

Chapter One

1. Introduction:-

Herbal galactogogues are widely used today in all cultures to stimulate milk production in both women and animals.

There is lack of information on effectiveness of these herbs in lactation.(watt et al .,1962)

Regarding fenugreek (*Trigonella foenum-graecum*) is considered to be a good herb used as a galactogogues, it is an annual herb that belongs to family leguminosae, sub-family papilionaceae, tribe Trifolieae. (kotb,1985)

The plant is cultivated in various parts, particularly in the middle east, India, North Africa, and South Europe. It is highly valued as a food for man and to promote lactation and lactation performance in women.

Fenugreek seeds are aromatic, bitter, carminative, galactogogues, and antibacterial. Bitter is mainly due to the oil, steroidal saponins and alkaloids, fenugreek seeds have 20 – 27% protein, 7 – 10% fat and 0.21 – 0.75% saponins. (El Ridi and shahat, 1944)

Sugars in fenugreek seeds are glucose, galactose and xylose. (Eozga, 2005) In traditional medicine, fenugreek increases milk and promote milk production in women.

In addition, fenugreek seeds were used to treat loss of appetite and to address weight.



Fig (1-1): Fenugreek Seeds



Fig (1-2): Fenugreek

1.1. Taxonomy of fenugreek

Fenugreek (*Trigonella foenum-graecum*) is an annual, self-pollinating legume. Most plants are diploid ($2n=16$) and often used as crops in agriculture (Petropoulos, 2000). Sinskaya (1961), Hutchinson (1964) and Heywood (1967), all have described plants belonging to the genus *Trigonella*. In general, the plants are scented and can be collectively categorized to their distinct botanical characteristics (Table1-1).

Taxonomy of the plant is as follows:

Kingdom	Plantae
Class	Magnolipsida
Order	Fabales
Family	Fabaceae
Genus	<i>Trigonella</i>
Species	<i>Foenum-graecum</i>

1.2. Botanical and physiological aspects of fenugreek

Germinated seeds from fenugreek form a seedling, which eventually develops into stems, flowers, pods and seeds (Petropoulos, 2002). Following swelling of the seed, the radical emerges from the seed coat, penetrates the soil and initiates primary root development. Release of cotyledons from seed husks soon follows and leads to growth of the first simple leaf, followed by development of the first trifoliate leaf (Petropoulos, 2002). Throughout the plant, the leaves are found in an alternate arrangement, are ovate in appearance and slightly toothed (Slinkard et al., 2006). Stems of the mature fenugreek plant are circular to slightly quadrangular, with a diameter of 0.5 -

1.0 cm. They are erect, hollow and may appear green or pinkish-green due to accumulation of anthocyanin (Petropoulos and Koulombis, 2002).

Flowering of fenugreek starts approximately 35-40 days from the date of sowing, and varies according to plant variety, climate and season in which the seeds were sowed (Petropoulos, 2002). The flowers sit in the leaf axils, are generally paired, but occasionally are solitary. There are two types of flower shoots; one that bears axillary flowers and follows an indeterminate growth habit and the other, referred to as 'blind shoots' that can carry both axillary and terminal flowers which eventually become tip bearers. There are two kinds of flowers; cleistogamous (closed) flowers and aneictogamous (open) flowers. Closed flowers are found on most plants. The keel of these plants remains closed throughout the flower's life, favoring self-pollination. Open flowers, in contrast, offer an abundance of opportunities for cross-pollination, as the corolla remains open continuously. However, these represent less than 1 % of fenugreek flowers (Petropoulos, 2002).

Seed pods from fenugreek plants are long, slender, sickle-shaped and pointed (with a sharp beak at the end). They appear brownish or yellowish brown and can be 10-19 cm in length and 0.2-0.6 cm in width (Ivimey-Cook, 1968 and Duke, 1986 cited in Petropoulos, 2002). Fenugreek plants can be single- (one pod per node) or double-odd (two pods per node projecting in opposite directions). Double-podded varieties appear to contain higher levels of bioactive compounds i.e. diosgenin (Petropoulos, 2002). Each seed-bearing branch of the plant can produce about 2-8 pods, each carrying roughly 10- 20 seeds (Petropoulos and Koulombis, 2002). Fenugreek seeds are surrounded by a seed coat (testa), which is separated from the embryo by the endosperm, the principal storage organ in the seed

(Spyropoulos, 2002). Between the seed coat and the endosperm, lies a single-cell layer of living tissue known as the aleurone layer. Galactomannan, a long chain polysaccharide makes up a large portion of the stored reserves in the seed, and is deposited as a cell wall thickening on the surface of cells in the endosperm. Deposition of galactomannan also occurs at the outer walls of the aleurone layer neighboring the seed coat (Spyropoulos, 2002). Seeds are generally about 3-6 mm long, 2-4 mm wide and 2 mm thick (Fazli and Hardman, 1968). The seeds have a rectangular or rhomboidal shape with grooves between the radicle and the cotyledon. They are generally yellowish-brown to golden yellow, but new cultivars that lack polyphenolic tannins, appear pale yellowish-white. The weight of a thousand seeds, a common seed quality determinant averages around 15 – 20 g (Slinkard et al., 2006).

Table (1-1) Characteristics of plants of the genus *Trigonella*.

Plant organs/tissues	Characteristics
Leaves	Trifoliate, usually toothed, nerves running out into teeth
Flowers	Solitary, pedunculate in axillary heads
Calyx	Teeth may be equal or unequal
Corolla	Yellow, blue or purple
Anthers	Uniform
Stigma	Terminal
Ovary	Sessile, ovules are numerous
Pods	Cylindrical or compressed, linear or oblong, indehiscent or dehiscing with a pronounced peak
Seeds	Tuberculate, cotyledons geniculate

Petropoulos (2002)

1.3. Historical uses of fenugreek

Fenugreek is one of the oldest known medicinal plants that have been documented in ancient herbal publications, religious scriptures, travel records and anecdotes dating back in human history (Lust, 1986). Seeds of the fenugreek plant were found in the tomb of the Egyptian Pharaoh, Tuthankhamun (1333 BC – 1324 BC) and leaves of the fenugreek plant were used as one of the components of holy smoke that the Egyptians used in fumigation and embalming rites (Fazli and Hardman, 1968). During the ancient Greek period, fenugreek was cultivated as a forage crop. In ancient Rome, it was used as an aid to induce labor during childbirth and delivery (Yoshikawa et al., 1997). Utilization of fenugreek in Chinese medicine was first introduced during the Song Dynasty (AD 1057). It was used in traditional Chinese medicine as a tonic and treatment for weakness and edema (tissue swelling due to excess lymph fluid) of the legs (Basch et al., 2003). Fenugreek was later introduced to Central Europe at the beginning of the 9th century but it was not until the 16th century when cultivation of the plant in England was recorded (Petropoulos, 2002).

1.4. Modern uses of fenugreek

One of the major uses of fenugreek seeds and leaves is for medicinal purposes. In India (Basch et al., 2003) it has been used as part of traditional medicine practices. Fenugreek contains a myriad of phytochemicals such as steroids, flavonoids and alkaloids, which have been identified, isolated and extracted by the pharmaceutical industry to serve as raw materials for the manufacture of hormonal and therapeutic drugs (Petropoulos, 2002). Polysaccharides form the mucilage (galactomannan) present in the plant, and

are finding wider applications in the food, pharmaceutical, cosmetics, paint and paper industries (Petropoulos, 2002) following the more commonly used locust bean and guar gums, all of which possess high viscosity and neutral ionic properties (Duke, 1986 cited in Petropoulos, 2002). However, fenugreek seed is most commonly used in everyday life as a spice and a seasoning in soups and curries (Duke, 1986 cited in Petropoulos, 2002). In India, fenugreek is consumed as a condiment, and is used as a coloring dye and as a medicinal stimulant to promote lactation in post-partum women and animals (Fazli and Hardman, 1968 cited in Petropoulos, 2002; Basch et al., 2003). Another alkaloid, trigonelline that has been extracted from fenugreek contributes to its distinctive odor. Trigonelline can be used in the manufacture of imitation maple syrup and artificial flavoring for licorice, vanilla, rum and butterscotch (Slinkard et al., 2006).

1.5. Fenugreek oils

Extractable oil from fenugreek represents about 6-8% of the seed weight and carries a fetid odor and bitter taste. Its fatty acid composition is listed in Table 1-2 (Sulieman et al., 2008). It is reported that the unsaponifiable portion of the oil (3.9 %) contains a lactation-stimulation factor (Srinivasan, 2006). Being strongly scented, the oil is used as an insect repellent for grains and cloths (Duke 1986). In cosmetics, traces of the oil are used in perfumes (Fazli and Hardman, 1968).

1.6. Fenugreek as a forage crop

The high forage value of fenugreek is attributed to its rich content of protein, vitamins, and amino acids along with its good digestibility in cattle. The seeds contain diosgenin, a growth and reproduction hormone. The

combination of the above factors in fenugreek is thought to improve growth rates and feed utilization efficiency in beef cattle [Alberta Agriculture and Rural Development, 2007]. In a study by Shah and Mir (2004), 20 % (w/w) fenugreek seed was supplemented into a dairy cattle diet and was reported to significantly improve the fatty acid profile in the milk produced an increase in the polyunsaturated fatty acid (i.e. linoleic, linolenic and conjugated linolenic acids) concentrations was observed. The study also found that the fenugreek-fed cattle had a 4 % reduction in blood cholesterol concentration as well as a 19 % decrease in milk cholesterol levels compared to controls, potentially extending health benefits to human consumers of the milk.

Table (1-2) Fatty acid composition of oil extracted from fenugreek seeds.

Fatty acid	Content (%)
Palmitic acid	11.0
Stearic acid	4.5
Arachidic acid	1.5
Oleic acid	16.7
Linoleic acid	43.2
α -Linolenic acid	22.0

Sulieman et al. (2008)

1.7. Fenugreek as a functional food

Presence of galactomannan in fenugreek seed accounts for approximately half of its dry weight and is recognized as the principal source of soluble dietary fiber in the plant (AAFC, 2005). Dietary fiber is known to have the potential to reduce risk of cardiovascular disease and to protect against some cancers through the reduction of lowdensity lipoprotein (LDL) and total cholesterol (AAFC, 2005). In Egypt, supplementation of wheat flour with a small percentage of fenugreek flour has been reported to enhance the nutritional quality of bread as well as its organoleptic characteristics (Bakr, 1997). Addition of fenugreek flour to more commonly used flours for bread making is a common practice in Egypt (Galal, 2001). Sharma and Chauhan (2000) reported improved physicochemical, nutritional and rheological properties of bread made from wheat flour supplemented with fenugreek flour. Galactomannan (mucilage or gum) in fenugreek acts as a thickener or stabilizer in foods such as soups, sauces and ice-cream (Garti et al., 1997, Balyan et al., 2001, Seghal et al., 2002). Currently, the food industry utilizes locust bean gum and guar gum as emulsifiers, viscosity-builders, thickeners and stabilizers. The relatively low cost and ease of growing fenugreek in abundance in Canada makes fenugreek gum a potential candidate for commercial use.

1.8. Fenugreek in Sudan:

Fenugreek is a common spice crop of forages with yields ranging between 150 and 275 kg/ha and seed yields between 500 and 3320 kg/ha.

In Sudan Fenugreek (Helba) is grown under irrigation in Khartoum , the Nile and Northern State.

Fenugreek (*Trigonella foenum*) are grown between the third week of the month October. to mid November.

Acre gives about 700-800/kg dry seeds may increase or decrease depending on the type of soil.

1.9. Chemical constituents in fenugreek:

Fenugreek seed contains approximately 4-10 % moisture, 6-8 % fat, 18-30 % protein and 4-5 % fiber (Sauvaire et al., 1976; Sharma et al., 1986b; Vats et al., 2003; Srinivasan, 2006), depending on varietal and ecological factors. Hemavathy and Prabhakar (1989) published a detailed report on the lipid composition of fenugreek seeds. In their report, total lipids extracted from dry seeds were 7.5 %, which agrees with other values given in the literature (Srinivasan, 2006). Fractionation of the lipids extracted indicates that the seed contains 84.1 % neutral lipids (composed mainly of triacylglycerols), 5.4 % glycolipids and 10.5 % phospholipids. A breakdown of the fatty acid composition shows that linoleic acid is the predominant fatty acid, followed by α -linolenic acid and oleic acid.

Some of the biological and pharmacological attributes of fenugreek are ascribed to the abundance of chemical constituents it contains; i.e., its steroids (saponins/sapogenins), alkaloids, polyphenolic compounds, volatile compounds and amino acids, in particular 4-hydroxyisoleucine (Skaltsa, 2002; Srinivasan, 2006).

The bitter flavor of fenugreek is most likely due to the presence of saponins (4 -8 %) and alkaloids (~ 1 %). Consumption of these constituents appears to contribute to gastric stimulation, increased acidity and improved appetite (Srinivasan, 2006).

1.9.1 Steroids

Fenugreek contains various steroidal saponins with diosgenin being the major component. Saponins are the aglycone portions of the plant-based steroid-derivative saponins, containing 6-C rings with 2 to 3 side chains substitutable either with methyl or hydroxyl groups (Skaltsa, 2002). Saponins are amphipatic glycosides (Fig. 2.1), containing both hydrophilic glycoside and lipophilic triterpene moieties, therefore capable of producing soap-like foaming properties.

Diosgenin, a 27-C steroidal compound (Fig. 2.) is currently used as a raw material for the manufacture of oral contraceptives and sex hormones by the pharmaceutical industry (Skaltsa, 2002). It is traditionally extracted from wild Mexican and Asian yam species, *Dioscorea*. Utilization of fenugreek seeds as an alternative source of diosgenin was proposed in the 1950s in recognition of the increasing demand for raw steroids (Marker et al., 1947 and Fazli and Hardman, 1968; Bhatnagar et al., 1975). It is important to note that fenugreek seeds contain no free saponins (Sauvaire and Baccou, 1978), and that they occur as complex glycosides (saponins) which are released following enzymatic treatment or acid hydrolysis (Blunden and Hardman, 1963 cited in Skaltsa, 2002). Fenugreek saponins also occur in various forms due to stereochemistry and functional groups substitution. Those that have been reported in extracts from fenugreek seeds are diosgenin, yamogenin, tigogenin, neotigogenin, yuccagenin, lilagenin, gitogenin, neogitogenin, sarsapogenin and smilagenin (Gupta et al., 1986b; Cornish et al., 1983; Skaltsa, 2002). Yamogenin is the (25S)-epimer of diosgenin (Fig. 2) and occurs at a ratio of 2:3 with diosgenin in the seed, acid-hydrolyzed extract (Skaltsa, 2002).

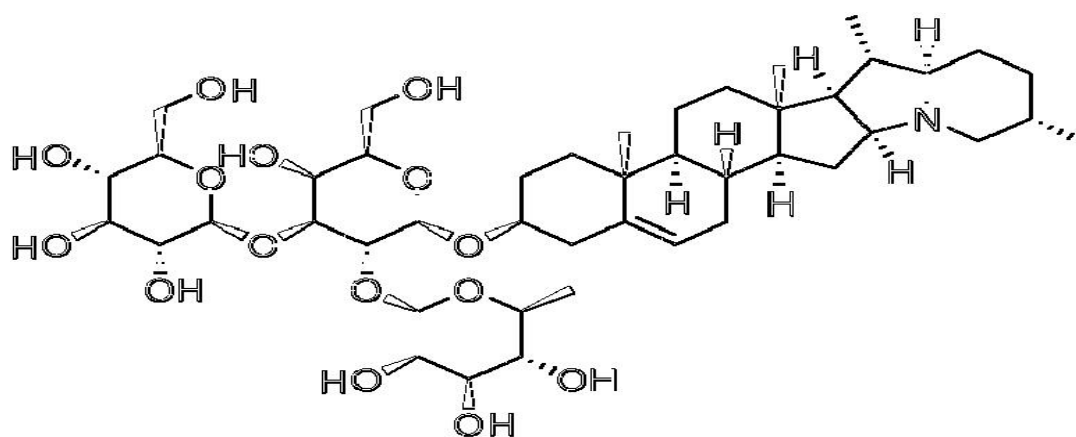


Figure (1-3): Chemical structure of solanine, exemplifying the amphipathic properties of a typical steroidal saponin.

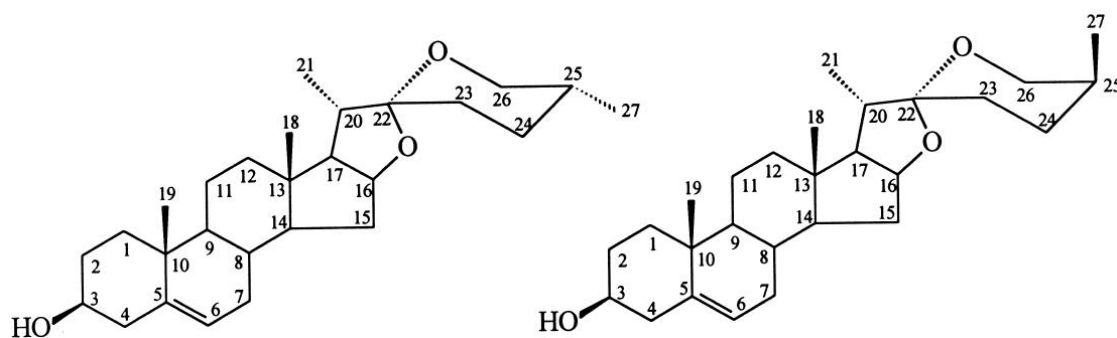


Figure (1-4): Chemical structures of diosgenin (A) and yamogenin (B) showing epimerism at C-25. Pires et al. (2002).

1.9.2 Polyphenolic compounds

Polyphenols possess anti-oxidative attributes, which may prevent some forms of chronic disease. Gupta and Nair (1999) reported that fenugreek is generally rich in polyphenols (100 mg g⁻¹). The phenylpropanoid pathway occurs ubiquitously across the plant kingdom and has been shown to be involved in the biosynthesis of important secondary plant metabolites such as flavonoids and lignins (Dixon and Sumner, 2003).

Flavonoids consist of several classes; i.e., the flavones, flavonones, flavonols, flavanols (flavan-3-ols), isoflavones, proanthocyanidins and anthocyanins. They exhibit strong antioxidative effects in vitro and have been largely associated with prevention of oxidative damage in biological systems, which can confer protection against cardiovascular disease and cancer (Erdman et al., 2007). Flavonoids have a basic three ring chemical structure; i.e., two aromatic rings (A and B) coupled with a three-carbon oxygenated heterocyclic ring (C) (Fig. 2.3). Among the many flavonoids reported in fenugreek, quercetin, luteolin, vitexin and kaempferol appear to be the most common (Parmar et al., 1982; Jain et al., 1992; Huang and Liang, 2000; Skaltsa, 2002). Quercetin and kaempferol are flavonols (Fig. 2.4); luteolin is a flavone while vitexin occurs as a glycosylated flavone.

Isoflavanoid phytoalexins are also reported to occur in fenugreek in the form of the pterocarpan, medicarpin (Fig. 2.7) and maackiaian (Ingham, 1981). These compounds play a key role in maintaining plant health in the event of microbial invasion; they are absent in healthy plants and their production is only induced upon microbial attack (Skaltsa, 2002).

Polyphenolics are also potent antioxidants that scavenge for free radicals and protect against oxidation. Common phenolic compounds isolated from fenugreek are scopoletin, coumarin, chlorogenic and caffeic and p-coumaric acids (Figs. 8 and 9) (Skaltsa, 2002; Acharya et al., 2007b). Aqueous extracts of germinated fenugreek seeds containing 17.5 mg mL⁻¹ quercetin and 64.4 mg mL⁻¹ gallic acid equivalents per gram of fenugreek powder and was shown to exhibit significant antioxidant activity and offered greater protection against oxidation compared to other extracts (Dixit et al., 2005).

The protective action of fenugreek seed polyphenols has been investigated in rats on lipid peroxidation using aqueous extracts and was found to exert gastroprotective effect on gastric ulcer (Pandian et al., 2002) and prevent ethanol-induced toxicity in the liver and the brain (Thirunavukkarasu et al., 2003). Recently, Kaviarasan et al. (2006) reported on the cytoprotective effect of a polyphenolic extract of fenugreek seeds against ethanol-induced damage in human Chang liver cells. The protective effects were found comparable to a silymarin, a known hepatoprotective agent.

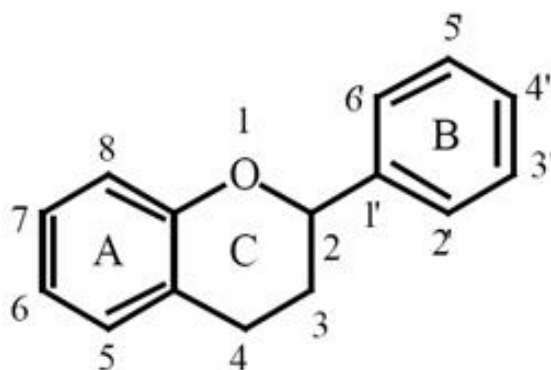


Figure (1-5): Basic chemical structure of a flavonoid.

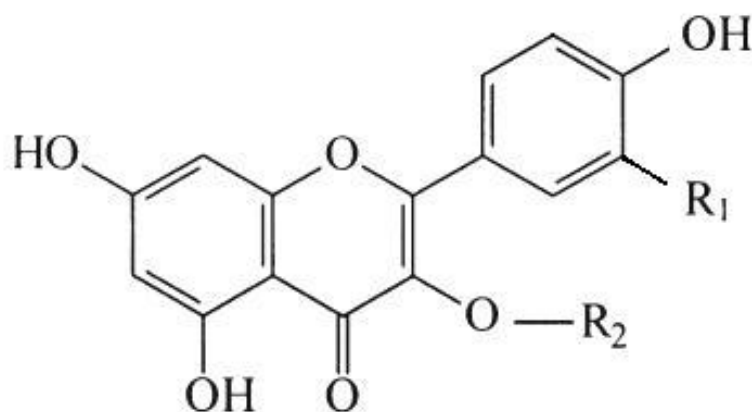


Figure (1-6): Chemical structure of a flavonol; quercetin when R₁ = OH, R₂ = H; kaempferol when both R₁ and R₂ = H. S Dubber and Kanfer (2004).

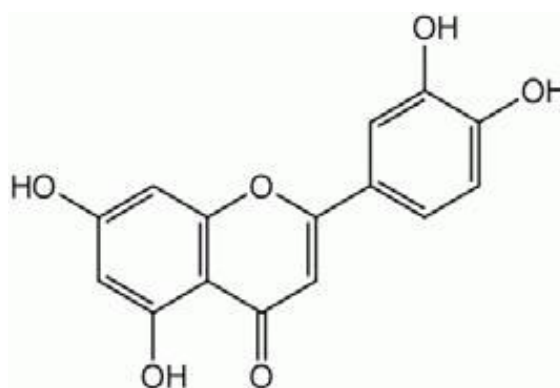


Figure (1-7): Chemical structure of luteolin, a flavone.

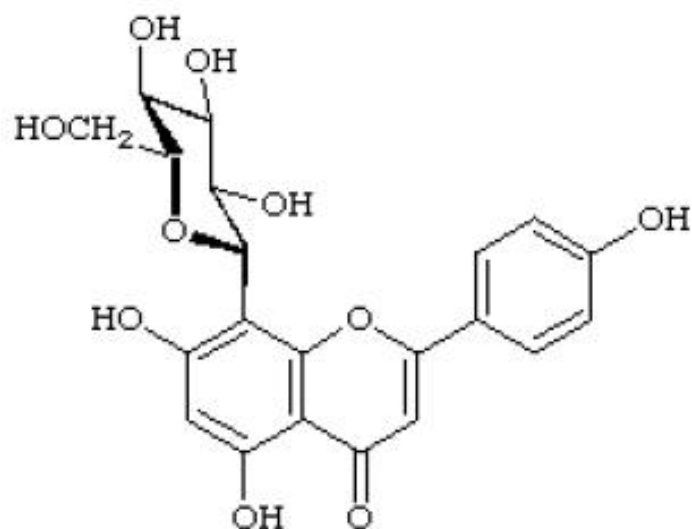


Figure (1-8): Chemical structure of vitexin (8-C- β -D-glucosyl 5, 7, 4'-trihydroxyflavone). SchemBlink (2008).

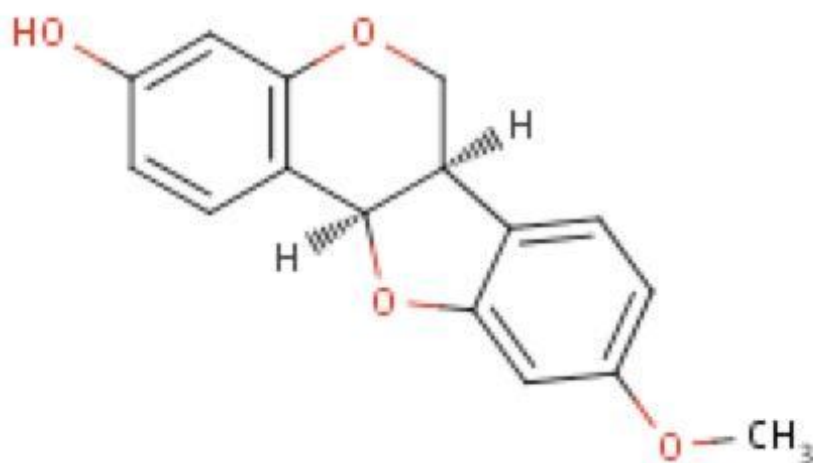
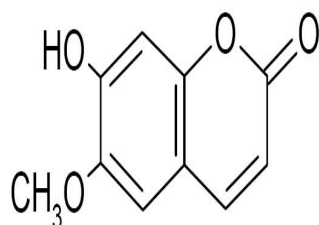


Figure (1-9): Chemical structure of the pterocarpan, medicarpin. Source: European Bioinformatics Institute (2008).

(A)



(B)

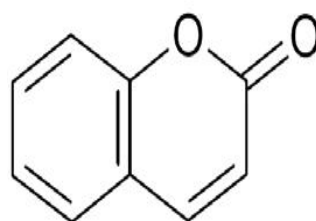
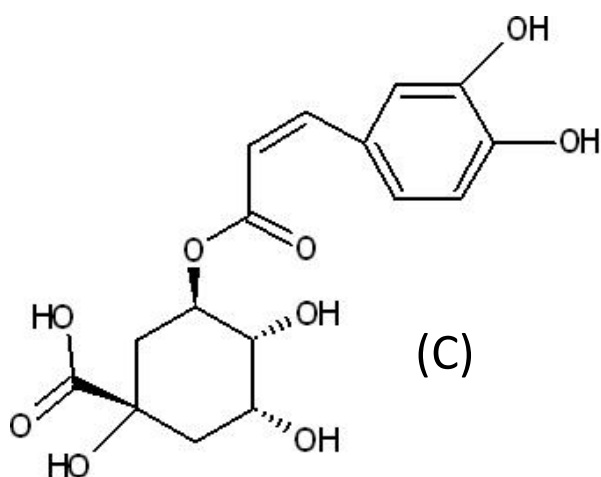
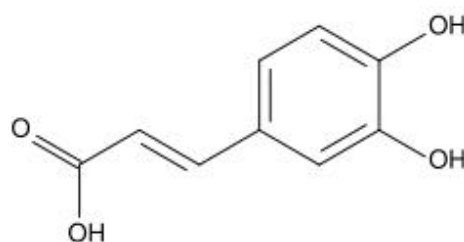


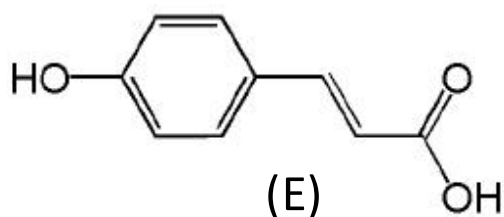
Figure (1-10): Chemical structures of (A) scopoletin and (B) coumarin.



(C)



(D)



(E)

Figure (1-11): Chemical structures of (C) chlorogenic acid, (D) caffeic acid and (E) *p*-coumaric acid.

1.9.3. Alkaloids

Trigonelline (Fig. 10), a methylbetaine derivative of nicotinic acid (Skaltsa, 2002) is one of the major alkaloids found in fenugreek seeds. This compound (chemical formula $C_7H_7NO_2$) has been reported to exert mild hypoglycemic (Shani et al., 1974; Marles and Farnworth, 1994) and anti-pellagra [pellagra is a disease caused by lack of dietary niacin (vitamin B3) and the amino acid tryptophan] effects (Bever and Zahnd, 1979).

1.9.4 Volatile components

Volatile constituents contribute to the aroma and flavor of fenugreek. Anethol (Fig. 11), an aromatic compound found mostly in anise, camphor and fennel, also occurs in fenugreek and produces a licorice-like aroma (Aggarwal and Shishodia, 2006; Acharya et al., 2007b). Mazza et al. (2002) have identified 175 volatile constituents in Sicilian fenugreek seeds, which include carbonyls, sesquiterpene hydrocarbons, alcohols, heterocyclic, and furan compounds. Sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone (Fig. 2.12) has been identified as the principal component contributing to the flavor of fenugreek (Hatanaka, 1992; Blank et al., 1997). These compounds together impart the burnt sugar, curry or maple syrup flavour, which is characteristic of fenugreek (Monastiri et al., 1997).

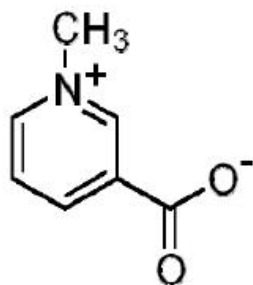


Figure (1-12): Chemical structure of trigonelline.

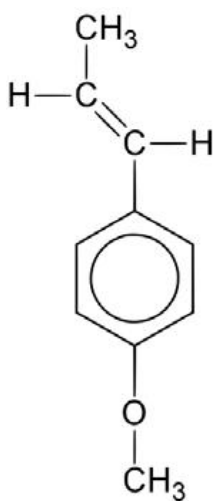


Figure (1-13): Chemical structure of anethol.

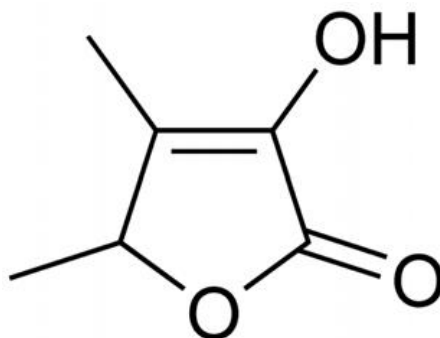


Figure: (1-14) Chemical structure of sotolone, the principal flavour constituent in fenugreek.

1.10. Bioactive Compounds and their Biochemistry

Various clinical (Bhardwaj et al., 1994; Sharma et al., 1996a; Vajifder et al., 2000; Sowmya and Rajyalakshmi, 1999; Abdel-Barry et al., 2000; Gupta et al., 2001) and animal studies (Sauvaire et al., 1991; Evans et al., 1992; Thirunavukkarasu et al., 2003; Anuradha and Ravikumar, 2001; Puri et al., 2002; McAnuff et al., 2002) conducted using fenugreek have identified numerous potential health benefits for consumption of fenugreek, and have drawn much attention to fenugreek as a potential functional food and natural health product or ingredient therein. Among the plethora of bioactive compounds found in fenugreek, the three major chemical constituents, galactomannan, diosgenin and 4-hydroxyisoleucine have, by far superseded the rest as being the most frequently studied health-promoting factors for humans.

1.10.1 Galactomannan

Galactomannan (Fig. 13) represents the major polysaccharide found in fenugreek seeds and accounts for approximately 17 – 50 % of the dry seed weight (Petropoulos, 1973 and Duke, 1986 cited in Petropoulos, 2002; Kochhar et al., 2006). It is an integral component of the cell wall which is found concentrated around the seed coat (Spyropoulos, 2002). Galactomannans are structurally composed of a 1 → 4 beta-D-mannosyl backbone substituted by a single galactose unit α -linked at the C-6 oxygen (Bhaumick, 2006). Fenugreek galactomannans are unique relative to other commonly used galactomannans such as those found in guar and locust beans. They contain a galactose to mannose ratio of 1:1. This high degree of galactose substitution renders the molecule relatively more soluble

compared to galactomannans from guar or locust bean, which has a galactose to mannose ratio of 1:2 and 1:4, respectively (Reid and Meier, 1970; Brummer et al., 2003).

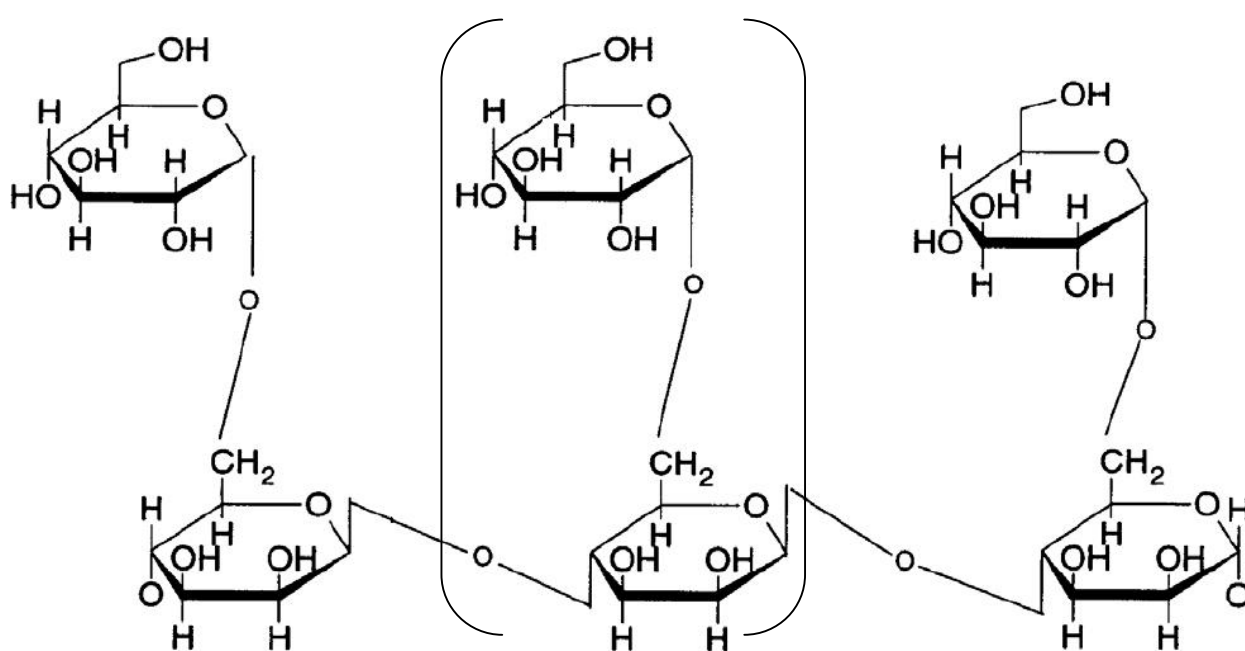


Figure (1-15): Chemical structure of fenugreek galactomannan (adapted from Bhaumick, 2006)

1.11. Fenugreek and Breast feeding

The literature of fenugreek use during lactation contains only a few anecdotal reports of fenugreek use during lactation. A nonblinded, anecdotal study of 1,200 women breastfeeding term infants found that nearly all of those who took fenugreek reported an increase in milk production within 24–72 hours after beginning the herb along with using an electric breast pump (K. Huggins, personal communication, July 1, 2013). These mothers noted that fenugreek could be discontinued once milk production was stimulated to an appropriate level. No negative side effects in these mothers or infants were reported. Huggins has found fenugreek to be a potent stimulator of breast milk production that appears safe for the mother and baby. However, this study was not reported in the literature. And there have been no studies with mothers of preterm infants, a population that is often concerned about producing enough milk (Hillervik-Lindquest, Hofvander, & Sjölin, 1991).

In summary, the benefits of breastfeeding are well established, specially for preterm infants. To date, there are no current published studies examining fenugreek's effect on breast milk volumes or PRL levels in mothers of preterm infants. Therefore, the purpose of this study was to determine if fenugreek increased breast milk volumes and PRL levels in mothers of preterm infants.

There are many purported natural galactagogues such as caffeine, hops, fenugreek, fennel seed, blessed thistle and alfalfa which have traditionally been used to increase breastmilk production, but none of these have reliable clinical evidence that they work for this purpose. At present, there is mainly anecdotal evidence that they are effective. A study found the

high use of herbal galactagogues by women previously having breastfed (43.3%) was surprising. This use may be related to the fact that 20–40% of breast-feeding women have perceived lactation deficiency.

None of these herbs have been studied to determine how well they stimulate lactation; nor has their safety in lactation been shown the attention it deserves, although there is at least some preliminary evidence supporting the utility of these herbs. Nonetheless, as shown in the box, Botanical Galactagogue Formulas, these herbs are common ingredients in herbal formulas used by many lactating women.

Fenugreek is especially popular for this use. Although many swear by it for increasing milk production, there is no reliable research demonstrating this effect. The mechanism of action is unknown, but there have been suggestions that fenugreek may affect milk production because the breast is a modified sweat gland, and the herb is known to stimulate sweat production.

1.12. Prolactin (PRL):

1.12.1. The pituitary and PRL:-

The pituitary gland is located at the base of the brain and is a hormone-secreting gland, compartmentalized into an anterior pituitary and a posterior pituitary, its endocrine function is regarded as critical for physiological homeostasis. The anterior pituitary secretes several essential hormones, of which PRL will mainly be considered here. PRL belongs to the family of polypeptide hormones, with a tertiary structure. (Forsyth and Wallis, 2002)

Prolactin (PRL) is a peptide hormone primarily synthesized and secreted by adenohypophysis. PRL is secreted into blood stream in response to the suckling stimulus on the maternal nipple. Most of the existing galactagogues act by increasing the production and release of PRL by the anterior pituitary gland.

The mechanisms of action of these drugs range from the direct stimulation of the adenohypophysis to the suppression of the hypothalamic secretion of PRL inhibitory factor (PIF) and stimulation of the hypothalamus to secrete PRL releasing hormone. In addition, as dopamine is a physiologic inhibitor of PRL release, some galactagogues act either by blocking hypothalamic dopaminergic receptors (e.g metoclopramide and domperidone) or by inhibiting dopamine-producing neurons (American academy, 2005).

The mechanism of breast milk production presented above provides only a simplified framework to understand the mechanisms of action of the many different galactagogues. Some authors suggest more complex relationship between blood PRL concentrations and milk synthesis.

PRL is also secreted by extra pituitary tissues where it has a variety of functions in mainly an autocrine or paracrine fashion. (Forsyth and Wallis,2002;Soares,2004)

1. 12.2. History:

PRL is a multifunctional hormone of 199 amino acids. That was first described 80 years ago as a pituitary derived hormone that induced milk production in rabbit mammary glands (Stricker and Greuter,1928;Bole et

al.,1998) and crop milk (feed for newly hatched birds) production in pigeons (13).

The pituitary factor inducing these effects was termed prolactin (Riddle et al.,1933).

Bole-feysot et al have summarized the broad biological functions of PRL in six categories:

1. Water and electrolyte balance,
2. Growth and development,
3. Endocrinology and metabolism,
4. Brain and behavior,
5. Reproduction,
6. Lactation.

The lactogenic effect of pituitary PRL is well established, in which PRL regulates food intake (Woodside,2007) and nutrient supply in the mammary glands (Neville et al.,1994). To shunt nutrients towards the mammary gland, nutrient utilization in non-mammary tissues is suppressed (Houssay et al.,1955).

1.12.3. Lactation and PRL:

During pregnancy the PRL level gradually increases to its peak concentration before parturition, reaching at least 100 µg/l. (Harrey and Hull,1997). The development of pituitary lactotrophs and induction of PRL.

Secretions are thought to be mainly regulated by placental-derived estradiol (Enjalbert et al.,1986).

Mammary differentiation is stimulated by PRL in concert with other peptide and steroid hormones (Samson et al.,2003), but milk production is constructed by progesterone from the placenta throughout pregnancy (Hauffa,2001). At parturition, placental hormones vanish, which allows the inhibition of milk synthesis, and sucking stimulus is the essential stimulus for sustained elevation of PRL (Fodor et al.,2006). It has been established that PRL is a prerequisite for milk production through findings that individuals with a rare condition of PRL deficiency are unable to lactate (Jansson et al.,1982).

In addition during breast-feeding the regulatory function of PRL is to ensure the provision of nutrients to be converted into the balanced constituents of milk to the mammary glands.

Peripheral mechanisms controlled by PRL direct them to the mammary glands instead of allowing them to be stored peripherally (Enjalbert et al.,1986). Regul

1.13. Techniques Used

1.13.1.ELISA

Enzyme – linked immunosorbent assay, called ELISA or, enzyme immunoassay, EIA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or antigen in a sample (Volter et al. 1980) .

The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries, in simple terms, in ELISA an unknown amount of antigen is affixed to the surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme and in the final step a substance is added so as the enzyme can convert to some detectable signal. Thus in this case of fluorescence ELISA, when light of the appropriate wavelength is shone upon the sample, any antigen/ antibody complexes will fluoresce so that the amount of antigen in the sample can be inferred through the magnitude of the fluorescence (Wilson and Walker, 1995).

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene micro titer plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bi-conjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates enabling much higher sensitivity (Switzer and Garritty L, 1999).

1.13.1.1. Applications:

Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in sample, it is a useful tool for determining serum antibody concentrations. It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs (Murray and Granner, 2000).

1.13.1.2. History :

Before the development of the EIA/ELISA, the only option for conducting an immunoassay was radioimmunoassay, the radioactivity provides the signal which indicates whether, a specific antigen or antibody is present in the sample (Chopra, et al. 1971).

Because radioactivity poses a potential health threat, a safer alternative was sought. A suitable alternative to radioimmunoassay would substitute a non-radioactive signal place of the radioactive signal when enzymes (such as peroxidases) react with appropriate substrates (such as tetramethylbenzidine). This causes a change in color, which is used as a signal. However, the signal has to be associated with the presence of antibody or antigen, which is why the enzyme has to be linked to an appropriate antibody. This linking process was independently developed by Stratis Avrameas and G.B. Pierce. Since it is necessary to remove any unbound antibody or antigen by washing the antibody or antigen has to be fixed to the surface of the container, i.e. the immunosorbent has to be

prepared. A technique to accomplish this was published by Wide and Jerker Porath in 1966 (Guarner et al., 1997).

1.13.1.3. Types of ELISA :

The types of ELISA are, Competitive ELISA in which a mixture of a known amount of enzyme – labeled antigen and an unknown amount of unlabeled antigen is allowed to react with a specific antibody attached to a solid phase. After the complex has been washed with a buffer, the enzyme substrate is added and the enzyme activity measured. The difference between this value and that of a sample lacking unlabeled antigen is a measure of the concentration of unlabeled antigen (Water and Boron, 2003) sandwich ELISA, measures the amount of antigens between two given antibody layers. The antigens contain at least two antigenic spots since at least two antibodies act in the sandwich. In performing this assay, the first antibody is purified and bound to a solid phase attached to the bottom of the plate. Then an antigen is added and allowed to combine with the bound antibody while the unbound products are removed.

Finally, the second antibody is bound to the antigen completing the sandwich ELISA. Is a method of directly labeling the antibody itself. The micro well plates which are coated with a sample containing the antigen and the labeled antibody are quantitated using a calorimetric or florescent end-point indirect ELISA is a two – step method that uses a primary and secondary labeled. The primary antibody is incubated with the antigen followed by incubation of the secondary antibody (Wilson and Walker, 1995).

Objectives:**This study was performed to :**

- 1- Determine the chemical composition of *trigornella foenum-graecum* .
- 2- Investigate the effect of oral administration of fenugreek seeds on prolactin level in Sudanese female
- 3- Determine its effect on milk production and body weight in Sudanese Females.

Chapter Two

2. Materials and Methods:-

2.1. Materials

- Fenugreek seed Powder.
- Concentrated sulphuric acid SP.Gr. 1.84 (96%)
- Mixed Indicator.
- Sodium Hydroxide, 40%
- Boric acid Solution, 2%.
- Sulphuric acid solution 0.1N.
- Oxalic acid , 0.1N.
- Phenolphthalein indicator , 0.1%
- Sodium Hydroxide , 0.1N Solution.
- Petroleum ether.
- Chloroform solution.
- Potassium Iodide.
- Sodium thiosulphate solution 0.1N.
- Potassium dichromate.
- Starch Indicator solution.

2.2. Methods

2.2.1. Determination of moisture content

Accurately 1 g of fenugreek sample was weighed in weighed dish and heated at 105 °C in an oven for 6 hours to a constant weight. Moisture content was then calculated as a percentage of the initial weight from the following equation:

$$\text{Moisture \%} = (w_2/w_1) \times 100$$

Where w_2 = weight of samples after heating.

w_1 = weight of samples before heating.

2.2.2. Determination of ash content:-

The total ash of test samples was determined to FAO paper No, (44),1990. A crucible was heated at 550 °C, cooled in a desiccators and weighted (w_1), accurately one gram of sample was weighted in the crucible (w_2) and ignited in muffle furnace at 550 °C for 6 hours, and cooled in desiccator and weighted (w_3) the total ash content was calculated from the equation: (AOAC,1990)

$$\text{Ash \%} = \frac{\text{weight of ash in grams}}{\text{weight of sample in grams}} \times 100$$

2.2.3. Determination of peroxide value:

5g of the sample was weighted and into a 250 ml Erlenmeyer flask with glass stopper and add 30 ml of the 3:2 acetic acid chloroform solution. Add 0.5 ml of saturated KI solution. Allow the solution to stand with occasional shaking for 1 minute, and then immediately add 30 ml of distilled

water. Titrate with 0.1N sodium thiosulfate, adding it gradually and with constant agitation, continue the titration until the yellow iodine color has almost disappeared, add about 2.0 ml of starch indicator solution. Continue the titration with constant agitation, especially near the end point, to liberate all of the iodine from the solvent layer. Add the thiosulfate solution drop wise until the blue colour just disappears.

$$\text{Peroxide value} = (S.b) \times N \times 100$$

2.2.4. Determination of fiber content:

Extracted 2 g of ground material with petroleum ether to remove fat. If fat content is below 1% extraction maybe omitted. After extraction with petroleum ether boil 2 g of dried material with 200 ml of sulfuric acid for 30 minutes with bumping chips. Filter and wash with boiling water until washing is no longer acidic. Boil with 200 ml of sodium hydroxide for 30 minutes. Filter and wash again with sulfuric acid, three 50 ml portion of water and lastly 25 ml alcohol. Remove the residue and transfer to ashing dish (preweighed dish w_1). Dry for two hours at $130 \pm$. Cool the dish in desiccator and weigh (w_2). Ignite for 30 minutes at 600°C , cool in a desiccator and reweigh (w_3).

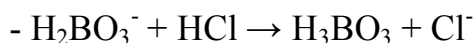
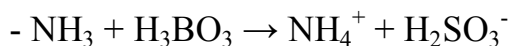
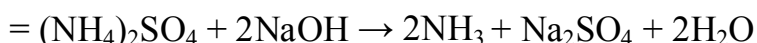
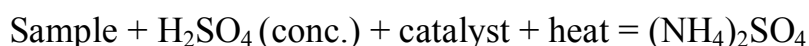
$$\text{Fiber content} = (w_2 - w_1) - (w_3 - w_1) / \text{weight of the sample } \%$$

2.2.5. Determination of total Nitrogen

The Kjeldahl method was developed over 100 years ago for determining the nitrogen content in organic and inorganic substances.

Although the technique and applications have been modified over the years, the basic principle introduced by Johan Kjeldahl is still the same (AOAC, 1995).

Kjeldahl method was used to determine the total nitrogen in trigonella foenum-graecum sample, the nitrogenous compound was digested with concentrated H_2SO_4 in the presence of the selenium and copper sulphate – Potassium sulphate catalyst to yield ammonia sulphate. An excess of sodium hydroxide was added and ammonia was distilled in steam absorbed in boric acid and titrated with HCl using methyl red as indicator, according to the following reaction:



2.2.6. Determination of Protein:

0.5 g of sample was weighted and transferred to Kjeldahl digestion flasks and one Kjeldahl tablet (copper sulphate, potassium sulphate catalyst) was added to each other. 10 cm³ concentrated nitrogen free sulphuric acid was added. The flask was then mounted in the digestion heating system which was previously set to 240 °C and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish green color was observed which indicates the completion of the digestion. The tubes were then allowed to attain room temperature. Their content was quantitatively transferred to Kjeldahl digestion apparatus

followed by addition of distilled water and 30% (w/v) sodium hydroxide. Steam distillation was then started and released, ammonia was absorbed in 25 cm³ of 2% boric acid. Back titration of the generated borate was then carried out versus 0.02M using methyl red as an indicator. Blank titration was carried in the same way.

$$\% \text{ protein} = N \times 6.25$$

2.2.7. Oil extraction:-

The ground sample 5.0 g was accurately weighed in an empty thimble of known weight plugged with a piece of cotton wool; then the thimble with the materials was placed in Soxhlet extractor. A dry and accurately weighed round bottomed flask was fitted to extractor; then petroleum ether was poured into the flask until it filled approximately two thirds of the flask.

The flask, the extractor and condenser were fitted together. Water was allowed to flow through the condenser and heat was continued 16 hours. The apparatus was carefully dismantled and the solvent in the flask was evaporated to dryness in an air bath at 150 °C.

$$\text{Oil \%} = \frac{W_2 - W_1}{s} \times 100$$

Where s = original weight of sample in grams

W₁ = weight of empty flask in grams

W₂ = weight of flask + oil in grams

2.2.8. Biochemical Tests:-

2.2.8.1. Sample Preparation:

Fenugreek seeds were purchased from the local market . They were cleaned and ground in a mill.

Fenugreek seeds powder was capsulated into capsules of 500 mg each At (Wafra Pharma Industry)

2.2.8.2. Protocol

The present study was carried out at Khartoum teaching hospital, Khartoum, Sudan. The experiments were conducted on 20 lactating Sudanese females the Females were clinically examined by a physician for freedom of disease and productive disorders. The females were weight and divided in two groups. Group (1) was untreated and was used as control. Group (2) was given trigonella foenum orally in a dose of 500 mg (capsules) 3 times dialy for 12 weeks. Prolactin was detected every 2 weeks using ELISA(Enzyme-Linked Immuosorbent Assay). The body Weight was meaured every 2 weeks for both groups. The data obtained from this study were subjected to statistical analysis using the SPSS .

Chapter Three

3. Results:

Table (3-1): Chemical composition of fenugreek powder

Sample	Moisture	Ash	Protein	Fiber	Oil	Peroxid value	Carbohydrate
Fenugreek powder	4.45%	3.44%	23.63%	4.68%	4.46%	8.25%	67.34%

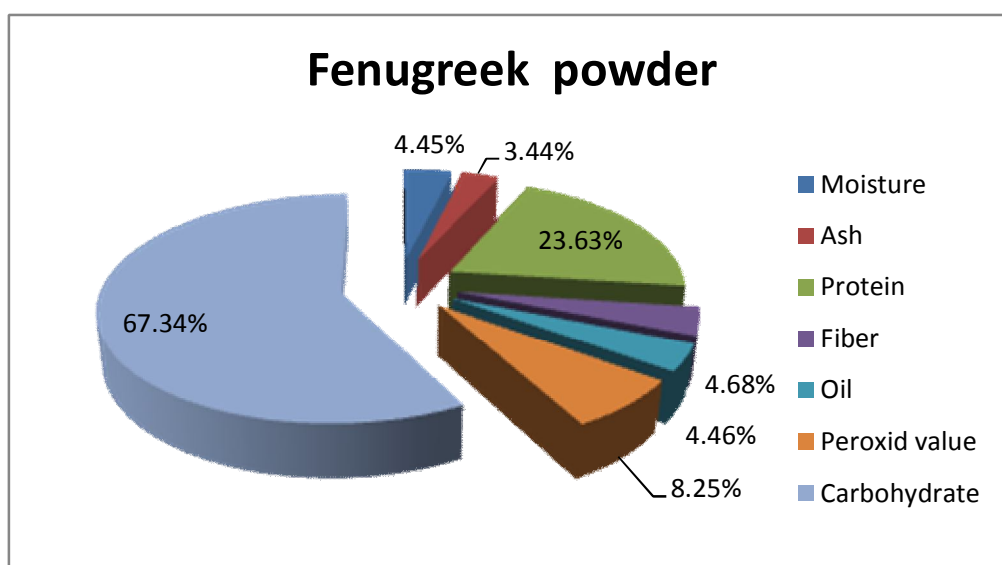


Fig (3-1): Chemical composition of Fenugreek powder

Table (3-2): Body wt in (kg) from week 1 to week 12 in controls

W1	W2	W4	W6	W8	W10	W12
80.2	80.55	80	81	81	80	80.5
72	73	73	74	73	74.5	74
70	70	70	72	73.21	74	73.2
72	72.5	72.1	71	71	71	71
68.2	68	68.2	67	67.5	67	67
72	75	75	75.3	75.1	75	75.2
78	78	78	78.5	78.3	78.2	78
68	70	71	71	72	72.3	72
68	69.12	68	68	69	69.1	69
73.56	75	75.1	75.2	75.1	75.1	75

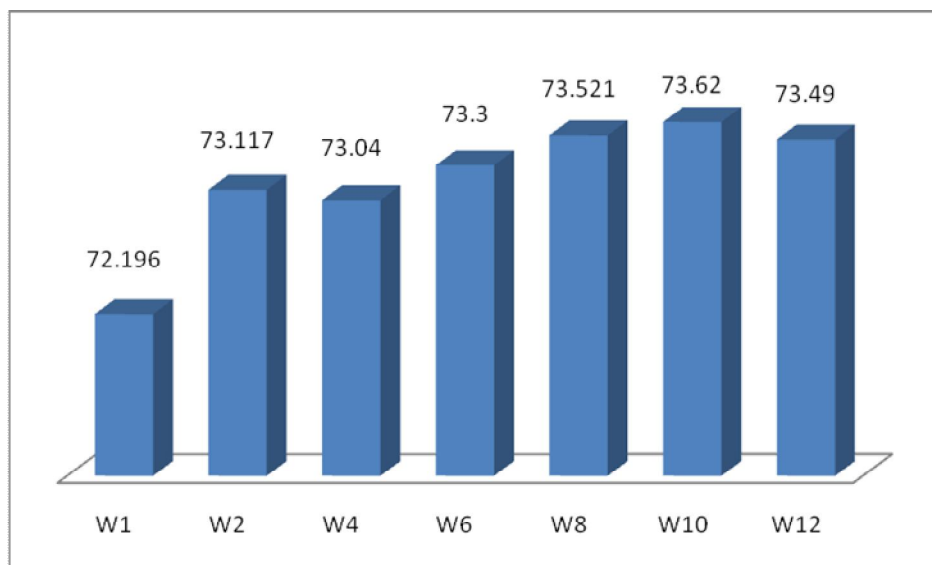


Fig (3-2): Body wt in (kg) from week 1 to week 12 in controls

Table (3-3): Body wt in (kg) after treatment with fenugreek

W1	W2	W4	W6	W8	W10	W12
71	72	73.5	74.2	75	76	77
80	80.5	82	82.2	83	83.5	84.2
69	70.2	72	72.3	73.5	74.6	75
70	71.1	72.3	73.7	74	75.5	75.6
76.5	78.2	79	80	80	80.5	81
80	82	83	84.6	85	85	85.5
67	68	68.5	69	70	71	72.3
70	71.5	72.6	73.1	74	74.5	75.5
76	77	77.2	77.8	79	79.5	79.5
80	82	83	83.3	84	84.7	85

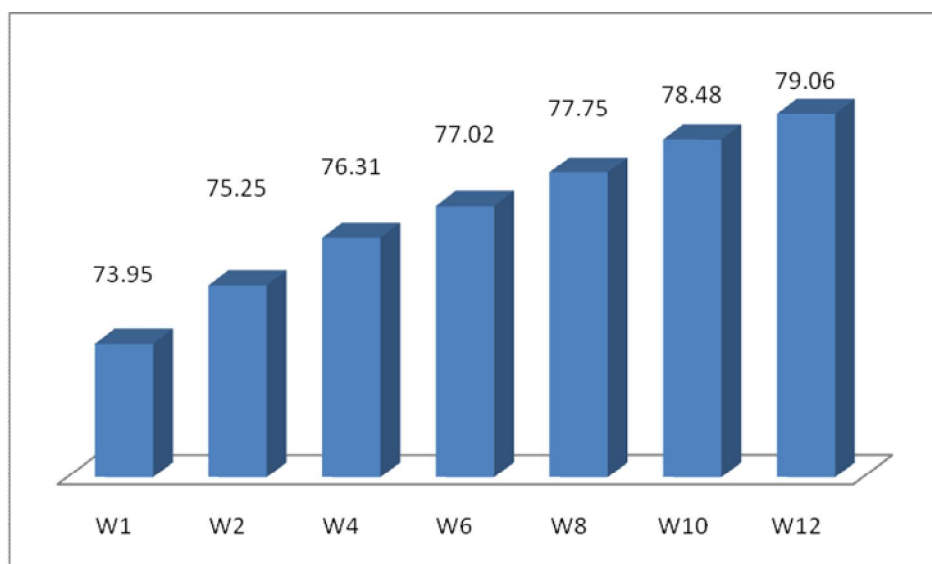


Fig (3-3): Body wt in (kg) after treatment with fenugreek

Table (3-4): Prolactin level in controls ($\mu\text{g}/\mu\text{l}$)

W1	W2	W4	W6	W8	W10	W12
8.0	8.54	8.2	7.9	8.3	8.4	8.4
8.1	7.5	7.2	8.0	8.12	8.50	8.4
7.8	8.0	8.41	8.35	8.5	8.1	8.0
8.34	8.35	8.5	8.5	8.42	8.5	8.4
8.1	7.95	8.0	8.1	8.0	8.2	8.0
7.5	8.0	8.1	7.9	7.9	8.0	7.2
8.0	8.0	8.2	7.7	7.5	7.2	7.0
7.25	7.21	7.3	7.70	7.50	7.4	7.5
7.9	8.0	7.81	7.5	7.7	7.7	7.4
8.0	8.6	8.5	8.4	8.25	8.0	8.1

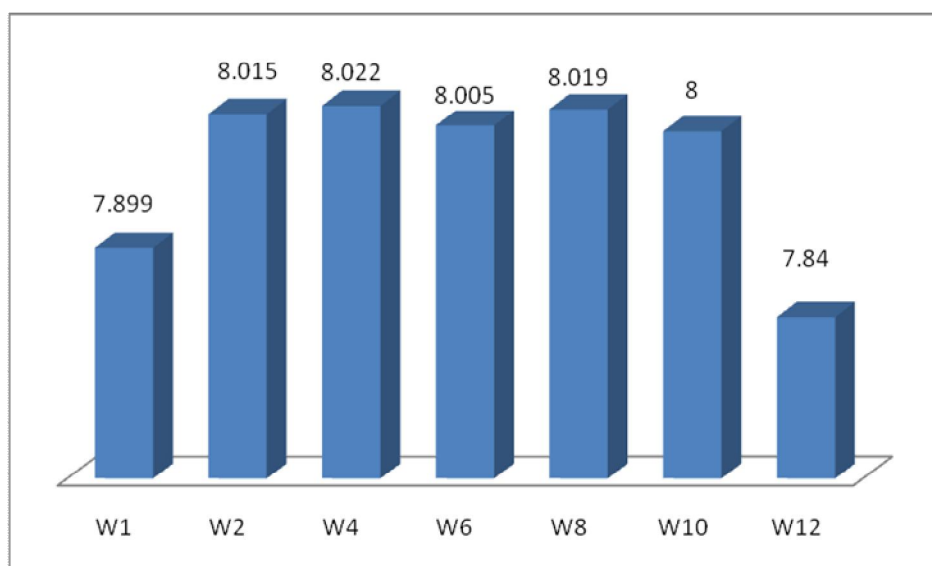


Fig (3-4): Prolactin level in controls ($\mu\text{g}/\mu\text{l}$)

Table (3-5): Prolactin level after treatment with fenugreek ($\mu\text{g}/\mu\text{l}$)

W1	W2	W4	W6	W8	W10	W12
8.0	8.1	9.0	8.9	9.2	9.45	9.6
7.0	8.0	8.5	9.1	9.5	9.2	9.52
8.1	8.5	9.0	9.2	10.1	10.4	10.6
7.0	7.21	8.0	8.5	9.0	9.3	9.5
7.56	8.2	9.1	9.8	10.0	10.5	10.51
8.0	9.0	9.54	10.0	10.2	11.0	11.8
7.0	7.5	8.0	9.3	10.0	10.5	11.2
8.0	9.2	9.56	9.8	10.0	10.6	11.0
8.2	9.0	9.86	10.1	11.1	12.0	13.0
7.4	8.3	9.4	10.4	11.2	11.6	12.5

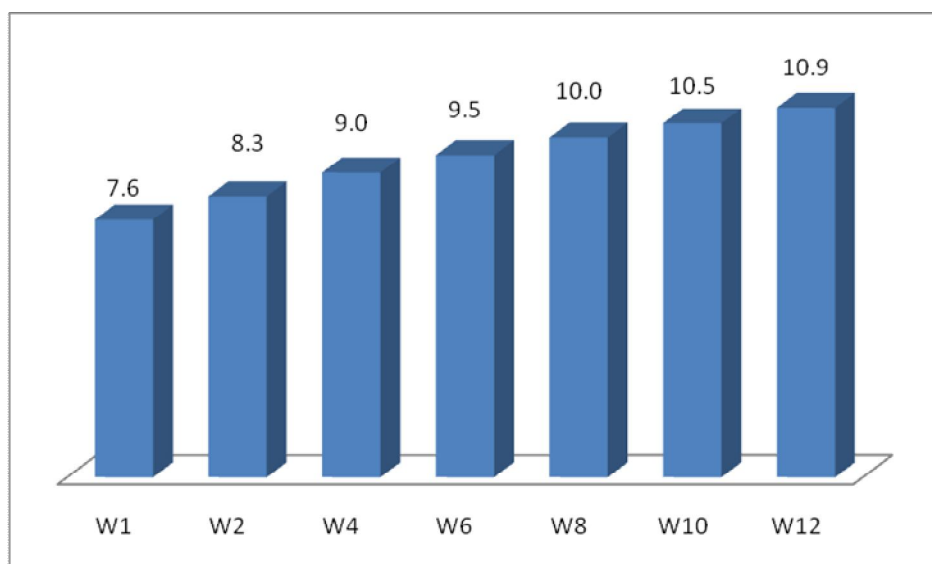


Fig (3-5): Prolactin level after treatment with fenugreek($\mu\text{g}/\mu\text{l}$)

Chapter Four

Discussion & Conclusion

4.1. Discussion:

In this study the chemical composition of fenugreek seed powder was found to be as follows: protein 23.63%, oil 6.46%, fiber 4.68%, ash 3.44%, moisture 4.45%, carbohydrate 67.34%, while other studies showed that protein 27.3%, oil 6.7%, fiber 6.7%, moisture 4.3%, ash 3.8%, carbohydrate 51.2%, (Nour, 1986). (Sauvaire et al., 1976; Sharma et al., 1986b; Vats et al., 2003; Srinivasan, 2006) found the protein 18-30%, oil 6-8%, moisture 4-10% depending on varietal and ecological factor.

The present data clearly demonstrated significant ($p < 0.05$) increase in the mean of body weights among the different women in the treated group and this could be explained to high content of carbohydrate in fenugreek seeds powder, which effecting in physiological status of women resulted in body weight gain. This result was higher than results obtained by different studies, and this shows that there was higher absorption of nutrient materials and may be attributed to stimulating action of fenugreek seeds on enhancing appetite and food intake.

This study also showed a significant increase in prolactin level ($p < 0.05$) in response to fenugreek administration in Sudanese females, this increase in prolactin level is known to have a strong galactopoietic effect on lactation performance.

4.2. Conclusion

The importance of human milk for infants is well recognized. The purpose of this study was to examine the use of fenugreek with breastfeeding mothers and its effect on milk production and prolactin levels. Fenugreek is one of the most common herbs mother use in an effort to increase milk volume. However, there is a paucity of research determining its efficiency and safety. This study concludes, that *trigonalla foenum graecum* is highly nutritive, with very high carbohydrate percentage, compared to other studies, and high protein content as well. This result leads to gain in weight and enhancement of milk production by its effect on increasing prolactin levels.

No change in health status or negative side effects were observed in either the mothers or infants.

Recommendations

More randomized controlled studies are needed for all galatogogues, in particular for herbal substances to establish efficacy, standard dosages and safety.

Future studies must include a larger and more diverse sample, diet history to evaluate caloric intake before and during the study period which might shed some light on why fenugreek is effective in increasing milk volumes.

References

1. A,E Ozgoz E, & Taser, F. (2005).“ Some physical and chemistry properties of fenugreek seeds. *Journal of food Engineering*, **71**: 37-43.
2. A3uri D., Prabhu K.M., Murthy P.S. (2002).“ Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds”. *Indian Journal of Physiology and Phamacology* **46**:457-462.
3. Abdel-Barry J.A., Abdel-Hassan l.A., iawad AM., Al-Hakiem M.H.H. (٢٠٠٠).“ Hypoglycemic effect of aqueous extract of the leaves of Trigonel'a foenum graecum healthy volunteers. *Eastern Mediterranean Health Journal* **6**: 83-88.
4. Acharya S.N., Thomas J.E., Basu S.K. (٢٠٠٧a).“ Methods for the improvement of plant medicinal properties, with particular reference to fenugreek (*Trigonella foenum graecum* L.), in:--S. N. Acharya and S. E. Thomas (Eds.),
5. Aggarwal B.B., Shishodia S. (٢٠٠٦).“ Molecular targetsof dietary agents for preventionand therapy of cancer. *Biochemical Pharmacology* **71**: 1397-1421.
6. Al-Rawi A and Chakravarty H (1988).“ *Trigonella foenum graecum*. In *Medicicine plants of Iraq*. Second edition. ALY aqda Press, Baghdah; 94.
7. Anuradha C.V., Ravikumar P. (٢٠٠١).“ Restoration on tissue antioxidants by fenugreek ‘٩ seeds (*Trigonel?afoenum-graecu,n*) in alloxan-diabetic rats”. *Indian Journal of Physiology and Phamacology* **45**:408-420.
8. American Academy of Pediatrics. (2005).“ Breast feeding and the use of human milk”. *Pediatrics*, **115**:496–506.

9. Basch E., Ulbricht C., Kuo G., Szapaly P., Smith M. (2003).“ Therapeutic applications of fenugreek. *Alternative Medicine Review* **8**:20-27.
10. Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW (1996).“: Extrapituitary prolactin distribution regulation, functions, and clinical aspects. *Endocr Rev* **17**:639-669.
11. Bhaumick D. (2006).“ An in vitro cholesterol and bile acid binding capacity of saponins extracted from fenugreek (*Trigonella foenum-graecum* L.) grown in Alberta, Canada”. M. Sc. Thesis, University of Alberta, Edmonton, Alberta.
12. Blank I., Lin J., Devaud S., Fumeaux R., Fay L.B. (1997).“ The principal flavor component of fenugreek (*Trigonella foenum-graecum* L.), in: S. J. Risch and C. T. Ho (Eds.), *Spices, Flavor Chemistry and Antioxidant Properties*, ACS Symposium Series No 660. pp. 12-28.
13. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998).“ Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* **19**:225-268.
14. Chopra IJ., Solomon and Barney J, (1971).“ Immunoassay, *Endocrinal.*, 33;87.
15. Dixit P., Ghaskadbi S., Mohan H. and Devasagayam T.P.A. (2005).“ Antioxidant properties of germinated fenugreek seeds. *Phytotherapy Research* **19**:977-983.
16. Dixon R.A., Sumner L.W. (2003).“ Legume natural products: Understanding and manipulating complex pathways for human and animal health. *Plant Physiology* **131**:878-885.

17. Dubber M.J. and Kanfer I. (2004).“ High-performance liquid chromatographic determination of selected flavonols in Ginkgo biloba solid oral dosage forms. *Journal of Pharmaceutical Science* **7(3)**:303-309.
- 18.El-Ridi Al. and Shahat Al. (1944).“ A preliminary note on a fat-soluble lactation factor, *J Egypt Med. Ass.* (27,): pp: 199-200.
- 19.Enjalbert A, Sladeczek F, Guillon G, Bertrand P, Shu C, Epelbaum J, Garcia-Sainz A, Jard S, Lombard C, Kordon C, et al (1986).“: Angiotensin II and dopamine modulate both cAMP and inositol phosphate productions in anterior pituitary cells. Involvement in prolactin secretion. *J Biol Chem* 261: 261: 4071-4075.
20. Erdman J.W., Balentine D., Arab L., Beecher El., Dwyer J.T., Folts J., Hamly J., Hollman P., Keen C.L., Mazza El., Messina M., Scalbert A., Vita J., Williamson G, Burrowes J. (2005).“ flavonoids and heart health: Proceedings of the ILSI North America Flavonoids Workshop, May 31-June 1, 2005, Washington, DC, American Society of Nutritional Science. PP. 718S-737S.
21. European Bioinformatics Institute (2008) Meclicarpin. Available at www.ebi.ac.uk/chebi/searchld.do?chebiid=CHEBI:100. Retrieved June 23, 2008.
22. Evans A.J., Hood R.L., Oakenfull D.G., Sidhu G.S. (1992).“ Relationship between structure and function of dietary fiber - a comparative study of the effects of 3 galactomannans on cholesterol metabolism in the rat. *British Journal of Nutrition* **68**:217-229.
23. Evolution. *J Mammary Gland Biol Neoplasia* **7**:291-312, 2002

24. Fazli FRY., Hardman R. (1971).“ Isolation and characterization of steroids and other constituents from *Trigonella foenum, graecum* L. *Phytochemistry* **10**:2497-2503.
25. Fodor M, Kordon C, Epelbaum J (2006).“ Anatomy of the hypophysiotropic somatostatinergic and growth hormone-releasing hormone system minireview. *Neurochem Res* **31**:137-143
26. Fotopoulos C. (2002).“ Marketing, in: El. A. Petropoulos (Ed.), *Fenugreek - The genus Trigonella*, Taylor and Francis, London and New York. pp. 183-195.
27. Galal O.M. (2001).“ The nutrition transition in Egypt: obesity, undernutrition and the food consumption context. *Public Health Nutrition* **5**:141-148.
28. Garti N. Madar Z., Aserin A., Sternheim B. (1997).“ Fenugreek galactomannans as food emulsifiers. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* **30**:305-311.
29. Guarner, J., Valdives , E., and A deen G.,(1997).“ comparison of two assays in a Mexican Population. *Amjclin Metab*, 59-74.
30. Harvey S, Hull KL (1997).“ Growth hormone. A paracrine growth factor? *Endocrine* **7**:267-279.
31. Hatanaka S. (1992).“ Amino acids from mushrooms. *Progress in the Chemistry of Organic Natural Products* **59**:1-140.
32. Hauffa BP (2001).“ Clinical implications of pulsatile hormone signals. *Growth Horm IGF Res* **11** Suppl A:S1-8.
33. Hormones/cytokines at the maternal-fetal interface (2004).“ *Reprod Biol Endocrinol* **2**:51.
34. Houssay BA, Anderson E, Bates RW, Li CH (1955).“ Diabetogenic action in prolactin. *Endocrinology* **57**:55-63.

35. Hillervik-Lindquest, C., Hofvander, Y., & Sjölin, S. (1991).“ Studies on perceived breast milk insufficiency. III. Consequences for breast milk consumption and growth. *ACTA PaediatricaScandnavica*, **80**: 297–303.
36. Huang W., Liang X. (2000).“ Determination of two flavone glycosides in the seeds of *Trigonellafoenum-graecum* L. from various production localities. *Journal of Plant Resources and Environment* **9**:53-54.
37. Irrigation Management Climate Information Network (2008).“ Available at <http://www.agric.gov.ab.ca/app49/imcin/met.jsp>. Retrieved Jan 16, 2009.
38. Jain S.C., Agrawal M., Vijayvergia R. (1992).“ Regulation of pharmaceutically active flavonoids in *Trigonella foenum-graecum* tissue cultures. *Fitoterapia* **65**:367-670.
39. Jansson JO, Albertsson-Wikland K, Eden S, Thorngren KG, Isaksson O (1982).“ Circumstantial evidence for a role of the secretory pattern of growth hormone in control of body growth. *Acta Endocrinol (Copenh)* **99**:24-30,
40. Jard S, Lombard C, Kordon C, et al (1986).“ Angiotensin II and dopamine modulate both cAMP and inositol phosphate productions in anterior pituitary cells. Involvement in prolactin secretion. *J Biol Chem* **261**:4071-4075.
41. Javan M., Ahmadiani A., Semnanian S., Kamalinejad M. (1997).“ Antinociceptive effects of *Trigonellafoenum-graecuni* leaves extract. *Journal of ethnopharmacology* **58**:125-129.
42. Kochhar A., Nagi M., Sachdeva R. (2006).“ Proximate composition, available carbohydrates, dietary fiber, and anti-nutritional factors of selected traditional medicinal plants. *Journal of Human Ecology* **19**:195-199.

43. Marles RJ., Farnsworth N.R. (1994).“ Antidiabetic plants and their active constituents. *Phytomedicine* **2**:137-189.
44. Marker RE., Wagner RB., Ulshafer, Wiubecker E.L., Goldsmith D.P.J., RuofC.H. (1947).“ New sources for sapogenins. *Journal of The American Chemical Society* **69**:2242.
45. Mazza G., Di Tommaso D., Foti S. (2002).“ Volatile constituents of sicilian fenugreek (*Trigonella foenum-graecum* L.) seeds. *Sciences De Aliments* **22**:249-264.
46. McAnuff MA., Omoruyi F.O., Morrison E.Y.S., Asemota H.N. (2002).“ Plasma and liver lipid distributions in streptozotocin-induced diabetic rats fed sapogenin extract of the Jamaican bitter yam (*Dioscorea polygonoides*). *Nutrition Research* **22**:1427- 143 4.
47. Monastiri K., Limame K., Kaabachi N., Kharrat H., Bousnina S., Pousse H., Radhouane M., Gueddiche M.N., Snoussi N. (1997).“ Fenugreek odour in maple syrup urine disease. pp. 614-615.
48. Mondal D.K., Yousuf B.M., Banu L.A., Ferdousi R., Khalil M., Shamim K.M. (2004).“ Effect of fenugreek seeds on the fasting blood glucose level in the streptozotocin induced diabetic rats. *Mymensingh Medical Journal* **13**:161-164.
49. Murry RK, Granner DK (2000).“ Harpers Biochemistry, Appetton and lange , Caifornia , **5**:555-564.
50. Neville MC, Casey C, Hay WW, Jr (1994).“ Endocrine regulation of nutrient flux in the lactating woman. Do the mechanisms differ from pregnancy? *Adv Exp Med Biol* **352**:85-98.
51. Pandia R.S., Anuradha CV., Viswanathan p. (2002).“ Gastroprotective effect of fenugreek seeds (*Trigonella foenuin-graecum*) on experimental gastric ulcer in rats. *Journal of Ethnopharmacology* **81**:393-397.

52. Parmar VS., Jha H.N., Sanduja SK., Sanduja R. (1982).“ Trigonocoumarin - a new coumarin from *Trigonella foenum-graecum*. *Zeitschrift Fur Naturforschung Section B- A Journal of Chemical Sciences* **37**:521-523.
53. Petropoulos G.A., Kouloumbis P. (2002).“ Botany, in: GA. Petropoulos (Ed.), *Fenugreek - The genus Trigonella*, Taylor and Francis, London and New York. pp. 9-17.
54. Petropoulos GA. (2002).“ *Fenugreek - The genus Trigonella*, Taylor and Francis, London and New York. pp. 255.
55. Reid J.S.G., Edwards ME., Dickson CA., Scott C., Gidley M.J. (2003).“ Tobacco transgenic lines that express fenugreek galactomannan galactosyltransferase constitutively have structurally altered galactomannans in their seed endosperm cell walls. *Plant Physiology* **131**:1487-1495.
56. Riddle O, Bates R, Dykshorn S (1933).“ The preparation, identification and assay of prolactin-a hormone of the anterior pituitary. *Am J Physiol* **105**:191-216.
57. Samson WK, Taylor MM, Baker JR (2003).“ Prolactin-releasing peptides. *Regul Pept* **114**:1-5.
58. Sauvaire Y., Baccou i.e., Besancon P. (1976).“ Nutritional value of proteins of a leguminous seed - fenugreek (*Trigonella foenum-graecum* L.). *Nutrition Reports International* **14**:527-537.
59. Sauvaire Y., Baccou J.C. (1978).“ L'obtention de la Diosgenine (25R)-Spirost-5-ene-3B-ol; problems de l'hydrolyse acide des saponines. *Journal of Natural Products* **41**:247-255.
60. Sauvaire Y., Ribes G., Baccou J.C., Loubatieresmariani M.M. (1991).“ Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. *Lipids* **26**: 191-197.

61. Sehgal O., Chauhan G.S., Kumbhar B.K. (2002).“ Physical and functional properties of mucilages from yellow mustard (*Sinapis alba* L.) and different varieties of fenugreek (*Trigonella foenum-graecum* L.) seeds. *Journal of Food Science and Technology-Mysore* **39**:367-370.
62. Shani J., Goldschmied A., Joseph B., Ahronson Z., Sulman F.G. (1974).“ Hypoglycaemic effect of *Trigonella foenum-graecum* and *Lupinus termis* (Leguminosae) seeds and their major alkaloids in alloxan-diabetic and normal rats. . *Archives Internationales de Pharmacodynamie et de Therapie* **210**:27-37.
63. Sharma R.D. (1986a).“ An evaluation of hypocholesterolemic factor of fenugreek seeds (*Trigonella foenum-graecum*) in rats. *Nutrition Reports International* **33**:669-677.
64. Sharma R.D. (1986b).“ Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. *Nutrition Research* **6**:1353-1364.
65. Sharma R.D., Sarkar A., I-lazra D.K., Misra B., Singh J.B., Maheshwari B.B., Sharma S.K. (1996b).“ Hypolipidaemic effect of fenugreek seeds: A chronic study in noninsulin dependent diabetic patients. *Phytotherapy Research* **10**:332-334.
66. Skaltsa H. (2002).“ Chemical constituents, in: O. A. Petropoulos (Ed.), *Fenugreek - The genus Trigonella*, Taylor and Francis, London and New York. pp. 132-161.
67. Slinkard A.E., McVicar R., Brenzil C., Pearse P., Panchuk K., Hartley S. (2006).“ Fenugreek in Saskatchewan, Saskatchewan Agriculture and Food.

68. Soares MJ (2004).“ The prolactin and growth hormone families: pregnancy-specific hormones/Cytokines at the maternal-fetal interface. *Reprod Biol Endocrinol* **2**:51.
69. Sowmya P., Rajyalakshmi P. (1999).“ Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. *Plant Foods for Human Nutrition* **53**:359-365.
70. Spyropoulos CO. (2002).“ Physiolo, in: O. A. Petropoulos (Ed.), *Fenugreek - The genus Trigonella*, Taylor and Francis, London and New York. PP. 18-25.
71. Srinivasan K. (2006).“ Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. *Food Reviews International* **22**:203-224.
72. Stricker P, Greuter R (1928).“ Action du lobe antérieur de l'hypophyse sur la montée laiteuse. *CR Soc Res* **99**:1978-1980.
73. Sulieman, A.M.E., Ali, A.O., Hemavathy, J. (2008),“ Lipid content and fatty acid composition of fenugreek (*Trigonella foenum-graecum* L.) seeds grown in Sudan. *International Journal of Food Science and Technology* **43**:(2): 380-382.
74. Vats V., Yadav S.P., Grover J.K. (2003).“ Effect of *Trigonella foenum-graecum* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *Journal of Ethnopharmacology* **85** :237-242.
75. Voltev, A; Bedwel, D.E. and Bart L.A,(1980).“ Enzyme- linked Immunosorbent assay, *Manual of clinical Immunological*, Washington, D.C.Am Soc, of Microbial, 359.
76. Walter F., Boron P. (2003).“ Enzyme- linked Immunosorbent assay Cambridge, 112.

77. Watt LM and Mc-Breyer-Brandwi J (1962).“ Leguminosac. In: (he medical and poisonous plants of southern arid eastern Africa. Second edition. Pub. Livingstone Ltd, Edinburgh and London; pp: 666-667 2
78. Kotb F (1985).“ Trigonella foenum-graecum: Medicinal - plants in Libya. Arab Encyclopedia House. First edition. :85-86
79. Wilson K., and Walker J.M,(1995).“ Principle and techniques of practical Biochemistry cambridge 4:91-100.
80. Woodside B(2007),“ Prolactin and the hyperphagia of lactation. Physiol Behav 91:375-382.
81. Yoshikawa M., Murakami T., Komatsu H., Murakami N., Yamahara J., Matsuda H. (1997).“ Medicinal foodstuffs .4. Fenugreek seed .1. Structures of trigoneosides Ia, Ib, Ha, lib, IIIa, and Ilib, new furostanol saponins from the seeds of Indian Trigonella foenum-graecum L. *Chemical & Pharmaceutical Bulletin* 45:81-87.