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RESEACH ARTICLE

EFFECT OF CAFFEINE ON STRESS-INDUCED HYPERGLYCAEMIA IN FEMALE WISTAR RATS

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Abstract

Background: Caffeine is a commonly used psycho-active agent present in cocoa, coffee, tea and a lot of beverages. It is believed to ameliorate depression and anxiety due to its ability to antagonize the adenosine receptors in the CNS. The aim of this study is to find out the effect of caffeine on stress-induced hyperglycemia using underwater trauma and predator model of stress in female Wistar rats. **Methods:** The rats were first exposed to a cat for 30 minutes, followed by 20 seconds forced swimming in a plastic water container. After the forced swimming, the rats were submerged in water for another 10 seconds followed by a single dose of caffeine given intraperitoneally at dosage of 10, 20 & 40 mg/kg respectively. A week later, the serum glucose level of the rats was assayed using a glucometer. **Results**: Stress was found to significantly increase the serum glucose level of the rats with significantly reduction in the elevated blood glucose level in the caffeine treated group compared to the rats in the control group. **Conclusion**: Caffeine reduces stress-induced in female Wistar rats.

Key words: Caffeine, stress, hyperglycaemia, underwater, predator

Introduction

Hyperglycaemia and insulin resistance are features of altered metabolism in critical illness that occur due to release of cortisol, catecholamines, and cytokines, as well as glucagon and growth hormone

(Fangming *et al*., 2014). Caffeine (1, 3, 7- trimethyl xanthine) is a commonly used psychoactive drug used to lessen the effects of fatigue and to increase physical activity. It is a non-selective adenosine (A) antagonist with primary functions in the brain at Al and A2A receptors leading to increase in CNS activity (Swarup *et al.*, 2020).

Caffeine is among the most commonly consumed psychoactive beverage globally (Crocq, 2022). Its consumption increases alertness, concentration, cognitive performance and physical strength (Abdoli *et al*., 2024). Caffeine in higher doses can cause symptoms such as digestive discomfort, insomnia, and restlessness however, despite these effects; consuming caffeine at normal levels can protect against dementia and type 2 diabetes mellitus/T2DM (Abdoli *et al*., 2024).

Caffeine is commonly found naturally in cocoa beans, coffee and tea leaves and is also added to a variety of food and beverages. Coffee is among the most frequently consumed beverages in the world, and has been linked to beneficial effects on diseases; specially type 2 diabetes mellitus (Reis *et al.*, 2018). Coffee is a common beverage composed of numerous bioactive substances including caffeine. Epidemiological studies link moderate consumption of coffee with a reduced risk of developing T2DM. This association has been shown in several studies with different populations showing a consistent dose-response effect (Yarmolinsky *et al.*, 2015; Santos & Lima, 2016; Reis

et al., 2018). Caffeine can easily cross the blood-brain barrier and stimulate the central nervous system (Santosh *et al.,* 2019). Energy mobilization is a primary result of the fight or flight response. Stress stimulates the release of various hormones, which can result in elevated blood glucose levels. Although this is of adaptive importance in a healthy organism (Richard *et all.,* 1992).

Stress-induced hyperglycemia is when plasma glucose levels exceed 200mg/dL, which has been documented in patients undergoing severe trauma or injury. Hyperglycemia is believed to be adaptive in stressful conditions, but long-term stress induced hyperglycemia is linked to poor clinical outcomes and increased risk of mortality (Fangming *et al.,* 2014). Stress leads to a state of hyperglycemia by inducing insulin resistance, and increased blood glucose through the release of counterregulatory hormones including catecholamines, cortisol, glucagon, and growth hormone, which alter glucose hemostasis in addition to increase in inflammatory cytokines (McCowen *et al.,* 2001; Plummer *et al.,* 2014; Vedantam *et al.,* 2022). As a result, hepatic gluconeogenesis is poorly controlled. In addition, glucose uptake by the skeletal muscle via the GLUT-4 transporter is also decreased (McCowen *et al.,* 2001). Increased gluconeogenesis and insulin resistance are the key factors in development of stress hyperglycaemia (McCowen *et al.,* 2001). Interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) cause insulin resistance and also suppress insulin release, in a concentration-dependent manner. Increased levels of IL-6 in the blood cause insulin resistance which promotes hyperglycemia by depleting glucose from hepatic stores. (Vedantam *et al.,* 2022). Therefore, the HPA axis, proinflammatory cytokines (TNF-α, IL-1 and IL-6) and the sympatho-adrenal system act together synergistically to create hyperglycemia in stressful conditions. The body's neuroendocrine response to stressful conditions is characterized by elevated glycogenolysis, gluconeogenesis, and insulin resistance (Argyropoulos, *et al.,* 2021; Marik and Bellomo, 2013). Cortisol increases the blood glucose concentration via activation of key enzymes involved in hepatic gluconeogenesis and inhibition of peripheral uptake of glucose by tissues such as the skeletal muscles. Epinephrine and norepinephrine due to stressful condition activate hepatic gluconeogenesis and glycogenolyssis (Kuo *et al.,* 2015; Marik & Bellomo, 2013; Gustavson *et al.,* 2003). Norepinephrine additionally increases the supply of glycerol to the liver via lipid breakdown. Glucose uptake into cells is facilitated by GLUT-1, GLUT-3 and GLUT-4 glucose transporters. Insulin increases glucose transport and reduce serum glucose via GLUT-4-mediated transport by accelerating the translocation of GLUT-4 from intracellular stores to the cell membrane. Stress and the inflammatory response led to decreased translocation of this GLUT-4 transporter to the cell membrane. (Marik & Bellomo, 2013).

The aim of this study is to determine the effect of stress and caffeine on serum glucose of female Wistar rats subjected to predator and underwater stress.

EXPERIMENTAL DESIGN

I. Animal grouping and caffeine administration:

Twenty-five female Wistar rats and one home bred cat were used for this study. The cat was used for the induction of predator stress on the rats. The twenty-five female Wistar rats were divided into five groups (n = 5) of five as follows:

Group 1: Control, which receive 0.1 ml/100g body weight of 0.9% normal saline intraperitoneally (Pechlivanova *et al.,* 2010).

Group 2: subjected to predator and underwater trauma (Zoladz *et al*., 2008a; 2018).

Group 3: subjected to stress (predator and underwater trauma) + 10 mg/kg caffeine intraperitoneally (Pechlivanova *et al*., 2010).

Group 4: subjected to stress (predator and underwater trauma) + 20 mg/kg caffeine intraperitoneally (Pechlivanova *et al.,* 2010).

Group 5: subjected to stress (predator and underwater trauma) + 40 mg/kg caffeine intraperitoneally (Pechlivanova *et al*., 2010).

On the 1st day of the experiment, the rats were subjected to predator and underwater trauma, followed by single dose of caffeine intraperitoneally after 30 minutes. The

blood samples were collected for biochemical analysis on the 8th day.

II. Stress induction:

a. Predator exposure

Rats were first exposed to a cat for 30 minutes by placing them in a plastic container with a perforated partition that prevents direct contact with the cat (Zoladz *et al*., 2008).

b. Forced swimming and underwater trauma

After the cat exposure, the rats were subjected to forced swimming in a plastic water bath. Each rat was placed in a water bath and allowed to swim for 20 seconds. After the forced swimming, the rats were submerged and allowed to struggle under the water for another 10 seconds (Moore *et al*., 2012).

III. Blood glucose determination

After one week, the rats were fasted overnight (12-7am), after which the blood samples were collected via cardiac puncture after euthanasia via chloroform inhalation and their fasting blood glucose level was determined using Accu-check® active (Roche, Mannheim, Germany) glucose test kit. Each test strip was inserted into the glucometer and a drop of the test blood was put at the sample application area of the test trip to display the reading on the glucometer screen. This is due to ability of glucose oxidase on the test strip to convert glucose in the blood sample to gluconic acid, which reacts with another compound on the strip called ferricyanide to ferrocyanide. This generates electronic current through the blood sample on the strip, which is read by the glucometer.

a. Rat forced swim test.

b. Rat submersion test.

c. Rat predator exposure.

Plate I: Underwater stress (a & b) and Predator models (c) of stress.

a = rat forced swimming, b = rat submerged in water & c = predator stress, showing rats exposed to cat.

d. Statistical analysis

The data obtained from the study was analysed using version 23.0 of *Statistical Package for the Social Sciences (*SPSS) software using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test and the results were expressed as mean ± standard error of the mean (Mean ± SEM), with the level of significance set at $p < 0.05$.

RESULTS

Effect of Stress and Caffeine on Blood Glucose Level of Female Wistar rats.

Figure 1 shows significant increase in the blood glucose level of female Wistar rats in the stress group (115.00 ± 5.17 mg/dl) compared to control group (75.60 ± 5.92 mg/dl). The elevated blood glucose was significantly (P=0.000) reduced in the 10 mg/kg caffeine group (70.60 ± 3.63 mg/dl), 20mg/kg (78.40 ± 2.04 mg/dl) and 40 gm/kg (78.40 ± 2.04 mg/dl) caffeine treated groups compared to the stress group (115.00 ± 5.17 mg/dl) group with much decrease at 10 mg/kg. (F = 19.117, P = 0.000).

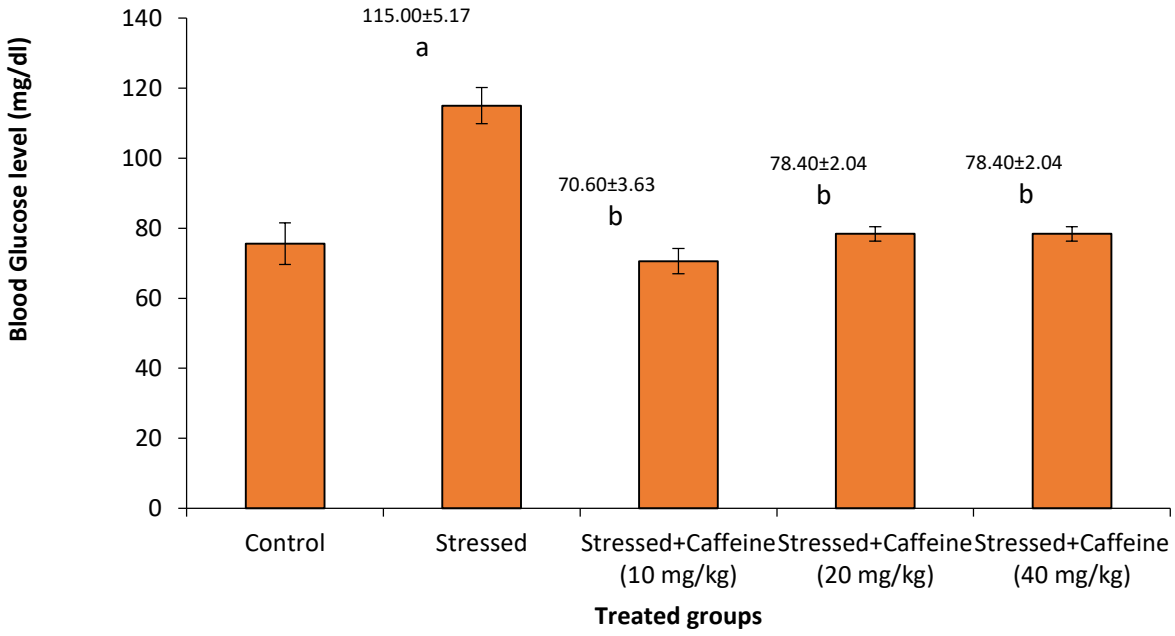

Figure 1: Effect of stress and caffeine on blood glucose level of female Wistar rats.

Superscripts [a] = significant increase compared to normal control and [b] = significant decrease compared to stress control group.

Table 1: Effect of Stress and Caffeine on blood glucose level of female Wistar rats.

Groups	Blood Glucose level (mg/dl)
Control	75.60±5.92
Stressed	115.00±5.17[a]
Stressed + Caffeine (10 mg/kg)	70.60±3.63[b]
Stressed + Caffeine (20 mg/kg)	78.40±2.04[b]
Stressed + Caffeine (40 mg/kg)	78.40±2.04[b]

F= 19.117, P= 0.000

Superscripts [a] = significant increase compared to normal control and [b] = significant decrease compared to stress control group.

DISCUSSION

In this study, the effect of caffeine post-treatment on plasma glucose levels in female Wistar rats subjected to predator and underwater stress was evaluated.

The present finding indicated hyperglycaemia in female Wistar rats subjected to predator and underwater stress and that caffeine treatment after stress session ameliorated the stress-induced hyperglycaemia. The glucose lowering effect can be due to caffeine's activation of the dopamine system or activation of AMP-activated protein kinase (AMPK) in hepatic cells and muscles, which causes increase in glucose transporters, causing increase in blood glucose transport in an insulin-independent manner. The glucose lowering effect seen with caffeine treatment is supported by the work of Fang Chong-Ye *et al*., (2015), who reported dose-dependent glucose lowering effect with caffeine treatment in mice, however, the effect seen in this study is not dose-dependent. Coffee/caffeine consumption has also been associated with a decreased risk of T2DM (Biessels, 2010). The rise in blood glucose of the female rats with stress is also supported by a previous study, which reported hyperglycemia in Wistar rats subjected to predator stress, but the glucose level return to normal level after stress, with lesser time in normal than diabetic rats (Jin-

Sun *et al*., 2013). Furthermore, long-term studies (2–16 weeks) showed that caffeinated coffee may improve glycaemic metabolism by reducing the glucose curve and increasing the insulin response (Reis *et al*., 2018)

On the contrary, it has been reported that consumption of weaker coffee and caffeine (4 weeks) is not associated with fasting glucose concentrations in women volunteers (Rob *et al*., 2004).

Therefore, the ameliorative effect seen with caffeine may be beneficial as previous studies have associated stress-induced hyperglycaemia in certain critical illness with high risk of developing type 2 diabetes (Vedantam *et al*., 2022). Another opposing explanation is that the hyperglycaemia ameliorative effect of caffeine may hinder the body's normal response to stress which include elevation of blood glucose to meet the body's energy demand. This makes it insufficient to conclude regarding the use of caffeine as anti-hyperglycaemic in critically ill patients.

CONCLUSION

Caffeine dose-independently reduces stress-induced hyperglycaemia in female Wistar rats. However, further studies should be aimed at determining the effect of caffeine on the various markers of stress response in order to determine its merits/demerits in critically ill patients.

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