

EFFECT OF FENUGREEK POWDER AND MACKEREL FISH POWDER ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF WHITE MALE AND FEMALE RABBITS

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ABSTRACT : This research included study the effect of replacement a part of the traditional feed of rabbits with fenugreek powder (4%) and mackerel fish (8%) for the period from 11/12/2019 to 20/12/2019 was studied on some physiological and / biochemical characteristics of 30 rabbits (15 males and 15 females) After dividing them into six groups, (5) rabbits for each group. The study showed that: i)The powder results showed a significant decrease in the (follicles stimulating hormone, luteinizing and testosterone hormone), and increase the level of (prolactin hormone) in groups treated with fenugreek powder ,and groups treated with mackerel fishes powder of both males and females compared to control group, and ii) the results of liver enzymes showed no significant differences in AST level in the both male groups (the fenugreek powder treated group, mackerel fishes powder treated group), while there was a significant decrease in the females groups for both treatments. The results showed a significant increase in ALT enzyme in the female groups (treated with fenugreek leaves powder and mackerel fishes powder). In the male groups, there was a significant decrease in ALT enzyme in the group treated with mackerel fishes powder, while there was no significant change in the group treated with fenugreek powder. For the enzyme ALP, there was no significant difference in the concentration of this enzyme in the both females groups (treated with fenugreek powder and treated with powder mackerel fishes powder), while the results showed a significant increase in the male groups for the same treatments.

Key words : Fenugreek, mackerel fish, prolactin hormone, Follicle Stimulating Hormone, AST.

INTRODUCTION

All medicinal plants play an important therapeutic role for many diseases by containing chemicals that have the physiological ability to treat diseases, or to reduce the incidence of many disease (Dabea and Khalidi, 1997). The fenugreek is one of the most important plants of the earlier, and has continued to be important to the present time by containing the important food, medical and pharmaceutical ingredients, including the various classics, which are the most important because it enters in the preparation of various industrial hormones (Newall *et al*, 1998; Barnes *et al*, 2002). The fenugreek has many medical uses, including increasing the milk supply after birth through the activation of the lactic glands, regulating the unstable and unstable menstrual periods in adult girls. It is also used to treat cases of anemia, general weakness of the body, treatment of pertussis and chest diseases, and reduce the Hemorrhoids pain and the use of diosgenin in the fenugreek as a raw material in the synthesis and

preparation of the sex hormones, which interfere in the effect of medical materials used in the preparation of cortisone, and its various derivatives that benefit in the treatment of thoracic diseases, and rheumatism (Chahat, 1986).

Fish is an essential source of animal protein for a large number of the world's population. It is the main dietary source of long-chain unsaturated fatty acids (omega-3), especially EPA and DHA, which is important for mental and physical health and plays an important role in the development of the infant's brain and eyes (EFSA, 2014; Ciriminna *et al*, 2017). The importance of polyunsaturated fatty acids (omega-3) in the protective effect on the heart has also been shown to act as an anti-heart beat disorder, anticoagulant, anti-inflammatory, antihypertensive, and anti- blood lipid factor (Geleijnse *et al*, 2002). One of the most widely traded food commodities in the world is fish, which is widely distributed in most countries of the world, including the

Indian Ocean, Indonesia, Pakistan, India and Thailand (Collete and Nauen, 1983; Devaraj *et al*, 1997; Mahdawi and Mehanna, 2010; Jayabalan *et al*, 2014). The package and fresh Mackerel are both good source of protein and other nutrients. According to US Department of Agriculture data, both fresh and canned mackerel have a similar amount of omega-3 fatty acids important to cardiovascular health.

MATERIALS AND METHODS

Animals used in the study

In this study, 30 female and male white rabbits were used with weights ranging from 1000-1500 mg. After they were purchased from the local markets, the animals were divided and distributed evenly in weight; to six groups and placed in wooden cages with dimensions of 125 × 60 × 50 cm) and wooden floor covered with sawdust, taking into consideration the cleanliness and sterilization of cages and the exchange of sawdust from time to time. The animals were subjected to laboratory conditions of a light cycle divided into 12 light and 12 hours of darkness. The experiment animals left for two weeks to adapt to the new conditions. To ensure that they were free from disease or pregnancy, food and water were given continuously and in sufficient amounts throughout the period of breeding and treatment. The food was as available in the local market after replacing part of it with fenugreek leaves powder with and mackerel fishes with keeping the same Calories of original feed. The material was well milled and mixed together and added to the water to become a cohesive paste, cut into small blocks and placed in the caffeinated food area, giving water and food continuously (Balducci and Roslind *et al*, 2001).

Design of experience

1. The experiment included, 30 laboratory rabbits (15 males and 15 females).

2. Divided into six groups (5) rabbits for each one, taking into account weight and are fed as follows:

Group 1: Control group for male rabbits was given only water and food.

Group 2: the male rabbits group which was given water and a feed mixed with fenugreek powder.

Group3: Male rabbits group was given water and a feed, mixed with mackerel fishes powder .

Group4 : Control group for female rabbits was given only water and food.

Group 5: The group of females rabbit was given water and feed mixed with fenugreek powder.

Group 6: The group of rabbit females was given water and a fees mixed with mackerel fishes powder.

Collection of study samples

After 30 days of experimentation, the food was cut off from the experimental animals for 24 hours with the removal of the sawdust during this period. The blood samples were then taken directly by Cardiac Puncture. 10 mL of blood was withdrawn and placed in the test tubes which are free of coagulant material, and then left for 15 minutes at room temperature. The serum was then separated by a centrifuge of 3000 cycles/minute for 15 minutes and the serum was kept under freezing in plastic tubes until the hormonal tests were carried out. Hormones are (follicles stimulating hormone, luteinizing hormones, testosterone and prolactin hormones).

Determination of Follicle Stimulating hormone

The FSH is estimated by following the steps in a leaflet, with the ready ELA kit and the instructions of the manufacturer; Bio Kit -U.S.A by ELISA technique (Vitt *et al*, 1998).

Determination of luteinizing hormone

LH was evaluated using the steps with to the LH ELA kit and according to the instructions of the manufacturer Bio Ki - USA using ELISA technique (Lenton *et al*, 1982).

Determination of prolactin hormone

Prolactin was evaluated using the steps with to the Prolactin ELA kit and according to the instructions of the manufacturer Bio Ki - USA using ELISA technique (Lenton *et al*, 2006).

Determination of testosterone hormone

The concentration of the testosterone hormone was measured by following the steps described in the attached leaflet with ready kits, as instructed by the manufacturer Bio Kit - USA.

Determination of Aspartate Aminotransferase activity AST (GOT)

The enzymatic method was used, the kit was used by the French company Biolabo (Tietz, 1999).

Determination of Alanine Aminotransferase activity ALT (GPT)

The enzymatic method was used, the kit was used by the French company Biolabo (Tietz, 1999).

Determination of serum alkaline phosphates activity ALP

The efficacy of serum alkaline phosphatase was estimated using the kit prepared by the French company Biolabo (Tietz, 1999).

RESULTS AND DISCUSSION

Effect of fenugreek powder leaves and mackerel fishes on levels of sex hormones:

Serum follicle stimulating hormone concentration of the male and female rabbits

The results of Fig. 1 showed a significant decrease in the concentration of follicle stimulating hormone in the male group treated with fenugreek leaves powder and mackerel fishes powder compared to control group. The results showed a significant decrease ($P \leq 0.05$) in FSH concentration in the female group treated with fenugreek leaves powder and Mackerel fishes powder compared to control group.

FSH is the primary regulator of fertility in animals (Haywood *et al*, 2002) and plays an important role in the process of semen formation, especially in the latter periods, and the evolution of the reproductive system, as it stimulates the growth of seminiferous tubules (Grover *et al*, 2005). Hormone LH, FSH may be the main cause of infertility because the cycle may occur without the occurrence of ovulation and this explains the incidence of infertility in some cases of early marriage. The high level of hormone prolactin may cause disorder in the menstrual cycle and irregularity and lack of ovulation because the hormone works directly on the hypothalamic gland reducing the number of times the hormone secretion is stimulated (GnRH) (Harborne *et al*, 2003). This is what Jameson and Melmed (2004) were found.

Serum LH concentration of males and females white rabbits

The results of Fig. 2 showed a significant decrease ($P \leq 0.05$) in the concentration of luteinizing hormone in the female group treated with fenugreek leaves powder and a high concentration of the hormone in the female group treated with mackerel fishes powder compared with the control group. The results showed significant decrease in the male group treated with fenugreek leaves powder, while there was no significant difference in group treated with mackerel fishes powder compared to the males control group.

This decrease in the LH hormone concentration in treatment animals with fenugreek powder correspond with the finding of Al-Roubai (2006). Maybe because the effect of the powder in the cells which are responsible of LH secretion in posterior parts of pituitary gland which is effect passively on hormone secretion. The increase of prolactin causes decrease in this hormone and inhibits the ovulation process, this is consistent with Nilsen *et al* (2001). The increase of LH levels in patients serum (women) because the decrease E2 and progesterone, it

is known that LH inhibition by the increase of E2 and progesterone by negative feedback mechanism.

Serum prolactin concentration of males and females white rabbits

The results of Fig. 3 showed significant increase in the concentration of prolactin hormone in females groups treated with Mackerel powder and fenugreek powder comparative with control group, for males group there was a significant difference in fenugreek and Mackerel comparative with control group. There was a difference between the two treatment for the fenugreek treatment group.

Several researchers (Jensen, 1992; Basch *et al*, 2003; Taylor, 2006) have reported that the fenugreek is a milk-producing factor, because it contains oil that stimulates the production of milk (lactation-stimulating oil) (Allen, 1981). This increase of hormone concentration occurs because the effect of the hormone to the effect of the negative fenugreek powder in the secretion of dopamine, which is one of the most important inhibitory factors of prolactin secretion, which is produced by the hypothalamic neurons, causing an increase in the secretion of this hormone.

The results of the current study corresponding with the results of Emokpase *et al* (2005), Jubouri (2006), Goswami *et al* (2009), Kazem (2010), Jawad (2012), who indicated a higher concentration of prolactin in women with impaired fertility than control. High concentrations of prolactin can inhibit the production of gonadotrophins in both males and females, although its secretion is very common in women (Gobilla *et al*, 2001). High concentrations of prolactin can also cause irregular menstruation and decrease in corpus luteum. Concentrations of androgens or male stimulants. Androgens that increase the concentration of blood prolactin lead to gradual changes in the lack of lutein phase and to the lack of ovulation and menopause due to the action of prolactin inhibitory secretion of GnRH hormone, the abnormal lutein phase in the menstrual cycle of women with high prolactin, is one of the reasons for lack of progesterone production and result infertility (1-3%) of infertility cases (Cavallaro *et al*, 2004).

Serum testosterone hormone concentration in males and females of white rabbits

The results of Fig. 4 showed a significant decrease ($P \leq 0.05$) in testosterone hormone in the male group treated with Mackerel fishes and the male group treated with fenugreek leaves powder compared to the control group. In the female group there was a significant difference of treatment with Mackerel fishes only

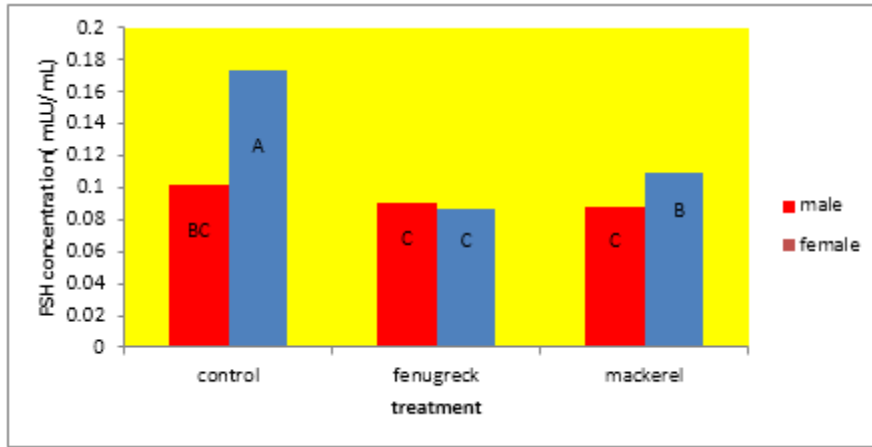


Fig. 1 : Demonstrates results of hormone measurement FSH.

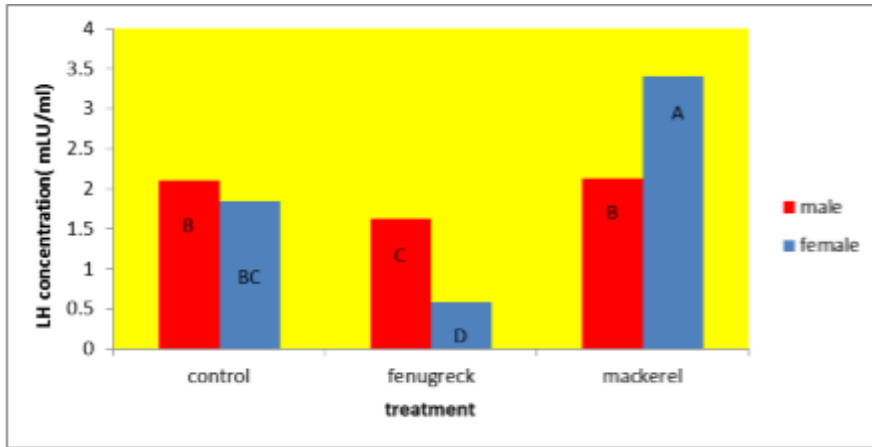


Fig. 2 : Demonstrates results of hormone measurement LH.

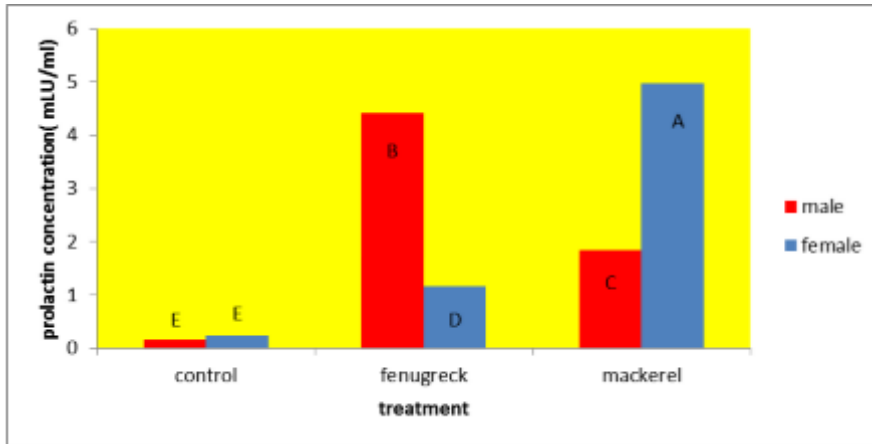


Fig. 3 : Demonstrates results of hormone measurement prolactin.

compared to the control group of.

The fenugreek has a negative effect on fertility in male white mice because of its effect on the production and secretion of lactic acid hormone (Baglan, 2006). This is consistent with the current study. And that the rise of the hormone FSH leads to a high level of testosterone (Speranda and Paptic, 2004), the relationship is positive between the (FSH and Testo. hormones), this explains why the decline of the hormone testo. through the current

study; due to low of FSH hormone. Yasilada (2008) and Cascella (2006) note that increased LH is considered as a cause of increased testosterone. Both LH and testosterone are increased in women with PCOS. This is demonstrated by the reduced testosterone in the current study due to low LH hormone, so the FSH, LH reduction is ultimately due to increased prolactin hormone, that is, the relationship between hormones (FSH, LH) and hormone (Testo) is a direct relationship, and between

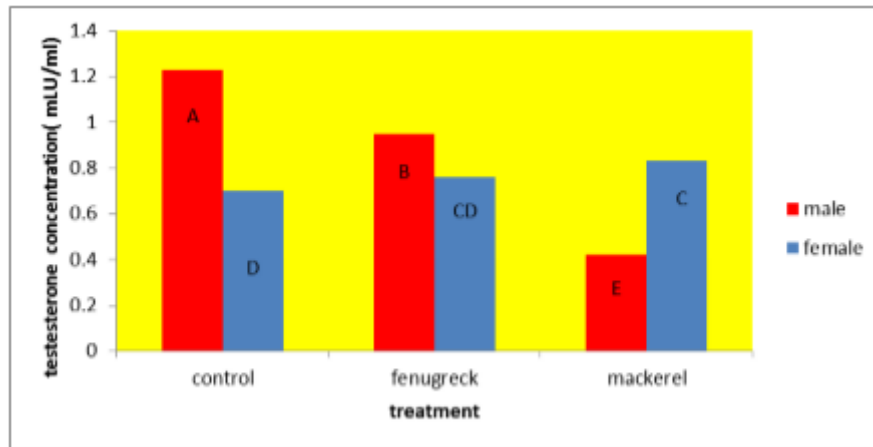


Fig. 4 : Demonstrates results of hormone measurement testosterone.

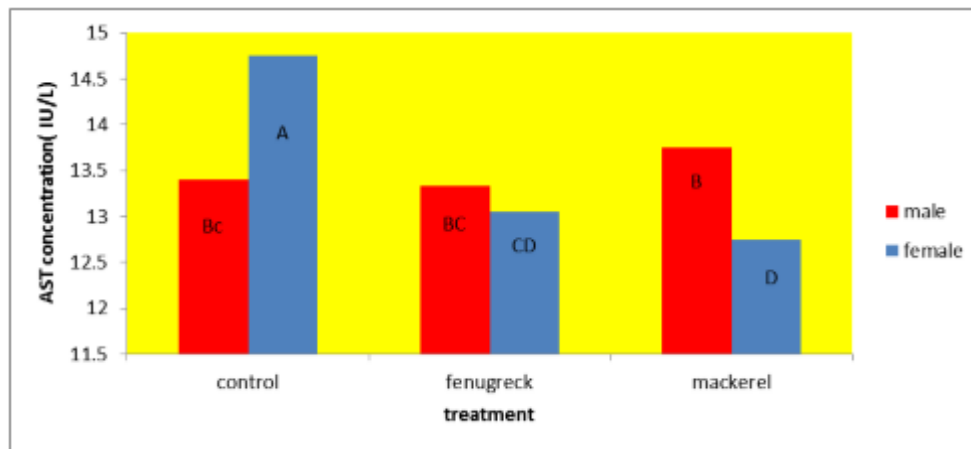


Fig. 5 : Demonstrates results of measurement AST.

(FSH, LH) and hormone (PRL) inverse relationship. There is also an inverse relationship testosterone hormone and prolactin hormone, as the increase in prolactin is associated with lower testosterone (Gill, 2008), which is consistent with the current study.

Effect of fenugreek leaves powder and mackerel fishes powder on the activity of liver enzymes

The activity of serum (AST) of males and females white rabbits

The results of Fig. 5 showed that although the male group did not show significant differences between the fenugreek and Mackerel fishes compared with the control, while in the female group showed a significant difference compared to control, although there were no significant differences between the two treatments.

No significant decrease in liver enzyme (AST) in male rabbits treated with fenugreek leaves powder showed that the fenugreek leaves powder used in the experiment had no toxic effect on hepatic cells, indicating that the cells in the hepatic tissue retained their form and no degradation occurred (AoAc, 1985), which showed that the use of fenugreek leaves powder during a specified

period does not affect the level of the enzyme (AST) in the homogeneity of the liver cells during the tests (enzymatic tests) and this result was identical to the study conducted by Muralidhara and others (AoAc, 1985), which they noted that hepatic enzymes remain almost at the level of proteins and cholesterol in the body when this powder was used even showed changes, but they are minor and insignificant, the decrease in the effectiveness of the enzyme in the females treated with fenugreek leaves powder may also be due to plant containment of compounds that have the potential to eliminate free radicals and reduce oxidative stress.

The results obtained indicate that the fenugreek leaves powder has no toxic effect on the liver tissue and the enzymes separated from it. The decrease in the level of the enzyme (AST) was shown to be due to the fish content of omega-3, which activate the process of oxidation of fatty acids in peroxisomes and mitochondria and reduce the production of fatty acids, triglycerides and VLDL-C (Lombardo and Chicco, 2006). Omega-3 acts on the production of anti-inflammatory fatty acids derived from Omega-3, namely resolpin E, D1 and proteectin that protect liver cells from oxidative stress (Gonzalez, 2009).

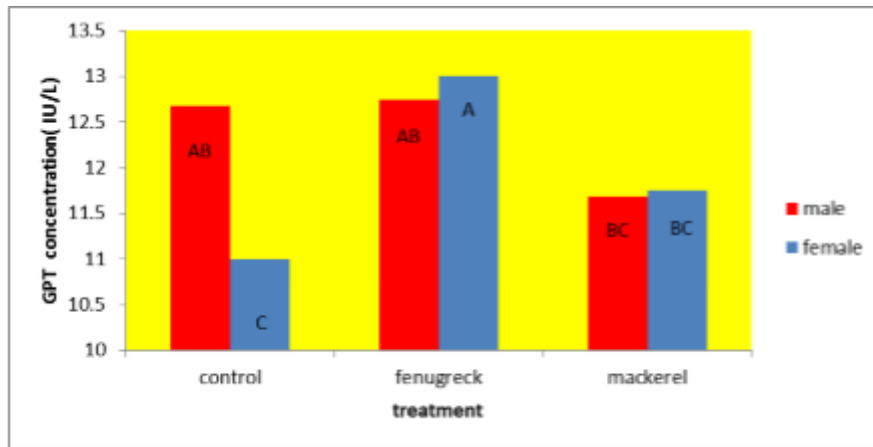


Fig. 6 : Demonstrates results of measurement ALT.

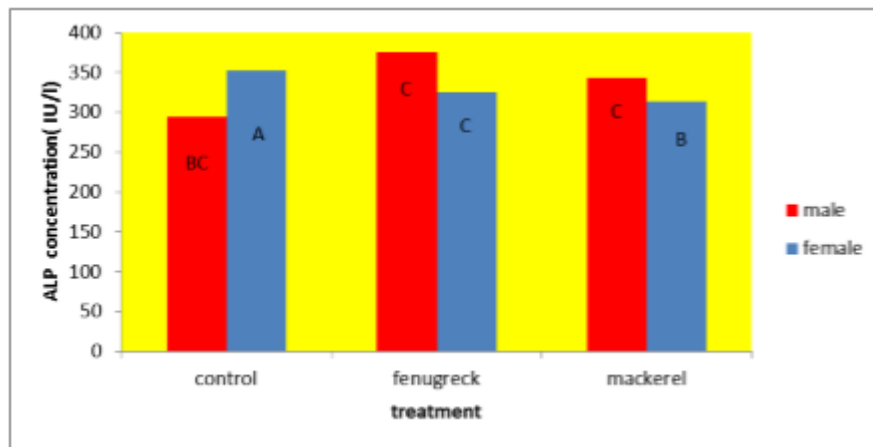


Fig. 7 : Demonstrates results of measurement ALP.

The activity of serum (ALT) of males and females white rabbits

The results of Fig. 6 showed a significant increase ($P \leq 0.05$) in the ALT enzyme in the female groups treated with fenugreek leaves powder and treated with Mackerel fishes compared to the control group. The treatment of the fenugreek leaves powder was higher than the mackerel treatment in the male group; the mackerel treatment was significantly lower than the control treatment. The treatment of the fenugreek leaves powder shows no significant difference compared to control.

The reason for the increase of ALT in the female group treated with fenugreek leaves powder due to the increase in milk production in the treatment resulted in increased requirements of more amino acids for milk production, which led to increase the activity of liver enzymes (Vihan, 1987). These results were consistent with what found (Abo El-Noor *et al*, 2007), when using 200 g of fenugreek seed in the feed / for each buffalo, and with what (Al-Rawi, 2011) found, who indicated a significant increase in the liver enzymes in the blood of ewes fed on the 1.5 and 3% of the fenugreek leaves powder, also the increase in milk production in the female

group treated with fish powder explained the increase in the effectiveness of this enzyme.

Effect of serum alkaline phosphatase enzyme (ALP) of males and females white rabbits

The results of Fig. 7 showed a significant increase ($P \leq 0.05$) in the ALP in the two groups of males treated with the fenugreek leaves powder and treated with mackerel compared to the control group. The group of females showed no significant difference between the treatments.

The alkaline phosphatase enzyme is hydrolyzing enzymes found in the liver, intestines, bones, heart and kidneys (Al-Saayady *et al*, 2007). And the absence of significant differences in the two groups of females treated with fenugreek leaves powder compared to control group shows that the fenugreek leaves powder that was used in the experiment did not have a toxic effect on the liver cells, indicating that cells in the hepatic tissue has retained their form and did not occur any analysis of hemolysis, this explain the elevation of enzyme (AST) in the homogeneity of liver cells during tests (enzymatic tests).

This result was identical to a study conducted by Muralidhara and others (AoAc, 1985), which showed that the use of fenugreek leaves powder during a specific period does not affect the level of hepatic enzymes, but remains almost the level of proteins and cholesterol in the body even if the changes showed but they are minor and ineffective, elevation of the effectiveness of ALP is usually due to many diseases such as liver or bile duct disease or gallstones. Elevation of ALP may indicate bone disease; such as Osteopenia or Osteoporosis. Blood disorders also increase ALP and some cancers (Martin, 1985; Fauci, 2008; Kumar, 2009).

Elevated levels of the ALP were also observed at 2-3 times during the prenatal period compared to the normal rate in non-pregnant women (Sembaj *et al*, 1999; Vongthavaravat *et al*, 2000). In addition, there is a contribution to the production of this enzyme from bone and other organs such as kidney and liver (Alonso, 2006).

CONCLUSION

1. The fenugreek and Mackerel fishes powder have a large capacity in the rise of milk hormone (Prolactin)
2. Large amounts of fenugreek and Mackerel fishes powder have a negative effect on fertility.
3. The results showed that the fenugreek and Mackerel fishes have no toxic effect on the liver.

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