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The Potential Influence of *Coleus forskolii*, Green Coffee and Green Tea on Body Weight, Reproductive Performance, Hematological Profiles in Mice

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ABSTRACT

Background: *Coleus forskolii*, green coffee and green tea have been shown to effect on body functions and health.

Methods: Twenty males and thirty female albino mice of 33.86 ±0.27g BW were classified into two groups; control group fed basal control diet versus treated group fed basal control diet containing multi-ingredient supplement (*Coleus forskohlii*, green coffee bean and green tea; 1.5% per supplement) for ten weeks. Initial and final body weights were recorded of control and supplemented groups. Germinal vesicle oocytes were harvested from ovaries after 48:0h of PMSG injection for investigating oocyte quality. Furthermore, female mice were mated with fertile males and pregnancy rate and offspring numbers were recorded. In addition, the male mice of both groups were sedated using 26.6 mg/kg BW xylazine for recording rectal temperature, pulse rate and SPO_2. Besides, blood samples were collected for complete blood picture and plasma biochemistry analyses.

Result: The results showed that multi-ingredient supplement caused significant decrease in body weight gain versus increase ($P<0.05$) of rectal temperature. Reproductive performances represented by oocyte quality and pregnancy rate were significantly decreased in multi-ingredient supplement group. Furthermore, blood cells and plasma profiles (urea, creatinine, glucose, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase) were decreased in multi-ingredient supplement groups except total protein if compared to control group. It could be concluded that multi-ingredient supplement resulted in significant decrease of body weight gain, reproductive performances and blood cell profiles as well.

Key words: *Coleus forskolin*, Glucose, Green coffee bean, Green tea, Oocyte, Reproductive.

INTRODUCTION

Dietary supplements are given to stimulate health and alleviate body dysfunctions in mammalian species (Mohammed, 2012, 2018, 2019; Mohammed and Al-Khamis, 2024; Mohammed *et al*., 2018, 2020, 2021, 2024a,b,c,d; Al Masruri *et al*., 2022a,b; Al-Saiady *et al*., 2024). *Coleus forskohlii*, green coffee bean and green tea were found to increase body weight loss, diabetes, thermogenesis and cardiovascular health (Litosch *et al.,* 1982; Godard *et al.,* 2005; Thom, 2007; Revuelta-Iniesta *et al.,* 2014; Huang *et al.,* 2014; Roshni and Rekha, 2024). Therefore, the potential effects of those ingredients were explored on changes of body weight gain, reproductive performances concerning oocyte quality and pregnancy rate, blood and plasma profiles (Mohammed *et al.,* 2024d,e).

Coleus forskohlii is a medicinal plant cultivated in India and other countries of Asia and eastern Africa (Tung *et al.,* 2021). The demand for *C. forskohlii* plant is increasing because it is importance in dietary and beverage supplements, pharmaceuticals and cosmetics usages (Roshni and Rekha, 2024). *C. forskohlii* plant was used for forskolin compound production (Mitra *et al.,* 2020; Kundur and Shyam, 2024). Dietary *C. forskolin* plant and forskolin compound were found to increase lipolysis, improve diabetes and cardiovascular health and increase blood flow to the brain (Amezcua *et al.,* 2022; Abbasi *et al.,* 2023; Roshni and Rekha, 2024).

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The effects of coffee extract on body functions were investigated in several studies including blood glucose and insulin values post exercise (Beam *et al.,* 2015). No significant effects of green coffee bean extract for blood glucose and insulin values were obtained. In addition, coffee extract effects on lipid metabolism were explored in several studies (Choi *et al.,* 2016; Takahashi *et al*., 2017; Farias-Pereira *et al.,* 2018). The doses were given in diet

(1-2%) or water (0.1-0.5%) or according to body weight (200-1000 mg/kg BW). The results showed decreases in body weight, glucose, triglycerides, cholesterol, HDL, LDL, insulin and leptin values versus increases of ATP levels.

In addition, green tea was shown to reduce appetite and increase thermogenesis. Besides, blood pressure and lipid profiles were changed due to green tea supplementation (Onakpoya *et al.,* 2014). Dietary green tea was significantly reduced systolic blood pressure, total cholesterol and LDL-cholesterol values. Collectively, the ingredients of *Coleus forskohlii* (Mohammed *et al.,* 2024), green coffee bean (Thom, 2007; Revuelta-Iniesta *et al.,* 2014) and green tea (Huang *et al.,* 2014) were known to increases lipolysis and thermogenesis *via* a variety of biological pathways (Litosch *et al.,* 1982; Godard *et al.,* 2005; Thom 2007; Revuelta-Iniesta *et al.,* 2014; Huang *et al.,* 2014). They have been associated with changing body composition (Barrea *et al.,* 2019) and may simultaneously influence the main pathways underlying obesity. Therefore, the aims of the current study were to explore the changes in body weight gain, rectal temperature, pulse rate and SPO_2 of control and multi-ingredient supplement groups. Besides, reproductive performance concerning oocyte quality and pregnancy rate were recorded as well. In addition, the changes in hematological profiles and plasma biochemistry values were investigated as well.

MATERIALS AND METHODS

The experimental procedures were approved by the ethical committee of King Faisal University [KFU-REC-2024-OCT-EA241670]. The *C. forskolin* dried leaves were obtained from farm located in Gizan area of KSA. The leaves were arranged in a single layer on a baking sheet of oven and they were exposed to 50-60°C for sixteen hours. In addition, green coffee bean (*Coffe arabica*) and green tea were purchased from the market. The experimental procedures were carried out in the experimental animal lab of Agriculture and Food Sciences College, KFU University.

Site of study and animal management

The current study was executed from August to October 2024 in animal lab of animal and fish production department. Twenty males and thirty females albino mice were used for the study (body weight; 33.86±0.27 g) (Fig 1). Mice were kept in groups of five mice per cage (40×24× 18 cm) of control and the treated groups. The animals were given pellet diet (Arasco, KSA), which composed of 2.90% fat, 22.0% protein, 3.20% fiber, 1.0% mixture of vitamins and minerals and energy of 3300.00 kcal/kg. The supplemented doses of *C. forskolin*, green coffee bean and green tea were 1.5% of each supplement according to the recommended doses of previous studies (Shimoda *et al.,* 2006; Murase *et al.,* 2010; Farias-Pereira *et al.,* 2018; Mohammed *et al.,* 2024 d,e). The ground basal diet was mixed with 1.5% powders of each supplemented ingredient and pelleted. Animals had free access to water and diets. Mice were kept controlled under 12 h light cycle and 12 h dark starting at 8 a.m. The relative humidity (%) and temperature (°C) values during the study were controlled to 25±2.50°C and 45±9.0%, respectively. The duration of study was twelve weeks.

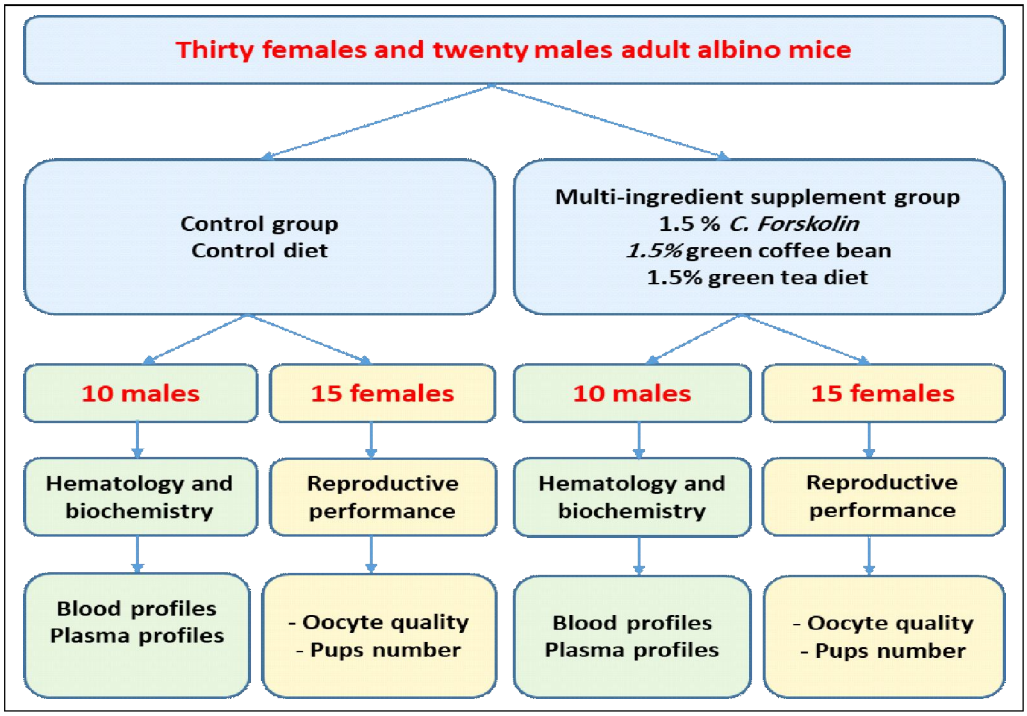


Fig 1: Experimental design of multi-ingredient supplement impact on thermo-tolerance parameters, blood and plasma profiles in DX anesthetized mice.

Monitoring oocyte quality and reproductive performance

Five females of each group were injected with 7.5 IU of PMSG to synchronize ovarian follicle development. The injected females were sacrificed through cervical dislocation 48h after PMSG injection. Ovaries were removed and germinal vesicle oocytes were released by puncturing of ovarian follicles with 30-G sterile needles under a stereomicroscope. The oocytes were released into TCM199 medium supplemented with BSA. The diameters of denuded GV oocytes were determined to the nearest micrometer using 0.01 mm eyepiece on a compound biological microscope (Mohammed, 2009; Mohammed and Al-Hozab, 2019). For reproductive performance, 10 females of each group were bred with male of proven fertility for eight weeks. The pregnant females and delivered pups were recorded.

Monitoring body weight, rectal temperature, heart rate, partial pressure of oxygen and values

Body weights (g) were recorded at the beginning and end of study (Sartorius balance, Azulmart-KSA). The male mice of both groups were sedated using 26.6 mg/kg BW xylazine for immediate recording of rectal temperature, pulse rate and SPO_2. Rectal temperatures were monitored using clinical thermometer (Citizen) (Mohammed, 2018; Mohammed *et al.,* 2024e,f). Pulse rate and partial pressure of oxygen were recorded using pulse oximeter device (CMS60D-VET).

Blood sample collection and analyses

Blood samples were collected from the orbital sinus of control and multi-ingredient supplement groups at the end of experiment (Hoff, 2000). The obtained blood samples were analyzed using automatic hematology analyzer (Mythic 5Vet PRO Hematology Analyzer) and biochemistry analyzer (Skyla VB1). The resulting blood parameters include red and white blood cell count, hematocrit and hemoglobin. The resulting biochemistry parameters include total proteins, liver enzymes, urea, creatinine and mineral values.

Statistical analysis

Body weight and rectal temperature, SPO_2 and pulse rate, oocyte quality and reproductive performance and blood and plasma profile values of control and multi-ingredient supplement groups were statistically analyzed using General Linear Model procedure of SAS (SAS 2008) according to the following model:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where:

μ = Mean.

Ti = Effects of multi-ingredient supplement.

e_{ij} = Standard error.

Duncan's multiple range test (1955) was used to compare between means of control and multi-ingredient supplement groups.

RESULTS AND DISCUSSION

Body weight and temperature, SPO_2 and pulse rate, oocyte quality and reproductive performance and blood and plasma biochemistry profiles of multi-ingredient supplement and control groups are presented in Table (1-4).

Body weight, rectal temperature, SPO_2 and pulse rate

Changes of body weight, rectal temperature, SPO_2 and pulse rate are presented in Table (1). Value of final body weight (g) was significantly decreased in multi-ingredient supplement group compared to that of control group. On the other hand, hyperthermia of rectal temperature (°C) was recorded in multi-ingredient supplement group. Additionally, pulse rate and SPO_2 were not differed between both groups.

Monitoring oocyte quality and reproductive performance

Values of oocyte quality and reproductive performance are presented in Table (2). Percentages of GV oocytes with diameter 70.0 and 80.0 µm were lower in multi-ingredient supplement group compared to those of control group versus the percentage of GV oocytes with diameter 60.0 µm if compared to control group. Simultaneously, pregnancy rate and number of pups per female were decreased ($P<0.001$) in multi-ingredient supplement group compared to control one.

Blood and plasma profiles

Values and blood and plasma profiles are presented in Table (3-4). Values of blood parameters were significantly improved in control group compared to multi-ingredient supplement group (Table 3). Regarding to plasma profiles, total protein values were increased whereas urea, creatinine, glucose, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenasevalues were significantly decreased in multi-ingredient supplement group versus control group.

Results of the current study are presented in Table (1-4) indicating the effects of dietary (1.5%) *C. forskohlii*, green coffee bean and green tea for each supplement on body weight, reproductive performance, hematological profiles in mice. Generally, the obtained results indicated significant decrease of body weight gain, blood and plasma profiles in addition to increase of rectal temperature and plasma total protein in the multi-ingredient supplement group compared to control group. Furthermore, the reproductive performances represented by oocyte quality and pregnancy rate and delivered pups were significantly decreased in the multi-ingredient supplement group compared to control group. These effects could be owing to the common ingredients in *C. forskohlii*, green coffee bean and green tea including botanicals (herbs and other plant components), caffeine, dietary fibers and minerals. Both *C. forskohlii,* green coffee bean and green tea contain various compounds, some of which can be potentially harmful if consumed in excess amount.

Dietary *C. forskohlii* and its extracted compound, forskolin, have been explored for a variety of potential health benefits (Amezcua *et al.,* 2022; Roshni and Rekha 2024). These benefits may include body weight loss, improved diabetes and cardiovascular health, increased muscle mass and enhanced blood flow to the brain (Abbasi *et al.,* 2023; Mohammed *et al.,* 2024d,e). The primary active ingredient in green coffee is chlorogenic acid. This compound is believed to be responsible for many of the potential health benefits as anti-obesity, regulating glucose and lipid metabolism, antioxidant and anti-inflammatory impacts (Tajik *et al.,* 2017; Yu *et al.,* 2022; Nguyen *et al.,* 2024). The primary active ingredient in green tea is a group of compounds called polyphenols, specifically catechins (Farhan, 2022). Among these catechins, epigallocatechin gallate is considered the most potent and abundant compound. These compounds are responsible for many of the health benefits attributed to green tea as antioxidant,

Table 1: Changes of body weight (g), rectal temperature (°C), pulse rate and SPO_2 (%) and glucose values (mg/dl) of multi-ingredient supplement and control groups in mice.

Parameters	Treat	Control	SE	P value
Initial BW, g	33.88	33.84	1.18	0.97
Final BW, G	38.75^b	43.10^a	1.55	<0.001
Rectal temperature, °C	36.68^a	36.46^b	0.11	<0.001
Pulse rate/min.	165.7	165.3	6.24	0.97
SPO_2, %	82.3	81.6	3.17	0.96

a, b: Values between groups with different superscripts significantly differ at P<0.05.
SE: Standard error.

Table 2: Changes of oocyte quality and reproductive performance of multi-ingredient supplement and control groups in mice.

Parameters	Treat	Control	SE	P value
Total collected oocytes/5 females	70.0^b	80.0^a	0.72	<0.01
No. collected oocytes/female	14.0^b	16.0^a	0.72	<0.02
Diameter, 60 µm	61.4^a	46.25^b	3.10	<0.001
Diameter, 70 µm	25.7^b	35.00^a	2.66	<0.001
Diameter, 80 µm	12.90^b	18.75^a	2.55	<0.001
Pregnancy rate, %	30.0^a (3/10)	80.0^a (8/10)	1.40	<0.001
Pups number/female	8.0^a	9.125^a	0.74	<0.01

a, b: Values between groups with different superscripts significantly differ at P<0.05.
SE: Standard error.

Table 3: Changes of blood profiles of multi-ingredient supplement and control groups in mice.

Parameters	Treat	Control	SE	P value
RBCs, 10^6/µl	4.50^b	6.40^a	0.18	<0.0001
PCV, %	22.7^b	29.7^a	1.21	<0.0001
Hb, g/dl	8.00^b	10.0^a	0.16	<0.0001
MCV, fl	50.6^a	46.4^b	3.41	<0.07
MCH, pg	18.0^a	15.8^b	1.12	<0.05
MCHC, g/dl	36.0	34.0	2.36	<0.07
RDW, %	14.9^b	17.0^a	0.98	<0.04
WBCs, 10^3/ml	5.25^b	8.73^a	1.32	<0.0001
Neutrophils, 10^3/µl	2.451^b	2.604^a	0.09	<0.01
Lymphocyte, 10^3/µl	2.002^b	4.505^a	0.14	<0.0001
Monocyte, 10^3/µl	0.437^b	1.219^a	0.001	<0.0001
Eosinophils, 10^3/µl	0.309^b	0.299^a	0.001	<0.06
Basophils, 10^3/µl	0.051^b	0.103^a	0.0002	<0.0001
PLT, 10^3/µl	1016^b	1293^a	15.19	<0.001

a, b: Values between groups with different superscripts significantly differ at P<0.05.
SE: Standard error; RBCs: Red blood cells; PCV: Packed cell volume; Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; WBCs: White blood cells; PLT: Platelet.

Table 4: Changes of profiles of multi-ingredient supplement and control groups in mice.

Parameters	Treat	Control	SE	P value
Total protein, g/dl	5.1[a]	4.9[b]	0.15	0.01
Urea, mg/dl	24.0[a]	28.0[a]	1.04	<0.0001
Creatinine, mg/dl	0.50[b]	0.70[a]	0.03	<0.0001
Glucose, mg/dl	81.0[b]	104.0[a]	2.50	<0.0001
Aspartate Aminotransferase, U/l	180.0	205.0	13.3	<0.06
Alanine Aminotransferase, U/l	216.0	261.0	13.5	<0.04
Lactate dehydrogenase, U/l	513	551	21.9	0.07
Iron, µmol/L	296	312	10.83	0.09

a, b: Values between groups with different superscripts significantly differ at P<0.05.
SE: Standard error.

weight management, improved diabetes and heart health properties (Du *et al.,* 2012; Legeay *et al.,* 2015; Mohammed and Al-Hozab 2019).

Collectively, the percentage 1.5% of each supplement resulted in lower body weight gain, hyperthermia and hypoglycemia if compared to basal control diet. Furthermore, values of blood cells and plasma profiles (urea, creatinine, glucose, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase) were decreased in multi-ingredient supplement groups except total protein if compared to control group. It seems that such supplements gave adverse effects in some parameters due to excess supplemented amount or harmful ingredients as caffeine, tannins and oxalates (Noonan and Savage 1999; Duda-Chodak and Tarko 2023). Tannins are polyphenols found in both green tea and green coffee offer some health benefits, while excessive tannin intake can interfere with iron absorption and may worsen digestive issues (Delimont *et al.,* 2017; Duda-Chodak and Tarko 2023). Excess caffeine can affect various aspects of female reproductive performances including slight decrease estrogen levels, possible delay in conception, increased risk of miscarriage, stillbirth and lower birth weight (Wen *et al.,* 2001; Karypidis *et al.,* 2006; Weng *et al.,* 2008; Greenwood *et al.,* 2010; Wikoff *et al.,* 2017; Bu *et al.,* 2020), where we found lower quality of GV oocytes and pregnancy rate in this study. In addition, the lower reproductive performance in supplement group could be also attributed to decrease of steroid hormone values due to the changes in lipid metabolism, low glucose level and body weight gain (Senosy *et al.,* 2018; Mohammed *et al.,* 2022).

CONCLUSION

It could be concluded that *C. forskohlii*, green coffee bean and green tea multi-ingredient supplement modulates body weight gain, thermo-tolerance response, blood and plasma profiles. On the other hand, multi-ingredient supplement resulted in negative effects reproductive efficiency represented by oocyte quality, pregnancy and delivered pups. Further studies are still required for exploring multi-ingredient supplement effects through the level of supplementation and the resulting physiological body functions and reproductive performance.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The Ethical Committee of Deanship of Scientific Research, King Faisal University, Saudi Arabia, approved all animal procedures, experimental animal care and handling techniques for experiments (KFU242726).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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