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Effect of Chronic Administration of Caffeine on Renal Function in Male Wistar Rats

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Abstract

Caffeine is one of the most widely consumed of all beverages in the World that is capable of stimulating the nervous system, producing alertness of short duration without much regards to organ affectations. Twenty-one (21) male Wistar rats were randomly divided into three (3) groups of 7 rats each as follows: Group I was the control and was given 0.5 ml of NaCl, Groups II and III were administered 0.38 mg/kg and 1.14 mg/kg of caffeine respectively for 14 days. Assessment of renal function was evaluated by collection of urine and blood samples for urinalysis and electrolytes, urea, and creatinine analysis respectively. Urinalysis result precisely showed that protein was positive (+) with a pH of 5.5 in the control group. The LD animals revealed bilirubin (2+), protein (+) with a pH of 5.0. The HD group showed bilirubin (2+), protein (3+) with a pH of 3.5. There was significant ($p<0.05$) increase in serum urea and creatinine of LD and HD. The serum sodium level was significantly ($p<0.05$) reduced in LD, while serum potassium level was significantly ($p<0.05$) increased in the HD group. Chronic consumption of caffeine should

be with moderation and at a very lower dose to prevent gradual untoward effects on functions of the kidneys, especially in an already compromised renal status.

Keywords: Caffeine, Creatinine, Urinalysis, Renal function, Urea, Electrolytes

Introduction

Caffeine (1,3,7-trimethylxanthine), a natural alkaloid found in vast number of tropical plant and food species is the most widely consumed of all beverages in the world (Heckman *et al.*, 2010) capable of stimulating the nervous system, producing a certain alertness of short duration. Metabolic studies suggest that caffeine may improve energy balance by reducing appetite and increasing the basal metabolic rate and food-induced thermogenesis, possibly through stimulation of the sympathetic nervous system and uncoupling of protein-1 expression in brown adipose tissue (Harpaz *et al.*, 2017; Schaik *et al.*, 2022). Some physiological effects associated with caffeine administration include central nervous system stimulation, acute elevation of blood pressure, increased metabolic rate and diuresis.

Evaluation of the functioning capacity of the kidneys may be ascertained through laboratory tests which can assist the physician in making an accurate diagnosis. Some of these investigative tools include the determination of the circulatory levels of non-protein nitrogenous substances, filtration capacity of the nephrons, excretion of endogenous and exogenous compounds by the kidney and the kidney's ability to maintain electrolytes and water balance. Serum and urine creatinine are essential in the evaluation of renal function, and the results from these measurements can be used to directly estimate the glomerular filtration rate (Naicker, 2012; Sudiarto et al., 2021).

Epidemiological studies have largely focused on the association between coffee intake and cardiovascular risk factors, including blood pressure and serum cholesterol levels, or the incidence of cardiovascular disease itself; nervous system and its parameters in mental health and pain; and other systems including the respiratory, gastrointestinal and skeletal systems (Marwicka *et al.*, 2014; Cappelletti *et al*., 2015; Turnbull *et al*., 2017; Rodak *et al.*, 2021). This study therefore aims to determine the effect of chronic administration of caffeine on renal functions in male Wistar rats.

Materials and Methods

Sources of Animals/Urine strips/Caffeine

Twenty-one (21) male Wistar rats with an average weight of 250 g ± 20 g were used in this study. These rats were purchased from the Animal Holding Unit of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State. Urine strip (Combi-10) was a product of Macherey-Nagel GmbH & Co. KG Valencienner, Germany, and was purchased at One Step Pharmacy/Medical Diagnostic outlet at Sawmill, Ilorin. Caffeine was gotten from Nescafe classic coffee tin of 50 g, (2g Nescafe coffee cup contains 90mg caffeine, Nescafe Australia), a product of Nestle Nigeria Company, Ilupeju, Lagos, Nigeria. It was purchased from Shoprite, Ilorin.

Animal Care and Ethical Approval

The animals were housed in metabolic cages in the animal house of the Faculty of Basic Medical Sciences under standard laboratory conditions The room was well ventilated and kept at a constant room temperature of about 25^0C ± 2^0C. They were given pelleted rat feeds and water *ad libitum*. The research was approved to be in compliance with internationally accepted laboratory animal use and care guidelines, and the guidelines for the institution research Ethical Review Committee of College of Health Sciences, University of Ilorin, Ilorin Nigeria.

Animal grouping

The 21 animals were randomly divided into three (3) groups of 7 rats each. Animals in Group I served as control group. Groups II and III were given caffeine orally through oral cannula at doses of 0.38 mg/kg body weight (low dose) and 1.14 mg/kg body weight (high dose) respectively for an experimental period of two weeks (14 days).

Experimental procedure

Coffee solution was prepared daily by weighing out appropriate weight of the coffee granules and dissolving in 0.5 ml of normal saline (0.9% NaCl). This was administered orally to the experimental groups using oral cannula for a period of 14 days.

Group I (control group) was given 0.5 ml NaCl only once daily. Group II (low dose) was given 0.38 mg/kg body weight of caffeine dissolved in 0.5 ml NaCl orally once daily, while Group III (high dose) was given 1.14 mg/kg of caffeine dissolved in 0.5 ml NaCl orally once daily.

Groups	Agent	Concentration	Route/frequency /duration
A (Control)	0.9% NaCl	0.5 ml NaCl	Oral/daily/2weeks
B (Low dose)	Caffeine	0.38 mg/kg	Oral/daily/2weeks
C (High dose)	Caffeine	1.14 mg/kg	Oral/daily/2weeks

After two weeks of administration, urinalysis was done with urine sample collected overnight with combi-10 urine strips on day 14. Following this, the animals were sacrificed under ketamine anaesthesia, and blood samples were collected by cardiac puncture into sodium heparin bottle from each rat. The blood samples were centrifuged at 3000 revolution for 10 minutes and serum was collected for analysis of serum urea, electrolytes (sodium, potassium) and creatinine using ion selective electrode, urease spectrophotometer, and Jaffe spectrophotometer methods.

Statistical Analysis

Statistical analysis was done with the aid of the Statistical Package for Social Sciences (SPSS) version 20.0. Differences in means were obtained using ANOVA and Ducan post hoc tests. All values reported in the study were expressed as MEAN±SEM (Standard error of Mean). Differences were taken to be significant at $P \leq 0.05$.

Results

Table 1.0: Serum electrolytes (creatinine, urea, sodium, potassium) level

Parameters	Control 0.9% NaCl	Low dose(caffeine) (0.38 mg/kg)	High dose (caffeine) (1.14 mg/kg)
Urea (mmol/l)	14.76±1.37	19.98±0.81*	30.73±1.69*[a]
Creatinine (mmol/l)	1.74±0.18	2.13±0.08*	3.03±0.21*[a]
Sodium (mmol/l)	160.96±4.36	141.95±1.86*	164.61±7.83[a]
Potassium (mmol/l)	5.12±0.55	5.43±0.52	6.08±0.28*[a]

Level of significance was considered as $p<0.05$.

*Caffeine compared to control
[a]High dose compared to low dose caffeine

The study revealed a significant increase in the serum urea levels of both LD and HD compared to the control. There was also significant increase in urea levels of HD compared to LD. In the same vein, there was significant increase in the serum creatinine levels of both LD and HD, while a significant increase in serum creatinine level of HD compared to LD was also observed. The study also showed a significant decrease in the serum level of sodium in LD caffeine administration compared to the control, while a significant increase in sodium level was noted in HD group compared to LD caffeine animals. However, there was no statistically significant difference in serum levels of sodium between HD and the control rats. The serum potassium levels showed significant increase in HD when compared to both control and LD animals. The dipstick urinalysis results summarily showed that protein was positive (+) with a pH of 5.5 in the control group. The LD animals revealed bilirubin (2+), protein (+) with a pH of 5.0. The HD group showed bilirubin (2+), protein (3+) with a pH of 3.5. All other parameters (glucose, ketone, nitrites, urobilinogen, blood, and leucocytes) were negative in all the three experimental groups

Discussion

Serum urea, creatinine, and electrolytes were assessed in this experiment to determine effects of chronic caffeine administration on renal functions. It was observed that serum urea levels were remarkably increased on chronic administration of low and high doses of caffeine. Urea is a metabolic product of protein, which is generally harmful to the body system and must be excreted. This study showed that ingesting caffeine at a low dose over long period actually increased serum urea but the level of increase was even doubled when ingested at a higher dose for long period. This could be an indication to gradual functional destruction of the renal system which could eventually snowball into morphological affectation of the kidneys, though not assessed in the current work, which usually follows functional derangement in most disease processes. It may also follow that care should be taken in chronic caffeine ingestion in an already compromised renal status as seen in renal failure. In this state, the recycling of urea that takes place between the tubule and medullary interstitium may be too enormous for this ADH-dependent process. This outcome is in tandem with the work of Elbendary *et al* in 2023 who

found out that caffeine in energy drinks increased plasma urea and uric acid levels in a human study carried out in Saudi Arabia. The work of Akande and Banjoko (2011) also corroborated the finding, having discovered a significant increase in serum urea in Sprague Dawley rats. Some authors have also shown that caffeine acts by increasing tubular reabsorption of urea, presumably in the distal part of the nephron, secondary to extracellular fluid (ECF) volume depletion (Jordá *et al.*, 1989).

In the same vein, increase serum creatinine levels observed in the study also follow a dose-dependent pattern in the LD and HD chronic caffeine administration when compared with the control group. This is in concordance with previous studies on the effect of caffeine on creatinine levels that have shown that caffeine had increasing effect on creatinine level (Musaiger & Zagzoog, 2014; Emmanuel *et al.*, 2017), while others reported decrease or no change in creatinine levels (Akande and Banjoko, 2011; Elbendary *et al.*, 2023). The disparities on the effects of caffeine may be attributed to the lack of uniform composition of caffeine containing beverages and energy drinks and differences in experimental duration as reflected in some of the previous studies (Hilmi *et. al.*, 2021) in which there was hypercreatinaemia in the first 14 days of the experiments, while a decrease was seen in the last two weeks of the study duration (Hilmi *et. al., 2021*).

The chronic administration of caffeine at LD in this study showed that there was significant relationship between caffeine and serum sodium level. This finding was supported by another researcher (Yu *et al.*, 2016) who suggested that chronic caffeine intake is associated with increased urinary sodium.

However, the slight decrease in serum sodium level following low dose chronic caffeine administration is likely due to the activation of renal adenosine monophosphate-activated protein kinase (AMPK) that inhibits epithelial sodium channel (ENaC) (define) activity of the kidneys of the rats which subsequently reduces sodium reabsorption in the proximal and distal nephron tubules by antagonism of adenosine A1 receptor. This results in increased renal fluid and sodium excretion. The increase serum sodium revealed in the HD caffeine administration was also supported by the Akande and Banjoko, 2011. They observed that the concentration of sodium in the serum level did not differ significantly when compared with the control group however; the increase was significant when compared to animals

given LD. The result of this present study showed no significant changes in serum potassium level in the LD group and an increase in HD when compared with control respectively. Additionally, a remarkable elevation of serum potassium was also recorded in the HD caffeine group compared to the LD group. The current finding of caffeine on serum potassium levels is not in tandem with the work of Yutaka, 2010 that examined coffee induced hypokalemia in humans.

Conclusion

The results from this study showed that, chronic caffeine intake might compromise renal functions in male Wistar rats, leading to hyperkalaemia, uraemia and hypercreatinaemia. Thus, moderate consumption of caffeine at low dose is advised, while frequent consumption of high dose of caffeine should be avoided to prevent gradual untoward effects on functions of the kidneys, especially in an already compromised renal status as seen in renal failure.

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