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Short Communication
In vivo, Lipid Profile Efficacy of Ethanol Extracts of *Stevia rebaudiana* Bertoni in Rabbits

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Abstract

Background and Objective: Contraceptive pills are chemical substances used as a means to prevent pregnancy, but they have several effects, including high lipid profile and in many cases, patients with heart and blood diseases cannot use it as a contraceptive helps in increasing the risk of cardiovascular diseases (CVD). A *Stevia* extract with high sweetening capacity due to its content of glycosides is used to reduce lipid profile and this study aimed to decrease lipid profile levels and lowering the risk factor in women using contraceptive drugs by stevia extracts. **Materials and Methods:** Sixteen rabbits have been used as a case-control study design due to their anatomical and physiological similarity to humans. The stevia leaves are extracted using Soxhlet apparatus of ethanol solvent. Statistical package (SPSS), were used for data analysis and management using independent sample t-test, test, comparison of means for lipid profile of Triglyceride (TG), Cholesterol, High-density lipoprotein (HDL-C), Low-density lipoprotein (LDL-C) between (di-contraceptive, mono-contraceptive and control groups). **Results:** The results showed increasing cholesterol and LDL-C during the combined oral contraceptive (COCP) and progesterone-only pills with decreased HDL-C level. A comparison of means before and after stevia used explains the elevated HDL-C and decreased LDL-C. **Conclusion:** The lipid profile levels should continuously be monitored during oral contraceptive intake and Stevia leaf powder extraction is suggested to reduce the risk of CVD.

Key words: Stevia-contraceptive, oral contraceptive, soxhlet apparatus, cardiovascular disease, LDL, HDL cholesterol, lipid

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oral Contraceptives (OCs) are widely used by females worldwide. It works mainly by the inhibition of ovulation. Furthermore, it induces an endometrium that is not receptive to ovum implantation and cervical mucus that becomes thick and hostile to sperm transport[1]. The first strategy to treat menstrual irregularity, acne and hirsutism includes Oral Contraceptive Pills (OCPs)[2-4] which help to control acne, heavy periods and endometriosis as well as reducing the risk of ovarian, uterine and colon cancers. Currently, there are three types of oral contraceptive pills: Combined estrogen-progesterone combined pills (combined oral contraceptive (COCP), progesterone-only that can be used while breastfeeding and continuous or extended use Pill[5-7]. On the other, there are many modern contraceptive methods uses by females like implants: Small, flexible rods or capsules placed under the skin of the upper arm; contains progestogen hormone only can be used for 3-5 years. Intrauterine device (IUD) copper-containing: Small flexible plastic device containing copper sleeves or wire that is inserted into the uterus[8]. Contraceptive use has increased in many parts of the world. In Palestine 21,035 women from the Gaza strip, used hormonal contraceptive pills and a rise of 53.8% from years ago[9], however, it's not the conditions for Asia and Latin America. In the period between 2008 and 2015, there was an increase in young women (15-49 years old) used a modern contraceptive method. For example, in Africa, the percentage increased from 23.6-28.5%, in Asia it has increased from 60.9-61.8%, but in Latin America and the Caribbean, it has stayed at 66.7%[10]. Although wide use between females many researchers showed statistically significant differences, found the changing in lipid profile among users of OCPs compared to non-users[11-14]. Currently, cardiovascular diseases are the main cause of death in Chile and worldwide[15,16]. In 2015, the World Health Organization reported that 17.7 million people died from cardiovascular diseases (equivalent to 31% of the total deaths in the world) and were responsible for 27% of the deaths in Chile. Projections indicate that the number of deaths due to cardiovascular diseases will increase to 23.3 million by 2030[17]. The pharmacological drugs used for the treatment of hyperlipidemia, are either too expensive or have certain adverse side effects[18,19]. Therefore, for good and safe treatment many traditional plants have been preferred as a natural source of drugs, *Stevia rebaudiana* Bertoni is a famous plant due to its sweet taste and beneficial effects in biochemical regulation and several studies have reported many health benefits associated with steviaconsumption[20-22]. It is a genus of plants native to subtropical and tropical regions of South and Central America. *Stevia rebaudiana* Bertoni has the highest sweetness potential due to its high content of diterpene glycosides named Stevioside and Rebaudioside. These compounds are steviol glycosides, which are formed by replacing the carboxyl hydrogen atom with glucose, xylose and rhamnose[23]. Generally in cultivated varieties, the main steviol glycosides are stevioside, rebaudioside A, rebaudioside C and Dulcoside A[24,25]; Stevioside is 250-300 times sweeter than sucrose but with a bitter aftertaste, while rebaudioside A is 350-450 times sweeter than sucrose without aftertaste[24,26]. The variety of *Stevia*, weather conditions and farming are factors that contribute to the amount and type of glycosides in the plant. For example, there is one variety of *Stevia* with low amounts of rebaudioside A and rebaudioside C, while in another variety the main component is rebaudioside C[27]. Moreover, even the same variety can have a higher content of glycosides if it is exposed to high solar radiation or grown with specific crop density[28] and several studies have reported many health benefits associated with stevia consumption.

The current study describes the efficiency of *Stevia rebaudiana* to control the lipid profile during contraceptive intake. Data presented here show that *Stevia* reduces the lipids level and may protect against cardiovascular complications.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacy and Biotechnology in Pharmacology and Clinical Biochemistry laboratories from October, 2018-June, 2019.

Experimental animals: Sixteen adult female rabbits (Flemish giant) of average age 4 months adult female this age is suitable for fertilization and ability to produce new generation; selected to be approximate with the same weight. Rabbits were divided into two groups of eight animals each, 1st group included healthy and those that received only distilled water. The 2nd group was divided into subgroups each included 4 rabbits treatment with mono-contraceptive and the others were 4 rabbits treatment with di-contraceptive, the extract of the leaf stevia plant is given to the 2nd after stop giving the contraceptives.

Drug preparation dose for rabbits: Weight 3 tablets of OCPs to 70 kg depend on the standard female weight and take the average the second step grinding 3 tablets by mortar and pestle then dissolved the average weight of powder in 100 mL volumetric flask finally, 100 mL solution a teach dry is obtained. Rabbits were given No. of milliliter suited with their weight according to the following equation.

Stevia preparation and extraction: Soxhlet method of ethanol extraction (70%) of collected stevia leaves are done, the plant materials are collected from herb store around the Gaza city, the fresh leaves are dried at room temperature away from the direct sunlight for 20 days after washed to remove the dirt adhered to the surface. its grounded, then 60 g of the grounded leaves were used in the extraction, which has been done using the Soxhlet for two days, the solvent was evaporated at room temperature by leaving it in the using rotary evaporator.

Analysis of serum lipid profile of rabbits: Random samples were measured within ten days of sample collection by "Enzymatic Colorimetric quantitative determination Method" on UV-Visible spectrophotometer Germany, Model by using standard DiaSys kit for TG, TC, HDL-C, LDL-C and standard and calibrator vial.

Statistical analysis: Data were computer analyzed using SPSS/PC (Statistical Package for the SocialScience Inc. Chicago, Illinois USA, version 22.0) statistical package. The main test used in this study was the paired sample t-test. The results in all the above-mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p<0.05$). The statistical tests were conducted on the sample for this study paired t-test and this test was conducted on the statistical SPSS version 22.

RESULTS AND DISCUSSION

The data of Table 1 illustrates that for healthy rabbit there are no significant differences in lifestyle and food of this group which similar to the lifestyle and food of other groups except that the other two groups treated with contraceptive drugs mono and COCPs, However, the table showed a statistically significant increase in the indicators of lipid treatments in experimental rabbits ($p<0.05$).

Data in Table 2 shows a small decreasing level in the value of lipid profile tests in the sample of untreated rabbits with stevia but without statistical significance and this indicates that there was no external factor affecting lipid profile changes other than stevia. While, the rabbits which were treated with stevia trials showed decreasing levels of lipid parameters except for the value of HDL, which recorded an increasing result. Furthermore, the decrease in the result was statistically significant for TG and LDL ($p = 0.001$ and 0.000) respectively.

Table 1: Lipid profile parameter among the three groups (healthy rabbits (n = 8) and rabbits that use monovalent contraceptives (n = 4) and the combined contraceptive (n = 4)

Parameters		TG	CHOL	LDL	HDL
Healthy rabbits	Before	132.9±19.1	84.9±18.4	41.4±20.3	20.7±5.8
	After	136.8±18.5	85.8±17.9	42.2±19.5	21.6±4.8
	t-test	0.420	0.098	0.076	0.354
	p-value	0.681	0.924	0.940	0.729
Rabbits with monovalent contraceptives	Before	108.9±8.5	77.9±9.9	26.6±5.7	37.3±4.5
	After	133.7±7.1	105.5±14.9	43.3±7.0	61.6±4.5
	t-test	5.357	8.920	11.475	6.829
	p-value	0.013	0.003	0.001	0.006
Rabbits with the combined contraceptive	Before	123.4±34.8	98.8±15.9	25.1±9.1	50.0±17.2
	After	146.6±32.1	116.2±14.2	37.8±9.5	63.3±19.5
	t-test	7.428	5.183	3.857	10.227
	p-value	0.005	0.014	0.031	0.002

*Mean difference is significant at $p<0.05$

Table 2: Lipid profile parameter among the two groups (healthy rabbits (n = 8) and rabbits that use contraceptives (n = 8))

Parameters		TG	CHOL	LDL	HDL
Rabbit without *Stevia* treatment	Before	136.8±18.5	85.8±17.9	42.2±19.5	21.6±4.8
	After	135.9±17.6	84.6±18.3	41.3±20.2	21.4±4.6
	t-test	0.104	0.132	0.094	0.102
	p-value	0.918	0.897	0.926	0.920
Rabbit treated with *Stevia*	Before	140.1±22.6	110.9±14.7	40.5±8.2	62.5±13.1
	After	132.2±23.7	107.0±17.6	28.8±8.9	62.8±12.2
	t-test	5.726	0.479	7.770	0.307
	p-value	0.001	0.356	0.000	0.767

Hyperlipidemia and CVD is a common public health problem in the world, the prevalence of which is rising in the users of OCPs in Palestine[9] as well as Asia and Latin America[10]. Hormonal contraceptive pills are in clinical practice for the control of birth however, hormonal replacement therapy had been reported to be associated with increased risk of cardiovascular diseases[29]. This study focused on the changes in lipid profile during users' types of OCPs treatments. Also, it was statistically significantly different between the groups. In a similar study[30], the users of COCs had been reported to be associated with a higher serum lipid profile than non-users contraceptive pills[31,32]. Concerning the lipid profile levels in our present study, an increase in LDL, TC and TG was found among rabbits treated with COCP. This increase was found to be in correlation with the duration of OCs intake but HDL-C is slightly decreased. It is important to perform additional studies to corroborate the possible natural drug for lowering the lipids and decrease the risk factor of CVD that comes from hyperlipidemia. The stevia plant is one of these choices. The sweet herb of stevia R. is fast becoming a source of high-potency sweetener, which produces a sweet taste that is found in the leaf of the plant but has no calorific value so it is well known as a therapeutic agent and for losing fat and weight[33,34]. In this study, the results are comparable with that in the research of Ahmad andUswa[20], George *et al.*[35] who reported that stevia extract may reduce the LDL-C, TC and TG levels in rabbits. Furthermore, the increase in the result for HDL-C was statistically significant between rabbit groups that were treated with stevia.

CONCLUSION

Although androgens hormones are present normally in women, the presence of it in the form of OCs may lead to a high concentration of lipid profile in the human body. The lipid profile levels should continuously be monitored during oral contraceptive intake. Stevia leaf powder extraction is suggesting have hypoglycemic and hypolipidemic activity can be used to reduce the risk of CVD in the future.

SIGNIFICANCE STATEMENT

This study describes the herbal use that can be beneficial for females during OCPs intake to eliminate the risk factor of CVD. The study uncovers the critical role of *Stevia rebaudiana* to decrease the lipid profile after androgen hormonal tablets use. Thus, a new theory on the combination of OCPs and Stevia is important.

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