram and thyme extract at two levels 250 and 500 mg/kg body weight in rats for biological experimental. Chemical constituents, minerals content and fractionation of polyphenol compounds using High Performance Liquid Chromatography (HPLC) were determined in lemongrass, marjoram and thyme. The results showed that these herbs had riches amounted from protein, mineral content and polyphenol compounds as natural antioxidants. At the end of experiential biological evaluation the sex organs (testis, seminal vesicle and vas deferens) weights, epididymis sperm count, hormonal profile, serum glucose, serum lipids, measurement of aromatase activity, assay of 3b-Hydroxysteroid dehydrogenase (3b-HSD) activity, acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated in diabetic rats had taken orally daily of lemongrass, marjoram and thyme extract at level 250 and 500 mg/kg b.w. compared with negative control (healthy rats) for four weeks. The results observed that no significant changes in sex organs between diabetic rats had taken from lemongrass, marjoram and thyme extract orally daily at level 500 mg/kg b. w. and negative control rats. From the results it could be noticed that increased serum levels of leptin, prolactin (PRL) and estrogen (E2), with increased serum dehydroepiandrosterone (DHEA) and testosterone (T) were also observed. Additionally, the results showed significant increase in epididymal sperm count, as well as in steroidogenic enzymes; 3b-hydroxysteroid dehydrogenase (3b- HSD), alkaline phosphatase (ALP), and acid phosphatase (ACP), with marked elevation in aromatase activity in testis of the diabetic rats. Oral administration of lemongrass, marjoram and thyme extracts at level 500 mg/kg b.w seemed to prevent overall mentioned alterations, as evident by elevated sperm count, in addition to improved testicular structure. From the obviously results it could be recommended that the lemongrass, marjoram and thyme extract had riches amounted polyphenol compounds and when give the diabetic rats lemongrass, marjoram and thyme extract at level 500mg/kg body weight may be beneficial for diabetic rats who have fertility problem, as their extracts produce antidiabetic activity and exhibit fertility enhancing properties in diabetic rats.

[Eman H. AL Garni and Dalia A. Hafez. **Effect of Extracts of Some Herbs on Fertility of Male Diabetic Rats.** *J Am Sci* 2015;11(11):165-175]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 17. doi:10.7537/marsjas111115.17.

Key word: Lemongrass - marjoram - Thyme- Infertility – Diabetics- Polyphenol compounds.

1. Introduction

Diabetes mellitus (DM) is a degenerative disease with alteration in carbohydrate homeostasis that affects male reproductive function at multiple levels particularly the endocrine control of spermatogenesis, spermatogenesis itself or by impairing penile erection and ejaculation (Sexton and Jarow, 1997). About 90% of diabetic patients generally experience sexual abnormalities such as sexual dysfunction, impotence and infertility (Amaral et al., 2006). Male infertility is a common threat nowadays and it has increased rapidly because of hyperglycemia (Andy et al., 2009).

The importance of fertility and procreation as a factor for survival of the human race is not hidden from anyone. According to documents the use of the herbs has a long history in order to fertility regulation. Nowadays the effect of plant-derived chemicals on the endocrine system and the activity of sexual organs have induced a great interest Talukder et al. (2011). Among the medicinal herbs in indigenous systems of medicine it can be pointed to the numerous health benefits of lemongrass, marjoram and thyme.

Lemongrass (Cymbopogon citratus), a plant of West Indian origin, is one of the few herbs from the grass family. Most research relates to its essential oil, of which citral, an oxygenated terpenoid, is a major component (Paranagama et al., 2003). In a comparison of 11 essential oils isolated from various plant sources, lemongrass oil ranked highly in terms of antioxidant activity radical scavenging and protection of lipids from peroxidation as well as antimicrobial activity against a range of food spoilage yeasts (Sacchetti et al., 2005). With a slightly different focus, Nakamura et al. (2003) found that

citral isolated from lemongrass induced the activity of the phase 2 enzyme, glutathione-S-transferase (GST), which plays important detoxification and anti-cancer roles in the body (Hedges and Lister, 2006).

Marjoram (Marjorana hortensis) was formerly classified as coming from a sister genus of oregano, but is now officially a species of oregano itself (McGee, 2004). In New Zealand the names are often used interchangeably, though marjoram (also known as sweet marjoram) differs from oregano in having a milder flavor.

Zheng and Wang (2001) found moderate levels of phenolics and antioxidant activity in thyme in comparison with other herbs. They also found a variation according to species, with common garden thyme ranking more highly in both respects than creeping or lemon thyme. There have been a few trials involving human cells. A further non-cell-based assay showed thymol to have significant scavenging activity, which the authors attributed to its phenolic structure. Thymol also showed significant anti-thrombotic activity *in vitro* and *in vivo* in mice (Yamamoto et al., 2005).

Regarding male fertility, increased levels of reactive oxygen species ROS have been correlated with decreased sperm motility (Eskenazi et al., 2003), increased sperm DNA damage (Armstrong et al., 1999), sperm cellular membrane lipid peroxidation (Aitken, 1995) and decreased efficacy of oocyte-sperm fusion (Agarwal et al., 2007).

Looking at animal studies, Baumber et al. (2000) observed a decrease in equine sperm motility associated with reactive oxygen species ROS. Bansal and Bilaspuri (2008 a and b) demonstrated that elevated ROS were responsible for the loss of motility and viability of bovine spermatozoa, as well as for increased lipid peroxidation, acrosome and total sperm abnormalities which subsequently affected sperm morphology. An important correlation between lipid peroxidation in the plasma membrane, production of toxic aldehydes such as malondialdehyde (MDA) and the loss of motility was found in caprine (Bucak et al., 2009) and boar (Cerolini et al., 2000) spermatozoa. Elevated ROS levels associated with a partial or complete loss of spermatozoa motility, ATP content and fertilising ability in fowl were reported by Wishart (1984). According to Bucak et al. (2007), excessive ROS impaired the motility and fertilisation capacity of ram semen.

The origin of ROS generation and the aetiologies of increased ROS in males with suboptimal sperm quality have only recently been elucidated, offering multiple targets for a potential therapy (Kefer et al., 2009). Antioxidant supplementation in infertility treatment is an interesting area to pursue, especially using a preventive approach. Also, the role of ROS in fertility and sub fertility is an area deserving a continued research (Sekhon et al., 2010).

The aim of this investigation was carried out to evaluate the chemical composition, minerals content and polyphenol fractions as natural antioxidants from the raw herbs as lemongrass, marjoram and thyme. Moreover studying effect the lemongrass, marjoram and thyme extract on male fertility in diabetic rats and measurement the levels of blood glucose parameter of serum lipids and testosterone hormones.

2. Materials and Methods

Materials:

The raw materials herbs as Lemongrass (Cymbopogon citratus), Marjoram (Marjorana hortensis) and Thyme (Thymus vulgaris) were obtained from west region at Kingdom Saudi Arabia. The raw materials herbs were milled in laboratory Mill Junior to a fine powder and kept in refrigerator at -5 ^{o}C until analysis.

Methods:

Proximate of chemical composition:

Protein, total lipids, crude fiber, ash content and total carbohydrates were determined raw materials herbs as lemongrass, marjoram and thyme using standard methods AOAC. (2005). Total dietary fiber was determined of the dried raw materials according to the methods described by Prosky (1988). Also, soluble and insoluble dietary fiber was determined with Lee and Prosky (1995).

Minerals content as calcium (Ca), iron (Fe), m (Mg), phosphorus (P), potassium (K), sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn) were determined in the diluted solution of ash raw materials using the atomic absorption spectrophotometer (3300 Perkin-Elme) as described in by AOAC (2005) method. While, phosphorus (P) was determined using the Unican SP 1800 Spectrophotometer at wave length 650 nm.

Preparation of raw materials extract:

The method of raw materials (lemongrass, marjoram and thyme) extraction was described by Shalaby and Hamowieh (2010). In this method, the dried powder lemongrass, marjoram and thyme (500 gm from each herb) were soaked separately in 2 liter of 90% ethyl alcohol overnight and extracted till complete exhaustion by percolation several times with ethanol as a solvent. The solvent was evaporated by using Rotary evaporator apparatus (made in Russia) connected to a vacuum pump and the temperature was adjusted to 50ċ till yielding a semisolid ethanolic extract. Known grams of the

obtained extract were dissolved in few drops of Tween 80 as a suspending agent, and then distilled water was added to prepare a solution of desired concentration.

Determination of polyphenol compounds fraction in some raw herbs materials:

Polyphenol compounds were fractionated in lemongrass, marjoram and thyme using High Performance Liquid Chromatography (HPLC) according to the method which described by Hakkinen et al. (1998). HPLC instrument (Hewlett Packard series 1100 HP). Column hypersil BDS 5 µm C 18 and Detector UV 254 nm. Flow rate 0.3 mL/min. Mobile phase A: (0.5 mL acetic acid: 99.5 mL distilled water). B: (0.5 mL acetic acid: 99.5 mL acetonitrile), temperature ambient 25°C.

Experimental biological evaluation:

Male adult rats (48 rats) weight ranging 170-180g were purchased from National Organization for Drug and Control Research, Giza, Egypt. Animals were housed in individual cages with screen bottoms and fed on basal diet for eight days. The basal diet consisted of corn starch 70%, casein 10% corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according AOAC (2005). After feeding on basal diet for eight days, rats were divided into two groups. The first group (6 rats) was fed on the basal diet and received distilled water for another four weeks (30 days) and considered as negative control. The second main group (42 rats) was fasted overnight and injected with strepto zootocin (was dissolved in 0.1M citric acid buffer and adjusted at pH 4.5) into the leg muscle (5mg /100g body weight) to induce diabetic rats according to Madar (1983). After 48 h of injection the second main group was divided into seven subgroups (6 rats for each). The first one (6 rats) was continued to be fed on basal diet and received distilled water for another four weeks (30 days) and considered as positive control. From the second to seven subgroups (6 rats for each) were fed on basal diet and the group two and three were taken orally daily from lemongrass extract at level 250 and 500 mg/kg. The group four and five were taken orally daily from marjoram extract at level 250 and 500 mg/kg and also the group sex and seven were taken orally daily from thyme extract at level 250 and 500 mg/kg.

At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm for 10 min to obtain the sera. After that, the sera were kept on a deep freezer at -20^{o}C until their biochemical measurements. Immediately after collecting blood, the two testes, epididymis, vas deferens and seminal vesicle from each rat were removed and weighed. The epididymis was homogenized in 5ml of 0.9% NaCl. Sperm counting was done using hemocytometer according to Adeeko and Dada (1998) and total number of sperm per gram of epididymis was then calculated.

The biochemical characteristics were serum testosterone (T) and dehydroepiandrosterone (DHEA) levels were evaluated, according to the methods of Tietz (1995) and Longcope (1996). Estrogen (E2) and prolactin (PRL) were estimated by Enzyme linked Fluorescent Assay (ELFA) technique as described by Dupont et al. (1991) and Sapin and Simon (2001). Leptin was estimated according to the method of Considine and Siha (1996). Serum glucose, insulin level, total lipids (TLs), total cholesterol (TC), triglycerides (TGs) and phospholipids (PLs) were determined according to knight et al. (1972), Yallow and Bauman (1983), Allain et al. (1974), Fossati and Prencipe (1982), Tietz (1986) and Connerty (1961), respectively.

Testicular enzyme activities in rats treated with different herbs:

The quantitative measurement of aromatase activity was performed by a solid phase enzyme-linked immunosorbent assay (ELISA), based on the sandwich principle, as described by Roselli (1998). Samples were incubated in micro titer plate wells precoated with biotin-conjugated antibody specific to aromatase. After incubation, a sandwich complex was formed and the unbound material was washed off. Next, Avid in peroxidase enzyme complex was added for detection of the bounded aromatase at 450 nm.

Portion from the testis was weighed and homogenized at 4 ^{o}C in 20% spectroscopic grade glycerol, containing 5 mmol potassium phosphate and 1 mmol EDTA. Resulting homogenate was centrifuged at 10,000 × g for 30min at 4 ^{o}C, and the supernatant was collected for assay of 3b-Hydroxysteroid dehydrogenase (3b-HSD) activity Talalay (1962). Testicular supernatant (1 ml) was mixed with 1 ml of sodium pyrophosphate buffer (pH 8.9), 40 mL of ethanol, containing 30 mg of dehydroepiendostero and 960 mL of 25% bovine serum albumin making a final incubation mixture of 3 ml. Next, 100 mL of 0.5 mM NAD was added for evaluating 3b-HSD activity at 340 nm against a blank (without NAD) Talalay (1962).

Acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated according to Kind and King (1954) and Tietz (1976).

Statistical analysis:

All chemical analyses were performed in three replicates and the results were statistically analysed.

Statistical analysis was performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $p \leq 0.05$ was used to establish the statistical significance.

3. Results and Discussion

Chemical composition and minerals content in raw materials:

Chemical constituents, total dietary fiber fractions and minerals content were determined in lemongrass, marjoram and thyme powder and the results are reported in Table (1). From the results it could be observed that the thyme had the highest in total protein (15.86%) followed by marjoram and lemongrass were 12.66 and 11.00%, respectively. Total lipids were higher in lemongrass 10.25% than marjoram and thyme (7.04 and 4.80%). Marjoram was the highest in ash content and crude fiber (12.10 and 15.31**%)** followed by thyme and lemongrass.

Total dietary fiber, soluble fiber and insoluble dietary fiber were determined in lemongrass, marjoram and thyme and the resultant showed that the marjoram had the highest contained in total dietary fiber, soluble fiber and insoluble dietary fiber (43.30, 13.43 and 29.87%) followed by lemongrass whilst the thyme was the lowest content in the fiber fractions.

Minerals content were determined in lemongrass, marjoram and thyme and the resultant showed that the marjoram had the highest contained in calcium, iron, magnesium, phosphorus, sodium and manganese were 199.0, 82.71, 346.0, 306.0, 77.0 and 5.43 mg/100g, respectively. Whereas, the lemongrass was the highest contained in potassium, zinc and copper were 669.0, 4.43 and 2.15 mg/100g and also, the thyme was the lowest in minerals content.

Furthermore, taken into consideration that potassium depresses while sodium enhances blood pressure, thus, high amount could be an important factor in presentation of hypertension Yoshimura et al. (1991). Calcium and phosphorous are associated with each other for development and proper functioning of bone, teeth and muscles Turan et al. (2003). Iron deficiency according to World Health Organization (WHO) affect about 3.7 billion people out of which 2 billion people are anemic Meng et al. (2005).

Table (1): Chemical composition (g/100g) and minerals content (mg/100g) of raw materials on dry weight basis:

Chemical analysis	Lemongrass	Marjoram	Thyme
Protein	11.00	12.66	15.86
Total lipid	10.25	7.04	4.80
Ash	7.15	12.10	9.14
Crude fiber	9.42	15.31	12.73
T.C.	62.18	52.89	57.47
TDF	40.80	43.30	14.0
SDF	12.54	13.43	4.59
INSDF	28.26	29.87	9.41
Calcium Ca mg/100g	157.6	199.00	105.0
Iron, Fe mg/100g	44.00	82.71	17.45
Magnesium, Mg mg/100g	270.0	346.0	160.0
Phosphorus, P mg/100g	200.0	306.0	106.0
Potassium, K mg/100g	669.0	522.0	609.0
Sodium, Na mg/100g	15.0	77.0	9.0
Zinc, Zn mg/100g	4.43	3.60	1.81
Copper, Cu mg/100g	2.15	1.133	0.555
Manganese, Mn mg/100g	4.667	5.433	1.719

Polyphenolic fractions of raw herbs materials:

Flavonoids compounds in lemongrass, marjoram and thyme were fractionated using HPLC apparatus and the results are reported in Table (2). From the results it can be noticed that the lemongrass, marjoram and thyme had contained the rutin, hisperidin, rosmarinic, quercitrin, querctin, narenginin, kaempferol, hispertins, flavones, narerigin and luteolin. The results observed that the marjoram was increased in rutin, hisperidin, flavones; narerigin and luteolin were 48.32, 551.51, 11.76, 309.96 and 8.79mg/100g, respectively whereas lemongrass had contained 77.02 and 28.47 mg/100g, in hispertins and kaempferol. Moreover, the thyme had increased in rosmarinic, quercitrin, querctin and narenginin were 67.21, 481.73, 48.52 and 19.42 mg/100g, respectively.

Whilst, phenols compounds in lemongrass, marjoram and thyme were gallic, catechin, pyrogalol, epicatechin, chlorogenic, catechol, p-hydroxy benzoic, vanillic, ellagic, benzoic, salicylic and O-cumaric acids using HPLC apparatus. The resultsin

the same table observed that the lemongrass was increased in catechin, epicatechin, ellagic and benzoic acids were 14.19, 41.75, 26.72 and 118.28 mg/100g, respectively. Whereas marjoram had the highest contained from phenol compounds pyrogalol, p-hydroxy benzoic, vanillic and O- cumaric were 63.14, 44.27, 17.76 and 54.64 mg/100, respectively. Moreover, the thyme had increased in gallic, chlorogenic, catechol and saliaylic acids were 4.15, 36.38, 50.95 and 48.91 mg/100g, respectively.

A range of phenolic compounds has been identified in oregano including rosmarinic, caffeic, and p-coumaric acids and caffeoyl derivatives, the phenolic monterpenes, carvacrol and thymol, and flavonoids, luteolin, apigenin myricetin and quercetin (Yanishlieva et al., 2006 and USDA, 2007). Although most research interest has centred upon oregano as an essential oil, and its constituent compounds carvacrol and thymol, which are believed to cause its antioxidant activity, it has been shown that water extracts, which isolate a range of different compounds, are rich in phenolic acids and flavonoids also have strong antioxidant activity (Triantaphyllou et al. 2001).

Table (2): Percent of total flavonoids and total phenols (mg/100g on dry weight) in ethanol extract from lemongrass, marjoram and thyme.

Compound		Lemongrass	Marjoram	Thyme
Flavonoid compounds	Rutin	35.06	48. 32	23.04
	Hisperidin	302.62	551.51	247.9
	Rosmarinic	22.78	12. 81	67.21
	Quercitrin	140.31	70. 42	481. 73
	Querctin	25.61	15. 27	48.52
	Narenginin	1.47	8.72	19.42
	Kaempferol	77.02	53.30	66.00
	Hispertins	28.74	9.47	23.0. 7
	Flavone	4.70	11.76	4.77
	Narerigin	--	309.96	181.72
	Luteolin	--	8.79	8.19
Phenolic compounds	Gallic	0.04	0.65	4. 15
	Catechin	14. 19	3.66	6. 49
	Pyrogalol	1. 87	63. 14	53. 39
	Epicatechin	41. 75	12.37	36.38
	Chlorogenic	3. 76	11.15	32. 08
	Catechol	5. 33	8.86	50. 95
	P_Hydroxy benzoic	12. 88	44. 27	21. 31
	Vanilic	8. 25	17.76	2. 34
	Ellagic	26. 72	1. 23	11. 42
	Benzoic	118. 28	85. 26	44. 08
	Salicylic	30. 73	36. 36	48.91
	O-Coumarin	52. 90	54. 64	32. 19

Effect of lemongrass, marjoram and thyme extract on sex organs and epididymis sperm count in diabetics rats:

Table (3) showed that the effect of lemongrass, marjoram and thyme extract at level 250 and 500 mg/kg b. w. were separately taken orally daily on diabetic rat. The results illustrated that the sex organs (testis, seminal vesicle and vas deferens) weights for diabetic rats taken orally 500 mg/kg b. w. showed non-significant changes compared to negative control rats. Whereas the epididymis sperm count was improve in diabetics rats which taken orally daily marjoram extract at level 500 mg/kg b.w. followed by thyme and lemongrass extract. These increased in sex organs and improvement for epididymis sperm count in diabetics rats which taken orally daily lemongrass, marjoram and thyme at level 500 mg/kg b.w. may be caused the herbs had contained riches in flavonoids and phenol compounds an natural antioxidant have been found beneficial human health.

The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone and other androgens. Testosterone stimulates growth and secretary activity of the reproductive organs Oberlander et al. (1994).

Flavonoids are a class of secondary plant phenolics having potential beneficial effects on human health with significant antioxidant and chelating properties in the human diet. Over the years, they have been found to be an important part of the human diet and are considered to be active principles in some medicinal plants. The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O2·-), hydroxyl (OH·), peroxyl

(ROO·) and alcohoxyl (RO·) radicals Wang et al. (2003).

Phenolic compounds and flavonoid contents are potential antioxidative phytochemicals that can act as metal ion chelators, free radical scavengers and reducing agents, thus offer human health benefits (Lum and Chong, 2012).

Table (3): Sex organs and epididymis sperm count in diabetic rats treated with different extract herbs.

Parameters	Control negative	Control positive	Lemongrass		Marjoram		Thyme	
			250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Testis weight (g)	1.43 ±0.02	1.32 ±0.02	1.35 ±0.03	1.46 ±0.04	1.39 ±0.03	1.47 ±0.02	1.37 ±0.04	1.45 ±0.02
Epididymis weight (g)	0.50 ±0.04	0.45 ±0.01	0.47 ±0.03	0.50 ±0.03	0.49 ±0.03	0.52 ±0.02	0.49 0.03±	0.52 ±0.03
Seminal vesicle weight (g)	0.80 ±0.04	0.70 ±0.04	0.73 ±0.05	0.80 ±0.04	0.75 ±0.07	0.83 ±0.06	0.74 ±0.04	0.82 ±0.08
Vas deferens weight (g)	0.23 ±0.20	0.18 ±0.01	0.20 ±0.01	0.24 ±0.01	0.22 ±0.01	0.24 ±0.02	0.22 ±0.02	0.24 ±0.02
Epididymis sperm count (10^4)/g	3.82 ±0.12	1.64 ±0.09	2.72 ±0.11	3.85 ±0.12	3.20 ±0.12	4.12 ±0.10	2.95 ±0.24	3.97 ±0.61

Table (4): Effect of different herbs extract on hormonal profile, serum glucose and serum lipids in diabetic rats.

Parameters	Control negative	Control positive	Lemongrass		Marjoram		Thyme	
			250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Serum Testosterone (ng/ml)	4.94 ±0.20	2.49 ±0.44	3.10 ±0.12	4.47 ±0.18	3.67 ±0.24	5.09 ±0.27	3.35 ±0.21	4.63 ±0.23
Serum DHEA (ng/ml)	5.27 ±0.34	2.39 ±0.23	3.74 ±0.23	5.15 ±0.33	4.54 ±0.32	5.73 ±0.19	4.18 0.27±	5.35 ±0.26
Serum Estradiol (ng/ml)	25.65 ±0.44	29.42 ±0.83	27.37 ±0.94	25.56 ±0.90	25.46 0.71±	24.84 ±0.57	26.12 ±0.65	24.84 ±038
Serum Leptin (ng/ml)	18.88 ±0.33	31.42 ±0.73	21.53 ±0.47	18.71 ±0.59	18.98 ±0.74	16.95 ±0.69	20.15 ±0.38	17.45 ±0.43
Serum Prolactin (ng/ml)	15.00 ±0.22	26.53 ±1.96	19.35 ±0.44	15.67 ±0.72	16.86 ±0.52	14.14 ±0.35	17.83 ±0.71	15.04 ±0.20
Serum TLs (mg/dl)	501.16 ±6.35	762.52 ±9.49	650.15 ±8.74	517.85 ±4.30	554.32 ±4.59	473.13 ±8.67	570.54 ±6.43	477.74 ±9.19
Serum TC (mg/dl)	93.75 ±1.48	125.49 ±2.39	112.12 ±1.84	92.48 ±1.88	100.48 ±1.43	88.19 ±0.96	105.64 ±1.25	90.46 ±1.17
Serum TGs (mg/dl)	113.98 ±1.48	154.11 ±4.75	135.32 ±1.45	114.73 ±1.87	125.24 ±1.20	110.35 ±1.50	128.25 ±2.04	112.45 ±2.68
Serum PLs (mg/dl)	16.08 ±0.43	21.67 ±0.67	19.12 ±0.43	16.16 ±0.52	17.54 ±0.37	14.01 ±0.97	18.11 ±0.36	14.38 ±0.68
Serum glucose level (mg/dl)	98.0 ±2.47	260.0 ±6.32	200.0 ±5.89	160.0 ±7.15	170.0 ±4.28	120.0 ±3.73	180.0 ±2.49	140.0 ±2.87
Serum insulin level (µU/ml)	6.85 ±0.24	3.76 ±0.34	4.28 ±0.25	5.67 ±0.42	4.96 ±0.41	6.44 ±0.53	4.60 ±0.38	6.15 ±0.41

Effect of lemongrass, marjoram and thyme extract on hormonal profile, serum glucose and serum lipids in diabetic rats.

The presented data in Table (4) show that the effect of lemongrass, marjoram and thyme extract at level 250 and 500 mg/kg b.w. on serum testosterone (T), dehydroepiandrosterone (DHEA) levels, Estrogen (E2), prolactin (PRL), leptin, serum glucose, insulin level, total lipids (TLs), total cholesterol (TC), triglycerides (TGs) and phospholipids (PLs) in diabetic rats and compared with negative control and positive control rats. From the resultant it could be observed that the marjoram extract at 500 mg/kg b. w. had significant preventing on hormonal profile in diabetic rats and non significant changes compared with negative control. Moreover the thyme extract is a good source of natural antioxidant and beneficial health at level 500 mg/kg b.w. in diabetic rats and non significant changes than negative control followed by

lemongrass extract at level 500 mg/kg b. w the results from diabetic rats have been equal negative control.

PRL is a hormone produced by the pituitary gland in mammals. It is a key hormone in controlling milk production, however apart from lactation; PRL is closely involved in several physiological actions, such as reproduction Freeman et al. (2000). Synthesis and release of PRL is controlled by dopaminergic inhibition mechanism. Normal serum concentrations of PRL have been shown to exert permissive roles on the male reproductive tract; however excessive PRL concentration is correlated with hypogonadism, impotence and infertility khatoon and Badawy (2013) probably through interfering with production of FSH and LH which in turn will affect the testicular function with decrease in T release khatoon and Badawy (2013).

Leptin is a protein hormone that is synthesized and secreted by adipocytes. Its physiological role is to regulate appetite and body weight, but due to excess adipose tissue in the obese subjects levels of leptin are often elevated leading to adverse effects, particularly on the male fertility Phillips and Tanphaichitr (2010). The most important of these effects is that increased levels of leptin may act as inhibitory signal for T synthesis through membrane receptors on testicular Leydig cells Khullar et al (2012). Leptin receptors are also present on the plasma membrane of sperms, suggesting that leptin may directly affect sperm production independent of changes in testosterone production Jope et al. (2003).

From the same table the results showed that the effect of lemongrass, marjoram and thyme extract at level 250 and 500 mg/kg b.w. on serum total lipids (TLs), total cholesterol (TC), triglycerides (TGs), phospholipids (PLs), serum glucose and insulin level in diabetic rats and compared with negative control rats. The resultant observed that the total lipids, total cholesterol, triglycerides and phospholipids were decreased using marjoram and thyme at level 500 mg/kg b. w. in diabetic rats 473.13, 88.19, 110.35 and 14.01 mg/dl in marjoram extract and 477.74, 90.46, 112.45 and 14.38 mg/dl in thyme extract compared with negative control rats was 501.16, 93.75, .98 and 16.08 mg/dl, and positive control was 762.52, 125.49, 154.11 and 21.67 mg/dl, respectively. Moreover, the serum glucose and insulin level were determined in diabetic rats and compared with negative control rats and the results observed that the marjoram, thyme and lemongrass extract at level 500 mg/kg b.w. had significant effects on diabetic rats. Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants. In addition, Plants have a long folklore of use in aiding fertility, including fertility-enhancing properties and aphrodisiacal qualities D'Cruz et al. (2010).

Table (5): Effect of different extract herbs on testicular enzyme activities in diabetic rats.

Parameters	Control negative	Control positive	Lemongrass		Marjoram		Thyme	
			250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Aromatase (ng/g)	5.70 ±0.36	9.71 ±0.54	6.41 ±0.36	4.29 ±0.46	6.54 ±0.25	5.79 ±0.65	6.50 ±0.41	5.11 ±0.59
3b-HSD (U/mg)	0.42 ±0.03	0.17 ±0.03	0.30 ±0.07	0.40 ±0.04	0.37 ±0.07	0.46 ±0.04	0.35 ±0.05	0.44 ±0.06
ALP (U/g)	38.04 ±1.91	21.14 ±1.49	30.25 ±2.14	39.32 ±2.76	31.45 ±2.32	38.74 ±1.50	31.56 ±2.53	38.49 ±1.13
ACP (U/g)	2.79 ±0.11	1.59 ±0.33	1.85 ±0.13	2.10 ±0.08	2.56 ±0.31	3.28 ±0.21	2.28 ±0.24	2.79 ±0.19

Effect of d lemongrass, marjoram and thyme extract on testicular enzyme activities in diabetic rats.

The quantitative measurement of aromatase activity, assay of 3b-Hydroxysteroid dehydrogenase (3b-HSD) activity, acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated in diabetic rats had taken orally daily of lemongrass, marjoram and thyme extract at level 250 and 500 mg/kg b.w. than negative control and the results are reported in Table (5). Results also revealed significantly increased activities of 3b-HSD, ACP and ALP, coupled with elevation of aromatase activity in testis of the diabetic rats compared to the control group. Administration of marjoram and thyme extract to positive control rats significantly improved all motioned enzymatic changes compared to positive control rats. Taken together, data obtained can thus indicate potential activity of marjoram, thyme and lemongrass extract at level 500 mg/kg b.w. against diabetics and associated biochemical changes however marjoram extract at level 500 mg/kg b.w. seemed most effective followed by thyme and lemongrass extract at the same concentration.

The aromatization of testosterone (T) is the key step in estrogen (E2) synthesis and is catalyzed by the

aromatase enzyme Jones et al. (2006). Aromatase enzyme is a member of cytochrome P450 superfamily foundmainly in the gonadal and the adipose tissues Santen et al. (2009). Consequently, a relation was found between the rise in aromatase activity and the increased E2 with decline of T, which is particularly responsible for developing infertility in the rat males, as notably demonstrated in the current study and by other investigations Hofny et al. (2010). Apart from the role of endocrine disruption, other pathogenic cascades could be implicated in promoting male infertility with diabetics. This may occur via defects of critical steroidogenic enzymes, including 3b-HSD, ALP and ACP. 3b-HSD controls T biosynthesis reactions through catalyzing conversion of DHEA to androstenedione in mitochondria, thereafter the process of biosynthesis of T is continued while moving into the endoplasmic reticulum in Leydig cells Liu et al (2012).

ALP helps in ionic movement across the cell membrane and is also associated with secretory and absorption process of the cell Bansal and Roy (1997). Decline in ALP activity may indicate a state of decreased steroidogenesis where the intera and interacellular transport were reduced as the metabolic reactions for steroidogenesis slowed down Latchoumycandane et al. (1997). ACP is an enzyme capable of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular ACP gene is up-regulated by androgens and is down-regulated by estrogens Yousef et al. (2001). Activities of ACP have been shown to rise when testicular steroidogenesis is increased. A decrease in ACP activity would thus reflect decreased testicular steroidogenesis and this may be correlated with the reduced secretion of gonadotrophins Latchoumycandane et al. (1997). Accordingly, the present findings of increased testicular activities of ACP, ALP, and 3b-HSD may be closely related to ability of testosterone production observed in the herbs extract fed orally rats.

4. Conclusions:

The present study suggests a beneficial effect of lemongrass, marjoram and thyme probably by its antioxidant and antidiabetic properties. As this antioxidant flavonoid is known to decrease the risk of degenerative diseases. It could be recommended that using dietary herbs rich in flavonoids and phenolic compounds could have beneficial effects on subjects with diabetes. Moreover these herbs extract at level 500 mg/kg b. w.showed that more effect to improve function properties of hormonal profile, lipids parameter and testicular enzyme activities in diabetic rats.

References

1. Adeeko, A. O. and Dada, O. A. (1998). Chloroquine reduces the fertilizing capacity of epididymal sperm in rats. Afr J Med Sci 1998;27:63-8.
2. Agarwal, A., Prabakaran, S. A. and Sikka, S. C. (2007). Clinical relevance of oxidative stress in patients with male factor infertility: evidence-based analysis. AUA Update Series 26, 1–12.
3. Aitken, R. J. (1995). Free radicals, lipid peroxidation, sperm function. Reprod. Fertil. Dev. 7, 659–668.
4. Allain, C. C., Poor, L. S. and Chan, S. O. (1974). Enzymatic determination of total serum cholesterol. Clinical Chem., 20 (4): 470-475.
5. Amaral, S., Moreno, A.J., Santos, M.S., Seiça, R. and Ramalho, J. (2006). Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. Theriogenology. 66: 2056–2067
6. Andy, P., Luiz, R., Marco, M. and Luciana, A. (2009). Relation between diabetes mellitus and male fertility. Einstein. 7: 407– 410.
7. AOAC (2005). Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed., Washington, D.C.
8. Armstrong, J. S., Rajasekaran, M., Chamulitrat, W., Gatti, P., Hellstrom, W. J. and Sikka, S. C. (1999). Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radic. Biol. Med. 26, 869–880.
9. Bansal, N. and Roy, K. S. (1997). Histoenzymic effects of chlorpyrifos toxicity on rabbit genitalia. Indian J Anim Sci 1997;67:125-7.
10. Bansal, A. K. and Bilaspuri, G. S. (2008a). Effect of ferrous sulphate and ascorbic acid on motility, viability and lipid peroxidation of crossbred cattle bull spermatozoa. Animal 2, 100–104.
11. Bansal, A. K. and Bilaspuri, G. S. (2008b). Effect of manganese on bovine sperm motility, viability, and lipid peroxidation in vitro. Anim. Reprod. CBRA 5, 90–96. Baumber, J., Ball, B. A., Gravance, C. G., Medina, V. and Davies-Morel, M. C. (2000). The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. J. Androl. 21, 895–902.
12. Bucak, M. N., Ateşşahin, A., Varişli, O., Yüce, A., Tekin, N. and Akçay, A. (2007). The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen microscopic and

oxidative stress parameters after freeze-thawing process. Theriogenology 67, 1060–1067.
13. Bucak, M. N., Sarıozkan, S., Tuncer, P. B., Ulutaş, P. A. and Akçadağ, H. I. (2009). Effect of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in Angora goat semen following cryopreservation. Small Rum. Res. 81, 90–95.
14. Cerolini, S., Maldjian, A., Surai, P. and Noble, R. (2000). Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim. Reprod. Sci. 58, 99–111.
15. Connerty, H. V., Briggs, A. R. and Eaton, E. H. (1961). Simplified determination of the lipid components of blood serum. Clin Chem 1961;7:37-53.
16. Cosidine, R. V. and Siha, M. K. (1996). Serum immunoreactive-leptin concentrations in normal weight and obese humans. N Engl J Med 1996;334:292-5.
17. D'Cruz, S.C., Vaithinathan, S., Jubendradass, R. and Mathur, P.P.(2010). Effects of plants and plant products on the testis. Asian J Androl 2010; 12: 468- 479.
18. Dupont, A., Dupont, B., Cusan, L., Tremblay, M., Rioux, J. and Cloutier, D. (1991). Comparative endocrinological and clinical effects of percutaneous estradiol and oral conjugated estrogens asreplacement therapy in menopausal women. Malturitas 1991;13:297-311.
19. Eskenazi, B., Wyrobek, A. J., Sloter, E., Kidd, S. A., Moore, L., Young, S. and Moore, D. (2003). The association of age and semen quality in healthy men. Hum. Reprod. 18, 447–454.
20. Fossati, P. and Prencipe, L. (1982). The determination of triglyceride using enzymatic methods. Clin. Chem., 28: 2077-2081.
21. Freeman, M.E., Kanyicska, B., Lerant, A. and Nagy, G. (2000). Prolactin: structure, function, and regulation of secretion. Physiol Rev 2000;80:1523-631.
22. Hakkinen, S. H., Karenlampi, S. O., Heinonen, I. M., Mykkanen, H. M. and Torronen, A. R. (1998). HPLC method for screening of flavonoids and phenolic acids in berries. J. Sci. Food Agric., 778: 543-551.
23. Hedges, L.J. and Lister, C.E. (2006). Nutritional attributes of *Brassica* vegetables. Crop and Food Research Confidential report no. 1618. Christchurch, New Zealand Institute for Crop & Food Research Ltd. 46 p.
24. Hofny, E.R., Ali, M.E., Abdel-Hafez, H.Z., Kamal, Eel-D., Mohamed, E.E. and Abd El-Azeem, H.G. (2010). Semen parameters and hormonal profile in obese fertile and infertile males. Fertil Steril 2010;94:581-4.
25. Jones, M.E., Boon, W.C., Proietto, J. and Simpson, E.R. (2006). Of mice and men: the evolving phenotype of aromatase deficiency. Trends Endocrinol Metab 2006;17:55-64.
26. Jope, T., Lammert, A., Kratzsch, J., Paasch, U. and Glander, H. J. (2003). Leptin and leptin receptor in human seminal plasma and in human spermatozoa. Int J Androl; 26:335-41.
27. Kefer, J. C., Agarwal, A. and Sabanegh, E. (2009). Role of antioxidants in the treatment of male infertility. Int. J. Urol. 16, 449–457.
28. khatoon, F. and Badawy, A. (2013). Association between BMI and prolactin levels in male patients. Sci J Med Clin Trial 2013:1-6.
29. Khullar, K., Agarwal1, A. and du Plessis, S. S. (2012). A hormonal, physical, and proteomic view of obesity-induced effects on male infertility and possible lifestyle modifications. Pac J Reprod 2012;1(2):161-8.
30. Kind, P. R. N. and King, E. J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Path 1954;7:322-6.
31. Knight, J. A., Anderson, S. and James, M. R. (1972). Chemical basis of the sulfophospho vanillin reaction for estimating total serum lipids. Clin. Chem., 18(3): 199-202.
32. Latchoumycandane, C., Gupta, S. K. and Mathur, P. P. (1997). Inhibitory effects of hypothyrodism on the testicular functions of postnatal rats. Biomed Lett 1997;56:171-7.
33. Lee, S. C. and L. Prosky (1995). International surrevy on dietary fiber definition, analysis and materials , JAOAC., 78 :22-36 .
34. Liu, G. L., Yu, F., Dai, D. Z., Zhang, G. L., Zhang, C. and Dai, Y. (2012). Endoplasmic reticulum stress mediating downregulated StAR and 3-beta- HSD and low plasma testosterone caused by hypoxia is attenuated by CPU86017-RS and nifedipine. J Biomed Sci 2012;8(19):4-9.
35. Longcope, C. (1996). Dehydroepiandrosterone metabolism. J Endocrinol;1 50:125-7.
36. Lum, M.S. and Chong, P.L. (2012). Potential antioxidant properties of pig-mented rice from sabah, Malaysia. IJANS, 1(2):29-38.
37. Mader, Z. (1983). Effect of brown rice and soybean dietary fiber on the control, glucose and lipid metabolism in diabetic rats. Am. M. J. Clin. Nutr., 388-393.
38. McGee, H. (2004). On food and cooking. Revised ed. New York, Scribner. 884 p.

39. Meng, F., Wei, Y. and Yang, X. (2005). Iron content and bioavailability in rice. J. Trace Element Med. Boil., 18:333-338.
40. Nakamura, Y., Miyamoto, M., Murakami, A., Ohigashi, H., Osawa, T. and Uchid, K. (2003). A phase II detoxification enzyme inducer from lemongrass: identification of citral and involvement of electrophilic reaction in the enzyme induction. Biochemical and Biophysical Research Communications, 302(3): 593-600.
41. Oberlander, G., Yeung, C.H. and Cooper, T.G. (1994). Induction of reversible infertility in male rats by oral Ornidazole and its effects on sperm motility and epididymal secretions. J Reprod Fertil 1994; 100: 551-559.
42. Paranagama, P.A., Abeysekera, K.H.T., Abeywickrama, K. and Nugaliyadde, L. (2003). Fungicidal and anti-aflatoxigenic effects of the essential oil of Cymbopogon citratus (DC.) Stapf. (lemongrass) against Aspergillus flavus Link. Isolated from stored rice. Letters in Applied Microbiology, 37(1): 86-90.
43. Phillips, K.P. and Tanphaichitr, N. (2010). Mechanisms of obesity-induced male infertility. Expert Rev Endocrinol Metab 2010;5:229-251.
44. Prosky , L., Asp, N.G., Schweizer, T. F. , Devries, J. W. and Furda, I. (1988). Determination of insoluble and soluble and total dietary fiber in Food products: Inter Laboratory study. J. Assoc. off. Anal. Chem., 71 :1017 – 1023.
45. Roselli, C. E. (1998). The effect of anaboliceandrogenic steroids on aromatase activity and androgen receptor binding in the rat preoptic area. Brain Res 1998;792:271-6.
46. Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 91(4): 621-632.
47. Santen, R.J., Brodie, H., Simpson, E.R., Siiteri, P.K. and Brodie, A. (2009). History of aromatase: saga of an important biological mediator and therapeutic target. Endocr Rev 2009;30:343-75.
48. Sapin, R. and Simon, C. (2001). False hyperprolactinemia corrected by the use of heterophillic antibody-blocking agent. Clin Chem 2001;47:2184-5.
49. SAS (2004). Statistical Analysis System. SAS User's Statistics SAS Institute Inc. Editors, Cary, NC.
50. Sekhon, L. H., Gupta, S., Kim, Y. and Agarwal, A. (2010). Female infertility and antioxidants. Curr. Women's Health Rev. 6, 84–95.
51. Sexton, W. J. and Jarow, J. P. (1997). Effect of diabetes mellitus upon male reproductive function. Urology. 49: 508–513.
52. Shalaby, M.A. and Hamowieh A.R. (2010). Safety and efficacy of Zingiber officinale roots on fertility of male diabetic rats. Food Chem. Toxicol. , 48(10): 2920-2924.
53. Talalay, P. (1962). Hydroxysteroid dehydrogenase. In: Colowick, Kaplan, editors. Methods in enzymology. New York: Academic Press; 1962. p. 51-6.
54. Talukder, S., Hossain, M., Sarker, S. and Khan, M. (2011). Investigation into effect of crude mixture of abrus precatorius seed on hypothalamopituitary gonadal axis and development of antifertility in male rats. Bangladesh J Agr Res 2011; 36(1): 103-9.
55. Taylor, B. A. and Phillips, S. J. (1996). Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics 1996;34:389-98.
56. Tietz, N. W. (1976). Fundamentals of clinical chemistry. Philadelphia: W.B. Sauders Co; 1976.
57. Tietz, N. W. (1986). Text Book of Clinical Chemistry. P.796.Saunders, W. B. Co., London-Pheladelphia.
58. Tietz, N. W. (1995). Clinical guide to laboratory tests. 3rd ed. Philadelphia, Pa: WB Saunders Co; 1995. p. 186-8.
59. Triantaphyllou, K., Blekas, G. and Boskou, D. (2001). Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae. International Journal of Food Sciences and Nutrition, 52(4): 313-317.
60. Turan, M., Kordali, S., Zengin, H., Dursun, A. and Sezen, Y. (2003). Macro and micro minerals content of some wild edible leaves consumed in Eastern Anatolia. Acta Agric. Sccandinavica, Section B, Plant Soil Sci., 53:L129-137.
61. USDA (2007). USDA Database for the Flavonoid Content of Selected Foods. Release 2.1. In, Agricultural Research Service.
62. Wang, S. Y., Chang, H. N., Lin, K. T., Lo, C. P., Yang, N. S. and Shyur, L. F. (2003). Antioxidant properties and phytochemical characteristics of extracts of Lactuca indica. J. Agri. Food Chem., 51: 1506-1512.
63. Wishart, G. J. (1984). The effect of anoxia on fowl spermatozoa: Recovery of fertilizing ability, motility, and cellular concentrations of potassium and ATP. Gamete Res. 10, 83–89.
64. Yallow, R. and Bauman, W.A. (1983). Plasma insulin in health and disease. In: Diabetes Mellitus: Theory and Practice. Editors:

Ellenberg, M. and Rifkin, H., Excerpta Medica, 15: 119 -120.
65. Yamamoto, J., Yamada, K., Naemura, A., Yamashita, T. and Arai, R. (2005). Testing various herbs for antithrombotic effect. Nutrition, 21(5): 580-587.
66. Yanishlieva, N.V., Marinova, E. and Pokorný, J. (2006). Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology, 108(9): 776-793.
67. Yoshimura, M., Takahashi, H. and Nakanishi, T. (1991). Role of sodium, potassium, calcium and magnesium on blood pressure regulation and antihypertensive dietary therapy. Jap. J. Nutr., 49:53-62.
68. Yousef, G.M., Diamandis, M., Jung, K. and Eleftherios, P. (2001). Molecular cloning of a novel human acid phoshatase gene that is highly expressed in the testes. Genomics 2001;74:385-95.
69. Zheng, W. and Wang, S.Y. 2001. Antioxidant Activity and Phenolic Compounds in Selected Herbs. Journal of Agricultural and Food Chemistry 49(11): 5165- 5170.

11/16/2015