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RESEARCH ARTICLE

Physiological Effect of Carob Seeds and Mulberry Leaves on Local Male Rabbits

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ABSTRACT

Carob and mulberry are medicinal plants that capable to alleviate or cure diseases and used traditionally as a remedy in a population or community. Even currently, the employment of medicinal plants as therapies has been increasing enormously due to numerous advantages such as less costly and acceptance among population due to a long history of use. The current study was aimed to detect the effect of carob seeds and mulberry leaves on levels of cholesterol, triglyceride, glucose and insulin in local male rabbits. Totally, 32 male rabbits were prepared and divided equally into four groups; C that feed basal pellets, G1 fed the pellets of mulberry leaves powder, G2 fed the pellets of carob seeds powder and G3 fed the pellets of mixed carob seeds and mulberry leaves powders. In comparison to control group, the findings of glucose, cholesterol, triglyceride and insulin were shown no significant differences in their values at zero time; whereas at 1 and 2 months, significant decreases in values of above mentioned markers were seen in all experimental groups (G1, G2, and G3). In comparison to zero time, values of glucose, cholesterol and insulin but not triglyceride were increased significantly in C group, while significant decreases in their values of experimental study groups (G1, G2 and G3) at 1 month and more apparently at 2 months were recorded. In conclusion, both plants were contributed effectively in supporting of health status, feeding of mulberry leaves appears more activity than carob seeds. Also, both plants were appeared as safe when be used as unconventional feed resources since no adverse effects or mortalities were occurred. However, furthermore studies are necessary to extract active substances existed in both plants, which could potentially used in pharmaceutical and food sectors.

1. INTRODUCTION

Medicinal plants are defined as those capable of alleviating or curing diseases and they have a traditional use as a remedy in a population or community. Its use is one of the oldest practices for treatment, cure and prevention of diseases (Zahra et al., 2020; Alhazmi et al., 2021). According to World Health Organization (WHO) it is estimated that almost 80% of the people living in the underdeveloped nations depend on traditional and complementary medicines for their basic health care (Qadir and Raja, 2021). Even currently, the employment of medicinal plants as therapies has been increasing enormously due to numerous advantages such as less costly and acceptance among population due to a long history of use (Araak, 2012; Al-Nasrawi, 2017; Barwary et al., 2019; Merkhan et al., 2019). However, it is worth mentioning that certain medicinal plants contain potentially dangerous substances and, for this reason, they should be used carefully, respecting their toxicological risks (Oladeji et al., 2024). Therefore, the use should have their pharmacological actions proven in scientific, pre-clinical and/or clinical tests (Rahimi et al., 2024).

Carob (Ceratonia siliqua) is one of the important nutritional and medicinal trees of the Middle East and Mediterranean basin, and in recent decades, it has been grown and cultivated in many other regions in the world (Shahrajabian and Sun, 2024). It belongs to the plant family and derives from

the Greek word "Kera". These fruits have keratomorphic shapes which are the Latin word siliqua that refers to the pods' hardness and shape (Ikram et al., 2023). Research of classical medicinal and related properties is ongoing; but in the last decade, there has been a notable increase in the studies of carob as food and nutrition source (Gioxari et al., 2022). It is important to emphasize that numerous studies were published to identify the optimal methods of cultivating this tree and to exploring the new activities (Clodoveo et al., 2022; Ahmadian et al., 2023; Martins-Loução et al., 2024). Along with these studies, many have investigated the effect of growth stressors such as dry farming (Correia and Pestana, 2018), or growth promoters like ultrasonic radiation (El Kahkahi et al., 2019), iron and other nutrients (Gama et al., 2020), and cytokinins (Costa-Pérez et al., 2023).

Mulberry (Morus alba), is a small to medium-sized tree in the family Moraceae, which native to temperate Asia and North America as fast deciduous grow plant with short proliferation under various environmental conditions (Zięć et al., 2021; Khafaji, 2022). Due to its chemical composition and pharmacological function, mulberry considers as one of the most conventional herbs used in medicine since time immemorial (Ma et al., 2022). Mulberry having exclusive therapeutic properties that are primarily due to their indigenous chemicals along with their anti-oxidant, anti-diabetic, anti-hypertensive, anti-cancer, hepatoprotective properties, and many more. Most important and relevant pharmaceutical ability of mulberry to improve human health and well-being is discussed by many authors (Jan et al., 2021; Vyry et al., 2024). The current study was aimed to detect the effect of carob seeds and mulberry leaves on levels of cholesterol, triglyceride, glucose and insulin in local male rabbits.

MATERIALS AND METHODS

Ethical approval

This study was licensed by the Scientific Committee of Department of Veterinary Public Health in the College of Veterinary Medicine (University of Baghdad, Baghdad, Iraq).

Preparation of carob and mulberry powders

Carob pods samples, collected from carob trees found in different local areas, were cleaned and the seeds were separated, dried (40°C / 24 hours) and grinded to get the fine powder. Then, the powder was mixed with wet pellets (15 gm carob / 1000 gm pellets) to form the pasty material that re-processed by the meat grinding machine to form new pellets having carob seeds powder (Guenaoui, 2020).

The mulberry leaves samples were collected from the mulberry trees of different local areas, cleaned, dried (80°C / 24 hours), and grinded to get the fine powder. Then, the powder was mixed with the wet pellets (15 gm mulberry leaves / 1000 gm pellets) to form the pasty material that re-processed by the meat grinding machine to form new pellets having mulberry leaves powder (Wen et al., 2019).

Pellets containing both carob seeds and mulberry leaves were prepared as following: 15 gm carob seed powder + 15 gm mulberry leaves powder / 1000 gm pellets. Then, the pasty material was re-processed by the meat grinding machine to form new pellets having powders of both carob seeds and mulberry leaves.

Study design

This study was designed to investigate feeding effect of carob seeds and mulberry leaves on health status of rabbit; therefore, a total of 32 male rabbits of 2-3 months age old were purchased, acclimated, and divided equally into four groups using plastic cages as following:

1. Group 1 as control (C): In which, study rabbits (Total No. 8) were received the basal pellets without additives and tap water.
2. Group 2 as experimental group 1 (G1): In which, study rabbits (Total No. 8) were received the pellets of mulberry leaves powder and tap water.
3. Group 3 as experimental group 2 (G2): In which, study rabbits (Total No. 8) were received the pellets of carob seeds powder and tap water.
4. Group 4 as experimental group 3 (G3): In which, study rabbits (Total No. 8) were received pellets of mixed carob seeds and mulberry leaves powders in addition to tap water.

Blood sampling and biochemical analysis

At zero time, 1 month and 2 months, the study animals of all groups were subjected to draining of venous blood into free-anticoagulant glass gel tubes. After centrifugation, sera were measured to detect the concentration glucose, cholesterol, and triglycerides following the manufacturer instructions of the specific test kits (BioSystems, Spain); while, insulin was assayed using the ELISA kit (Calbiotech, Germany).

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference groups in study parameters. Least significant difference (LSD) and One-Way ANOVA were used to estimate significant differences between the values (Means ± Standard Error) of the study groups at $P<0.05$ (Gharban and Yousif, 2020).

RESULTS

Throughout study groups, the findings of glucose were differed insignificantly ($P>0.05$) at zero time, while it's decreased significantly ($P<0.05$) in experimentally groups at 1 month and 2 months. Concerning study period, glucose was increased significantly ($P<0.05$) at 1 month and 2 months when compared to value of zero time, and decreased significantly ($P<0.05$) in experimentally G1 and G3 at 1 month and 2 months but at 2 months only for G2 (Table 1, Figure 1).

Table (1): Effect of carob seeds powder and mulberry leaves powder on glucose (mg/dl) in sera of male rabbits

Group	Time		
	Zero time	1 Month	2 Months
C	**B** 143.90 ± 2.72 **a**	**A** 154.17 ± 1.58 **a**	**A** 153.76 ± 1.94 **a**
G1	**A** 142.93 ± 2.52 **a**	**B** 130.37 ± 2.83 **c**	**C** 121.32 ± 1.59 **b**
G2	**A** 144.06 ± 3.14 **a**	**A** 137.94 ± 3.26 **b**	**B** 123.63 ± 2.13 **b**
G3	**A** 143.71 ± 2.92 **a**	**B** 120.72 ± 0.86 **d**	**C** 104.72 ± 2.82 **c**
LSD	**6.94**		

Different small vertical and large horizontal letters refer to significant differences ($P<0.05$)

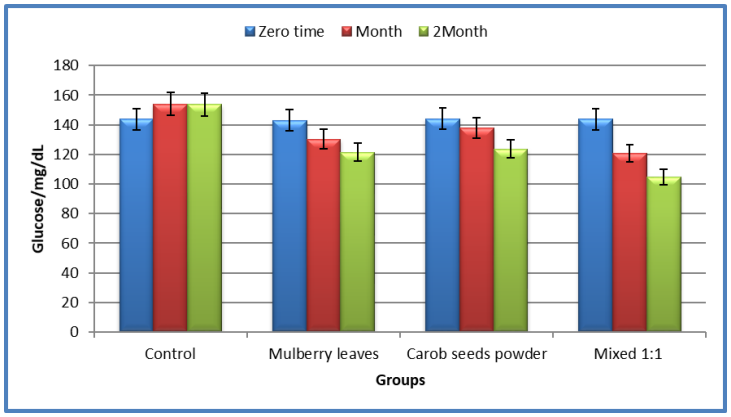

Figure (1): Effect of carob seeds powder and mulberry leaves powder on glucose in sera of male rabbits

In comparison to C group, the findings of experimentally study groups (G1, G2 and G3) showed that there was insignificant variation ($P>0.05$) in values of cholesterol at zero time; however, it decreased significantly ($P<0.05$) at G2 and more apparently in G1 and G3. Throughout the study period, values of cholesterol were increased significantly ($P<0.05$) at 1 month and 2 months when compared to zero time; but reduced significantly ($P<0.05$) in experimentally study groups at 1 month and more apparently at 2 months when compared to those of zero time (Table 2, Figure 2).

Table (2): Effect of carob seeds powder and mulberry leaves powder on cholesterol (mg/dl) in sera of male rabbits

Group	Time		
	Zero time	1 Month	2 Months
C	**B** 163.97 ± 2.15 **a**	**A** 174.78 ± 2.26 **a**	**A** 175.84 ± 2.20 **a**
G1	**A** 169.62 ± 2.23 **a**	**B** 142.84 ± 2.13 **c**	**C** 133.68 ± 2.16 **c**
G2	**A** 165.50 ± 3.16 **a**	**B** 157.26 ± 3.70 **b**	**C** 147.20 ± 3.23 **b**
G3	**A** 165.85 ± 2.23 **a**	**B** 147.04 ± 2.13 **c**	**C** 138.72 ± 1.70 **c**
LSD	7.05		

Different small vertical and large horizontal letters refer to significant differences (P<0.05)

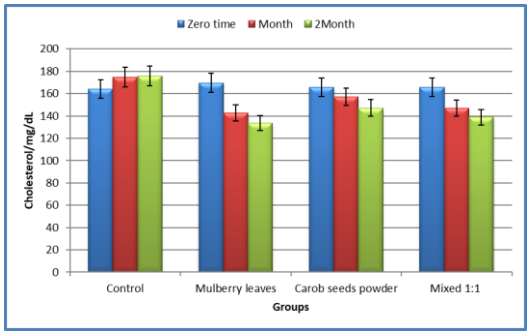

Figure (2): Effect of carob seeds powder and mulberry leaves powder on cholesterol in sera of male rabbits

Compared to values of C group, the findings of triglyceride among all experimentally groups (G1, G2, and G3) were varied insignificantly (P>0.05) at zero time, but decreased significantly (P<0.05) at 1 month and 2 months. Related to study period, values of triglyceride in C group were shown no significant variation (P>0.05) at zero time, 1 month and 2 months; whereas, significant decreases (P<0.05) were recorded in all experimentally study groups (G1, G2 and G3) at 1 month and more apparently at 2 months (Table 3, Figure 3).

Table (3): Effect of carob seeds powder and mulberry leaves powder on triglyceride (mg/dl) in sera of male rabbits

Group	Time		
	Zero time	1 Month	2 Months
C	**A** 181.67 ± 1.36 **a**	**A** 184.02 ± 1.35 **a**	**A** 184.50 ± 1.66 **a**
G1	**A** 179.16 ± 2.40 **a**	**B** 145.56 ± 2.35 **d**	**C** 125.83 ± 2.87 **c**
G2	**A** 180.93 ± 3.20 **a**	**B** 169.15 ± 3.87 **b**	**C** 140.36 ± 3.54 **b**
G3	**A** 177.14 ± 2.94 **a**	**B** 160.82 ± 3.91 **c**	**C** 134.36 ± 2.17 **b**
LSD	7.81		

Different small vertical and large horizontal letters refer to significant differences (P<0.05)

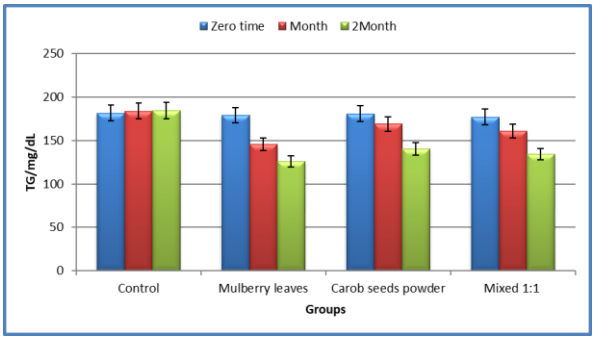

Figure (3): Effect of carob seeds powder and mulberry leaves powder on triglyceride in sera of male rabbits

Comparatively, the findings of insulin at zero time were not differed significantly (P>0.05) in G1 and G2 but decreased in G3; while at 1 month and 2 months, values of all experimentally groups (G1, G2 and G3) were reduced significantly (P<0.05) when compared to results of C group. For study period, insulin was increased significant (P<0.05) at 1 month and 2 months when compared to zero time; whereas, values of experimentally study groups were reduced significantly (P<0.05) at 1 month and months in G1 and G2, and at 2 months in G3 (Table 4, Figure 4).

Table (4): Effect of carob seeds powder and mulberry leaves powder on insulin hormone (mg/dl) in sera of male rabbits

Group	Time		
	Zero time	1 Month	2 Months
C	**B** 7.60 ± 0.11 **a**	**A** 8.62 ± 0.10 **a**	**A** 8.64 ± 0.10 **a**
G1	**A** 7.60 ± 0.14 **a**	**B** 7.05 ± 0.16 **b**	**C** 6.61 ± 0.17 **b**
G2	**A** 7.77 ± 0.12 **a**	**B** 6.98 ± 0.12 **b**	**C** 6.54 ± 0.11 **b**
G3	**A** 7.00 ± 0.10 **b**	**A** 6.70 ± 0.11 **b**	**B** 5.83 ± 0.15 **c**
LSD	**0.36**		

Different small vertical and large horizontal letters refer to significant differences (P<0.05)

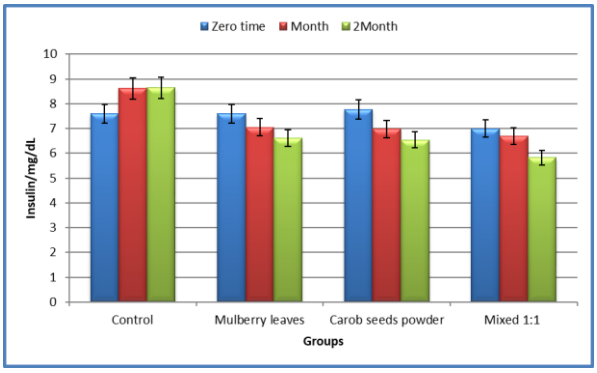

Figure (4): Effect of carob seeds powder and mulberry leaves powder on insulin hormone in sera of male rabbits

DISCUSSION

Research of classical medicinal and related properties is ongoing, but in the last three years, there has been a notable increase in the studies of medicinal plants as nutrition and drug sources (AL-Bayaty, 2006; Odhaib et al., 2021; Mohammed and Ayoub, 2022; Shnrwe and Ahmed, 2022). The findings of the current study revealed that the utilizing of each carob seeds, mulberry leaves or both as feed additives decreased significantly the levels of blood glucose, insulin, cholesterol, and triglyceride in particular at the end of experiment.

Bañuls et al. (2016) found that consumption of a carob pod inositol-enriched beverage in prediabetic subjects produces a response that is dependent on body mass index, with a clear improvement of insulin resistance and postprandial and nocturnal glycemia in non-obese subjects and a marked anti-inflammatory response in obese subjects. Macho-González et al. (2017) demonstrated the hypoglycemic properties of carob by modifying and decreasing of carbohydrate digestion and absorption, highlighting its potential role as a suitable nutritional strategy in diabetic patients. Rtibi et al. (2017) confirmed that carob has the ability to inhibit intestinal glucose absorption, improve glucose tolerance and protect against alloxan-induced diabetes in rat suggesting its use as a food supplement in hyperglycemia and diabetes treatments. Qasem et al. (2018) recorded that carob can inhibit α-amylase and α-glucosidase at low doses; but the high dose of carob exhibits an in vivo antihyperglycemic activity. Using two types of carob extracts, El-Haskoury et al. (2019) determined that ethyl acetate extract has more hypoglycemic effect than aqueous extract, and both extracts prevent the increment in blood glucose level. Macho-González et al. (2020) concluded that carob extract was strongly counterbalanced diabetic dyslipidemia by improving insulin signaling,

suggesting its being an adequate functional ingredient for type 2 diabetic patients. Navarro et al. (2022) showed that the administration of carob syrup to healthy individuals have resulted in less persistent glucose excursions, a lower insulin response to the hyperglycemia produced by its ingestion, and an enhanced glucagon / insulin ratio, compared to those ingested glucose. Also, researchers in the article have been seen that daily administration of the carob syrup to rats for 10 days lowered fat depots in the liver, reduced liver glycogen, promoted fat oxidation, and was devoid of toxic effects.

Relation to cholesterol and triglyceride, Zunft et al. (2003) reported that consumption of a 15-g/day carob fiber preparation for 6 weeks lowered serum total cholesterol (7.8 and 9.1%) and LDL cholesterol (12.2 and 11.0 %) in hypercholesterolemic subjects. Similarly, Ruiz-Roso et al. (2010) indicated that the consumption of insoluble carob fiber rich in polyphenols lowers the total and LDL cholesterol in hypercholestrolemic human patients suggesting it roles in prevention and treatment of hyperlipemia. Hassanein et al. (2015) seen that serum cholesterol and triglyceride levels were highly and significantly decreased in groups fed with the carob powder 10 and 20 % as compared with the control-positive group. El Rabey et al. (2017) concluded that carob extracts supplementation has a protective effect against hyperlipidemia and improved the histological alteration in heart and liver tissues of rats. El-Haskoury et al. (2019) determined that ethyl acetate extract was significantly ameliorated the lipid and liver function disorders induced by diabetes.

In recent decade, many studies have shown that mulberry leaf has good hypoglycemic effects (Wei et al., 2018). Clinically, mulberry leaf or mulberry leaf-based prescriptions is commonly used for the treatment of diabetic patients with satisfactory results (Qian et al., 2015, Tian, 2019). Modern pharmacological studies have also shown that various extracts and compounds from mulberry leaf can inhibit the increase of blood glucose and treat diabetes mellitus (Ge et al., 2018; Zhang et al., 2022; Liu et al., 2023). Zhang et al. (2024) confirmed that mulberry leaf multi-components exert hypoglycemic effects through regulation of the PI-3K/Akt insulin signaling pathway in diabetic rats. Mulberry is commonly used as an alternative medicine and reported to contain many antioxidative flavonoid compounds that having free radical scavenging effects in addition to presence many phenolics that can reduce cardiovascular disease (Wen et al., 2019; Hu et al., 2021).

Mulberry has also been reported to have hypoglycemic, hypolipidemic and antioxidant effects (Chen et al., 2023). Mulberry showed high antioxidant activity in LDL oxidation assay (Shi et al., 2023). Mahboubi (2019) discovered the inhibitory effects of mulberry on digestive enzymes and adipocyte differentiation, with its stimulatory effects on energy expenditures, and lipid metabolism in obese patients. Chaiwong et al. (2021) mentioned that dried mulberry prevented hyperphagia, body weight gain, and visceral fat accumulation, lowered blood glucose, plasma triglyceride and elevated plasma HDL beyond baseline. Li et al. (2024) summarized the efficacy of phytochemicals, molecular mechanisms and product applications of mulberry leaf in the prevention and treatment of obesity and related metabolic diseases. Phytochemical composition and nutritional potentials of mulberry varieties have studied worldwide (Ma et al., 2022). Mulberry leaves are used as infusion in Asian countries most common in Japan and Korea due to the presence of steroids, flavonoids, amino acids, vitamins, triterpenes and other trace elements show valuable effects (Sabuj, 2016; Ozturk et al., 2023).

Conclusions

In this study, both plants were contributed effectively in supporting of health status, feeding of mulberry leaves appears more activity than carob seeds. Also, both plants were appeared as safe when be used as unconventional feed resources since no adverse effects or mortalities were occurred. However, furthermore studies are necessary to extract active substances existed in both plants, which could potentially used in pharmaceutical and food sectors.

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