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Potential Impacts of Dried Green *Coleus forskolin* Leaves on Thermo-tolerance Parameters, Blood and Plasma Profiles in Anesthetized Mice

A.A. Mohammed[1], M.S. El Sayed[2], I. AlGherair[1], S. Al-Suwaiegh[1]

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ABSTRACT

Background: Transient negative side effects of hypothermia, hyperglycemia*and* bradycardia due to general anesthesia using diazepam and xylazine drugs has been reported in mice.

Methods: Twenty albino male mice of 31.52±0.25 g body weight were classified into two groups; control group (G1) fed basal control diet versus *C. forskolin* group fed basal control diet containing *C. forskolin* leaves (G2; 1.5%) for four weeks. The mice were general anesthetized using xylazine and diazepam (XD) drugs (13.3 mg/kg BW diazepam and 26.6 mg/kg BW xylazine). Changes of rectal temperature (°C), glucose (mg/dl), pulse rate (pulse/min.) and partial pressure of oxygen (SPO_2) were recorded at 0.0, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 5.0 h and 7.0 h over general anesthesia. In addition, blood samples were collected and were subjected to blood and plasma analyses.

Result: The results indicated that dietary *C. forskolin* caused significant decrease (P<0.05) in glucose values in addition to significant increase (P<0.05) in rectal temperature, pulse rate and SPO2 during the period hypothermia, hyperglycemia and bradycardia over general anesthesia. In addition, values of blood (RBCs, WBCs, PCV and Hb) and plasma parameters (TP, BUN, creatinine, AST and ALT, LDH) were normal due to *C. forskolin* supplementation compared to con
trol diet. It could be concluded that 1.5% *C. forskolin* supplementation modulates thermo-tolerance responses, blood glucose indices, blood and plasma metabolites over general anesthesia.

Key words: Anesthesia, Blood, *Coleus forskolin,* Hyperglycemia, Hypothermia.

INTRODUCTION

Coleus forskohlii is an Indian-origin medicinal plant cultivated in India and other countries of Asia and eastern Africa (Tung *et al.,* 2021). There is an increasing commercial demand of *C. forskohlii* plant from industries for dietary supplements, food, beverages, pharmaceuticals and cosmetics usages (Roshni and Rekha, 2024). The *C. forskohlii* plant has recently gained popularity for production of forskolin compound (Mitra *et al.,* 2020; Kundur and Shyam, 2024).

Dietary *C. forskolin* and its extract, forskolin (Godard *et al.,* 2005; Amezcua *et al.,* 2022; Roshni and Rekha, 2024), were used for a wide range of potential functions including body weight loss, improve diabetes and cardiovascular health, increase muscle mass and blood flow to the brain (Abbasi *et al.,* 2023). Therefore, it is expected to restore the transient negative effects of XD general anesthesia over dietary *C. forskolin* supplementation. Generally, forskolin is considered safe for most people when taken in a recommended dose. The *C. forskolin* leaves dose (1.5%) of the current study were chosen as spice to food or drinks. Wang *et al.* (2009) and Kanne *et al.* (2015) explored the chemical constituents of Coleus *forskohlii*. Twelve compounds were isolated and identified including forskolin and rosmarinic acid. It was found through HPLC to determine the polyphenol content of leaf extracts of *C. forskohlii* that rosmarinic acid had the highest concentration (Kundur and Shyam, 2024). Both forskolin and rosmarinic acid had effects on body functions (Abbasi *et al.,* 2023; Hwang *et al.,* 2025). Rosmarinic acid alleviates septic acute respiratory distress syndrome, improved skeletal muscle mass and strength and radical scavenging activity (Vo *et al.,* 2024; Zeng *et al.,* 2024; Hwang *et al.,* 2025).

The potential impacts of dietary supplements on body functions and therapeutic of diseases were explored in several studies (Mohammed *et al.,* 2020; 2024a,b,c). Appropriate dietary strategies were given for mitigation of

[1]Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, P.O. Box 402, Al-Ahsa 31982, KSA.

[2]Avian Research Center, King Faisal University, P.O. Box 402, Al-Ahsa 31982, KSA.

Corresponding Author: A.A. Mohammed, Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, P.O. Box 402, Al-Ahsa 31982, KSA. Email: aamohammed@kfu.edu.sa.

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hyperglycemia and hypothermia over general anesthesia in mammalian species (Mohammed, 2012; 2018; 2019; Mohammed *et al.,* 2012; 2018; Al Masruri *et al.,* 2022). Effects of diet and dietary supplementation including *C. forskohlii* leaves on thermo-tolerance parameters, blood and plasma indices have been reported (Mohammed *et al.,* 2020; 2024a,b,c). *C. forskohlii* is an important plant rich in certain micro and macronutrients, which are important in nutritional and medicinal purposes for both animals and humans in tested dose (Mohammed, 2024c).

Therefore, the aims of this study were to investigate the changes in rectal temperature, pulse rate, SPO2 and blood glucose levels before (0 min) and 30 min, 1 h, 2 h, 3 h, 5 h and 7 h after general anesthesia using DX drugs of control and *Coleus forskolin* groups. In addition, the changes in hematological (RBCs, HGB, HCT and WBCs) and plasma biochemistry (total protein, albumin, glucose and blood urea nitrogen) is investigated.

MATERIALS AND METHODS

The experimental procedure was approved by the ethical committee of King Faisal University [Ref. No. KFU-REC-2024-AUG-EA241670]. The *Coleus forskolin* leaves were purchased from farm located in Gazan area of Saudi Arabia. The experimental procedures were carried out in the experimental animal lab of Agriculture and Food Sciences College of KFU University.

Site of study and animal management

The study was carried out during the period from May to August 2024 of animal and fish department animal lab. Twenty albino mice of adult males were used for the experiment (body weight; 34.52±1.2 g) (Fig 1). Mice were kept in groups of five animals in transparent cages (40 × 24 × 18 cm) of control and the *C. forskolin* groups. Mice were fed commercial pellet basal diet (Arasco, KSA), which composed of 2.90% fat, 3.20% fiber, 22.0% protein, 1.0% mixture of minerals and vitamins*and* 3300.0 kcal/kg energy. The ground basal diet was mixed with powder of *C. forskolin* leaves (w/w; 1.50%) and and pelleted (Mohammed *et al*., 2024c). The chemical composition of *C. forskolin* leaves includes 91.23% dry matter, 8.77% moisture, 13.05% crude protein, 12.02% crude fiber, 27.96% non-free extract and 3.31% ether extract. 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 temperature (°C) and relative humidity (%) during the experiment were controlled to

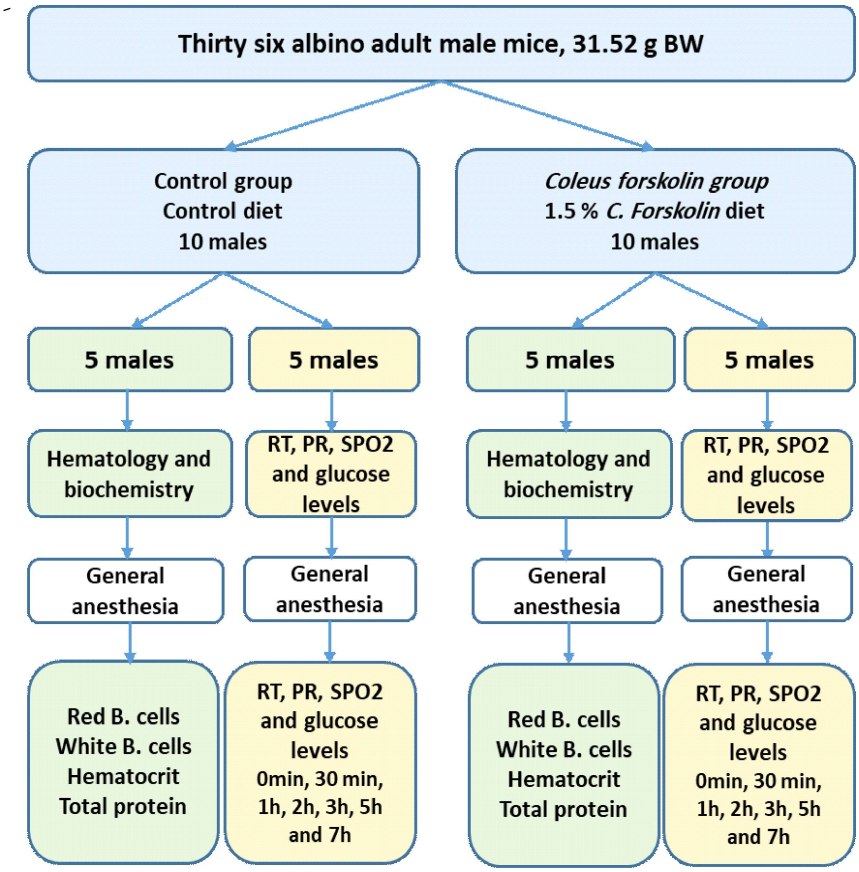


Fig 1: Experimental design of *C. forskolin* influences on thermo-tolerance parameters, blood and plasma profiles in anesthetized mice.

25.0±2.50°C and 50.0±8.0%, respectively. The *C. forskolin* feeding lasts for four weeks.

General anesthesia through xylazine and diazepam injection

After four weeks of feeding, fasting males for six hours were injected intraperitoneal with combination of 13.3 mg/kg body weight of diazepam (Neuril, Memphis Co. Egypt) and 26.6 mg/kg body weight of xylazine (Xylaject, Adwia Co. Egypt) for safe XD general anesthesia in mice (Mohammed, 2012; Mohammed *et al*., 2012, 2018; Al Masruri *et al*., 2022). The mice of control group injected intraperitoneal with 0.2 ml physiological saline. The general anesthesia injected dosage of xylazine and diazepam in this study was chosen according to the drugs' safety margin of our previous studies (Mohammed 2012, 2018, 2019; Mohammed *et al.,* 2012, 2018).

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

Rectal temperature, partial pressure of oxygen, heart rate and blood glucose values were monitored before (0 min) and 20 min, 40 min, 1 h, 2 h, 3 h and 4 h of DX general anesthesia of control and Moringa groups. During these chosen specific intervals, the changes in the recorded parameters were more pronounced in both control and treated groups (Mohammed, 2012; Mohammed *et al.,* 2012; 2018; Al Masruri *et al*., 2022). Rectal temperatures were recorded using clinical thermometer (Citizen). Pulse rate and partial pressure of oxygen (PO2) were recorded using pulse oximeter (CMS60D-VET Handheld Veterinary Pulse Oximeter) (Mohammed, 2018). Blood glucose values were recorded using blood glucose meter (Contour TS 4052 Basel, Switzerland) (Mohammed *et al.,* 2024c). The top of tail was punctured and the drop of blood put on strips for recording blood glucose values.

Blood sample collection and analyses

Blood samples were collected from the orbital sinus (Hoff 2000) at the end of experiment from mice of control and the *C. forskolin* groups. The collected blood samples were analyzed for blood chemistry analyzer (Skyla VB1). The readable blood parameters include red and white blood cells, hematocrit and hemoglobin. The readable plasma parameters include total proteins, liver enzymes, blood urea and creatinine and iron values.

Statistical analysis

Rectal temperature, glucose, pulse rate, partial pressure of oxygen, blood and plasma biochemistry values after xylazine and diazepam general anaesthesia were statistically analyzed using General Linear Model procedure of SAS (SAS 2008) according to the following model:

$$Yij = \mu + Ti + eij$$

Where,
μ = Mean.
Ti = Effect of *C. forskolin* (1.50%).
eij = Standard error.

Duncan's multiple range test (1955) was used to compare between means of control and *C. forskolin* groups.

Results and Discussion

Rectal temperature, heart rate, partial pressure of oxygen and glucose values of control and *C. forskolin* groups recorded before (0 min) and 30 min, 1 h, 2 h and 3 h, 5 h and 7 h of DX general anesthesia are presented in Table (1-4). Hematological and biochemical parameters of control and *C. forskolin* groups 7 h after general anesthesia using xylazine and diazepam drugs are presented in Table (5-6). The changes in rectal temperature values of control and *C.* forskolin groups recorded at 0.0, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 5.0 h and 7.0 h over general anesthesia are presented in Table (1). The significant hyperthermia was found in *C. forskolin* group compared to control one over general anesthesia.

The changes in glucose (mg/dl) values of control and *C. forskolin* groups recorded at 0.0, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 5.0 h and 7.0 h over general anesthesia are presented in Table (2). The significant hypoglycemia was found at 0.0 h and 3.0h in *C. forskolin* group compared to control one over general anesthesia.

The changes in pulse rate (pulse/min.) values of control and *C.* forskolin groups recorded at 0.0, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 5.0 h and 7.0 h over XD general anesthesia are presented in Table (3). During the recorded intervals,

Table 1: Rectal temperature (°C) changes over dietary *Coleus forskolin* supplementation (1.5%) in general anesthetized mice.

Rectal temperature, °C	Control	*C. forskolin*	SE	P value
0.0 h	36.92	37.12	0.15	0.04
0.5 h	35.36	35.48	0.18	0.05
1 h	31.9	32.3	0.18	0.001
2 h	32.1	32.6	0.19	0.001
3 h	32.8	33.1	0.17	0.001
5 h	33.90	34.1	0.20	0.001
7 h	34.90	36.15	0.21	0.001

SE: Standard error.

the pulse rates were restored earlier to normal values in *C. forskolin*. The restoration of the pulse rate was significant at 5 h and 7 h intervals. This gradual trend may indicate a stimulatory effect of *C. forskolin*. on cardiovascular parameters.

The changes in partial pressure of oxygen values of control and *C. forskolin* groups recorded at 0.0, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 5.0 h and 7.0 h over general anesthesia are presented in Table (4). The significant increase of pulse rate was found in *C. forskolin* group compared to control one over general anesthesia.

The blood and biochemical parameters of control and *C. forskolin* groups are presented in Table (5-6). Generally, the data indicated increased of WBCs and PCV (P>0.05) due to *C. forskolin* supplementation. In addition, plasma profiles concerning total protein, blood urea, creatinine, aspartate and alanine transferases were not observed in *C. forskolin* except significant decrease of urea (P=0.05) and significant increase of Lactate dehydrogenase (P= 0.001).

Results of the current study are presented in Table (1-6) indicating the effects of dietary *C. forskolin* leaves (1.5%) in general anesthetized mice. Generally, the data indicated that the values of thermos-tolerance, blood and metabolites in the *C. forskolin* group were modulated positively after general anesthesia compared to control one. Thus, *C. forskolin* supplementation (1.50%) alleviated the transient negative side effects concerning hypothermia, hyperglycemia and bradycardia through changes in blood and plasma metabolites and mineral profiles.

Dietary *C. forskolin* and its extract, forskolin (Amezcua *et al*., 2022; Roshni and Rekha, 2024), were used for a wide range of potential functions including body weight loss, improve diabetes and cardiovascular health, increase muscle mass and blood flow to the brain (Abbasi *et al*., 2023). Wang *et al.* (2009) and Kanne *et al.* (2015) explored the chemical constituents of *Coleus forskohlii*. Twelve compounds were isolated and identified from *C. forskolin* including forskolin and rosmarinic acid (Kundur and Shyam 2024).

Table 2: Glucose (mg/dl) changes over dietary *Coleus forskolin* supplementation (1.5%) in general anesthetized mice.

Glucose, mg/dl	Control	C. Forskolin	SE	P value
0.0 h	120.0	114.0	3.03	0.05
0.5 h	175.6	171.0	6.63	0.10
1 h	316.6	314.0	11.0	0.57
2 h	374.6	365.2	12.0	0.60
3 h	262.0	244.0	13.0	0.05
5 h	170.3	168.2	11.9	0.90
7 h	110.0	112.0	13.0	0.80

SE: Standard error.

Table 3: Pulse rate (pulse/min.) changes over dietary *Coleus forskolin* supplementation (1.5%) in general anesthetized mice.

Pulse rate, pulse/min.	Control	C. Forskolin	SE	P value
0.0 h	120.0	123.0	5.24	0.08
0.5 h	110.9	115.8	6.26	0.07
1 h	109.0	108.5	9.65	0.10
2 h	102.0	106.0	12.39	0.12
3 h	103.6	108.0	13.52	0.08
5 h	105.66	116.0	6.36	0.05
7 h	116.0	127.0	5.34	0.04

SE: Standard error.

Table 4: Partial pressure of oxygen (SPO2) changes over dietary *Coleus forskolin* supplementation (1.5%) in general anesthetized mice.

SPO2	Control	C. Forskolin	SE	P value
0.0 h	88.0	90.0	3.90	0.08
0.5 h	71.6	75.6	3.94	0.05
1 h	76.6	80.25	5.55	0.001
2 h	66.3	81.7	5.14	0.001
3 h	72.3	83.0	5.83	0.001
5 h	74.3	85.0	4.14	0.001
7 h	81.0	89.0	3.94	0.001

SE: Standard error.

Generally, forskolin is considered safe for most people when taken in a recommended dose. The percentage of *C. forskolin* leaves (1.5%) of the current study were chosen as spice to food or drinks.

Rectal temperature, heart rate, partial pressure of oxygen and glucose values of control and *C. forskolin* groups were recorded before (0 min) and 30 min, 1 h, 2 h and 3 h, 5 h and 7 h after DX general anesthesia (Table 1-4). The positive changes of the aforementioned parameters in *C. forskolin* group compared to control one indicates the importance of *C. forskolin* dose (1.5%) to alleviate the transient negative effects of DX general anaesthesia (Bristow *et al.,* 1984; Mohammed 2018; Al Masruri *et al.,* 2022). *C. forskolin* effects on thermo-tolerance parameters were reported in several studies (Mohammed *et al.,* 2024c). The effects of *C. forskolin* on thermo-tolerance parameters might be due to several factors including antioxidative properties and regulating pathways involved in the metabolism (Khatun *et al.,* 2011; Sameeh, 2023). In addition, these significant changes in thermo-tolerance parameters could be attributed to *C. forskolin* constituents including forskolin. Forskolin works by activating adenylate cyclase, increases the level of cAMP, which in turn plays pivotal roles in many cellular processes, including cell growth*and* metabolism (Alasbahi and Melzig 2012; Shaikh and Finlayson 2012; Rakhmanova *et al.,* 2023). The increase of rectal temperature of *C. forskolin* group compared to control group is owing to forskolin effect on thermogenesis (Abbasi *et al.,* 2023). In addition, Bristow *et al.* (1984) found that forskolin was a potent and a powerful activator of human myocardial adenylate cyclase and produced maximal effects. Furthermore, thermogenesis over *forskolin* supplementation has been confirmed (Mohammed *et al.,* 2024c) due to lipolysis and beta-oxidation (Zhang *et al.,* 2019).

Hematological and biochemical parameters of control and *C. forskolin* groups 7 h after general anesthesia using xylazine and diazepam drugs are presented in Table (5-6). The blood circulation and its profiles including blood and plasma components represents one of the most critical aspects of body health and functions. Antioxidants in *C. forskolin* are free radical scavengers, which protect the body defense system against excessively produced free radicals and stabilize health status of the stressed animal. Such natural antioxidants might involve in a number of oxidation and reduction reactions in the body. Blood glucose values were decreased in *C. forskolin* group might be due to forskolin effect as indicated in several studies (Abbasi *et al.,* 2023; Skelin Klemen *et al.,* 2023; Mohammed *et al.,* 2024c). Effects of *C. forskohlii* supplementation on hematological profiles in mildly overweight women were examined (Henderson *et al.,* 2005). The authors found none significant changes in red and white blood cells, blood lipids, muscle and liver enzymes and electrolytes as indicated in our study. It is important to mention that lactate dehydrogenase (LDH) value was increased ($P<0.001$) in *C. forskolin* group by about 20.0% compared to control group. This could be attributed to the earlier restoration of body functions to normal values in *C. forskolin* group compared to control one. Generally, lactate dehydrogenase catalyses the conversion of lactate to pyruvate and is commonly used as an indicator of tissue damage. LDH is found in a variety of body tissues. Serum LDH activity is tissue non-specific, although muscle, liver and erythrocytes may be the major source of high activity. Therefore, it is helpful to separate LDH into its different isoenzyme forms by electrophoresis to differentiate the LDH source (not explored in this study). On the other hand, the low LDH levels may be associated with major depressive disorder and suicidal behaviors (Yao *et al.,* 2022). Finally, the changes of blood and plasma profiles after *C. forskolin* supplementation and anesthesia because of the potential health effects of *C. forskolin* in modulating metabolism through rosmarinic acid, antioxidant and forskolin components.

Table 5: Blood profile changes over dietary *Coleus forskolin* supplementation (1.5%) in eneral anesthetized mice.

Parameters	Control	*C. forskolin*	SE	P value
WBCs, 10^3/ml	7.30	7.75	1.32	0.06
RBCs, 10^9/ml	8.33	8.16	0.68	0.10
PCV, %	40.06	40.26	3.41	0.16
Hb, g/dl	12.9	13.26	0.86	0.07

SE: Standard error.

Table 6: Plasma metabolites changes over dietary *Coleus forskolin* supplementation (1.5%) in general anesthetized mice.

Parameters	Control	*C.forskolin*	SE	P value
Total protein, g/dl	5.54	5.4	0.25	0.06
Urea, mg/dl	26.3	23.5	1.04	0.05
Creatinine, mg/dl	0.58	0.60	0.03	0.13
Aspartate aminotransferase, U/l	176.0	179.0	13.3	0.14
Alanine aminotransferase, U/l	219.0	223.0	13.5	0.18
Lactate dehydrogenase, U/l	519	623	31.9	0.001

SE: Standard error.

CONCLUSION

It could be concluded that *C. forskolin* supplementation (1.5%) modulates thermo-tolerance responses, blood glucose values, blood and plasma metabolites over general anesthesia. The occurrence of hyperthermia and hypoglycemia after general anesthesia was confirmed. In addition, green *C. forskolin* leaves might be used as feed additive since no negative effects was detected neither on blood or serum profiles. Further studies are required for exploring *C. forskolin* effects through the level of supplementation and the physiological body conditions during pre-partum and post-partum periods.

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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 enimal care and handling techniques for experiments (KFU 241670)

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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