Original Research Article

Comparative Effect of Chilli Pepper (*Capsicum frutescens*) Extract and Capsaicin on Some Haematological Parameters and Serum Electrolytes in Albino Wistar Rats

ABSTRACT

Aim: Capsaicin is the active ingredient in chilli pepper, and is responsible for the pungency of chilli pepper. This study compared the effect of ethanolic extract of chilli pepper fruit and capsaicin on haematological parameters and serum electrolytes in female albino Wistar rats on the background that they are widely consumed in foods.

Methodology: Fifteen female Wistar rats (140 – 200 g b.w) fed with rat feed and water *ad libitum* were divided into three groups (n = 5) thus: control, chilli pepper and capsaicin groups. The three groups were treated with daily oral administration of 0.2 mL normal saline, chilli pepper extract (5 mg/100g b.w) and capsaicin (3 mg/100g b.w) respectively, for 30 days. Blood samples were collected from each animal via cardiac puncture for assessment of haematological parameters and serum concentration of electrolytes.

Results: Red blood cell (RBC) count, haemoglobin (Hb) concentration and packed cell volume (PCV) in both treated groups were significantly ($p<0.01$) reduced compared with control. PCV was significantly ($p<0.05$) reduced in capsaicin group compared with the chilli pepper group. Platelet count and platelet large cell ratio (P-LCR) were significantly reduced ($p<0.01$) in capsaicin group compared with the control. Serum Na^+, Cl^-, and urea concentrations showed no significant ($p>0.05$) difference among groups, but creatinine level decreased significantly ($p<0.05$) in the treated groups compared with the control. Serum HCO_3^- increased while K^+ decreased significantly ($p<0.05$) in capsaicin treated group compared with the control. Furthermore, serum K^+ increased ($p<0.05$) in chilli pepper group, compared with the control.

Conclusion: Capsaicin and chilli pepper did not cause serious electrolyte imbalance, but reduced red cell indices. Additionally, capsaicin altered platelet parameters. Therefore, we suggest that capsaicin might be detrimental to individuals with bleeding and/or blood coagulation disorders.

Keywords: Capsaicin, creatinine, chilli pepper, haematology, serum electrolytes, urea

1. INTRODUCTION

Chilli pepper is a diploid and self-pollinating crop. It is a member of the family of plants known as *Solanaceae.* It belongs to the genus *Capsicum* and has five species namely: *frutescens, pubescens, baccatum, annuum* and *chinense* [1]. Chilli pepper is one of the world's most widely grown spice and a major ingredient in most foods worldwide. It is used as the active substance in defence repellent, ornaments, colouring agents and for therapeutic purposes [2]. It has a pungent sensation due to the presence of a group of compounds called capsaicinoids [3]. Chilli pepper increases gastric activities and enhances blood flow and is used to relieve pain associated with child delivery [4]. However, reports have shown that high doses of chilli pepper can also lead to gastric erosion [5]. Chilli pepper promotes the health of diabetic patients by reducing disaccharides in the intestinal lumen through α-glucosidase and α-amylase inactivation [6]. Crude ethanolic extract of chilli pepper may be effective in reducing renal insufficiency as it has been reported to decrease serum creatinine and urea and increase total protein levels [7]. A study conducted by Elamin *et al.*[8] on broilers showed that chilli pepper significantly reduced serum cholesterol, abdominal fat, and AST enzyme activity, but serum total protein, urea, ALP, calcium (Ca) and phosphorus (P) were unaffected.

Phytochemical screening by Dougnon *et al.*[9] revealed the presence of sterols, alkaloids, mucilage, polyterpenes, and reducing compounds in the powder of chilli pepper (*Capsicum frutescens)*. This chilli pepper powder fed to Hubbard broilers at 5 g and 10 g/kg diet did not significantly change haematological

parameters and creatinine levels. Another study by Shahverdi *et al.*[10] showed that 1 % chilli pepper powder used as broilers' feed additive improved overall performance but decreased haemoglobin concentration and packed cell volume.

The active ingredient (i.e. the main capsaicinoid) in chilli pepper is capsaicin, and is responsible for the irritation and pungency of various hot peppers [11]. Capsaicin can be extracted from chilli pepper using different solvents and is also available in synthetic form. It acts by binding to transient receptor potential vanilloid-1 (TRPV1), formerly called vanilloid receptor. Basically, TRPV1 is located in nociceptive neurons and widely distributed in brain tissues, intestines, liver, keratinocytes of epidermis and macrophages. TRPV1 was first discovered on the beta cells of pancrease. It was discovered that capsaicin could activate TRPV1 with resultant increase in insulin secretion [12]. Capsaicin, administered to healthy and diabetic rats taking high iron diet has been reported to reduce levels of haemoglobin, cholesterol and triglycerides [13]. Capsaicin has been reported to decrease plasma glucose concentration [14, 15] and inhibit glucose absorption [16]. Reports have shown that capsaicin is used to treat osteoarthritis, post-herpatic neuralgia and diabetic neuropathy [17]. It has been reported to have antimicrobial activity [2, 3], cardioprotective effect [18], anti-inflammatory effect [19] and anticancer activity [20, 21]. Capsaicin also reduces the severity of headaches [19]. Additionally, it has been reported that capsaicin has anti-obesity effect [22, 23], but the potential side-effects limits its clinical application [24]. Capsaicin has been reported to increase gastric acid secretion and mucosal blood flow [25]. Nishihara *et al.* [26] reported that low dose of capsaicinoids showed gastroprotective effect, while Wang *et al.* [27] reported that a high dose may be detrimental to the gastrointestinal tract since it damages capsaicin-sensitive afferent nerves and causes exhaustion of neurotransmitters. In spite of the numerous researches carried out with chilli pepper and capsaicin, very little or none has documented and compared the effect of ethanolic extract of chilli pepper fruit and capsaicin on haematological parameters and serum electrolytes. Therefore, the present study was carried out to assess and compare the effect of administration of ethanolic extract of chilli pepper (*Capsicum frutescens)* fruit and capsaicin to experimental rats on haematological parameters and serum electrolytes.

2. MATERIAL AND METHODS

2.1 Extract Preparation

Chilli pepper (*Capsicum frutescens*), bought from Watt market, Calabar, Nigeria, were washed with tap water and dried at 35^{o}C. The dried pepper fruits (including the wall, seed and placenta) were ground to powder using an electric grinder. Grinded chilli pepper (300g) was extracted in 2L of 95 % ethanol by maceration for 72 hours. The extract was filtered using Whatman No1 filter paper and the filtrate was evaporated to dryness in a rotary evaporator, lyophilized and thereafter preserved for use.

2.2 Experimental Animals

Fifteen female albino Wistar rats (140 – 200 g body weight) handled according to Helsinki's (1964) laid down principles, were used for the study. They were bought from Department of Agriculture, University of Calabar, Nigeria, and housed in well ventilated wooden cages in the animal house of the Department of Physiology, University of Calabar. The animals were given rat feed and water *ad libitum* and exposed to 12/12 hours light/dark cycle. All animals were allowed for seven days to acclimatize before treatment began.

2.3 Animal Grouping and Extract Administration

The fifteen (15) rats were randomly assigned into three (3) groups (n = 5) thus: control, chilli pepper and capsaicin groups. All groups had access to rat feed and water. The control group was treated with 0.2 mL normal saline. Chilli pepper was administered at 5mg/100g body weight daily while capsaicin (Sigma-Aldrich, St. Louis, MO, USA), was administered at 3 mg/100g body weight daily. All treatments lasted for 30 days.

2.4 Collection of blood sample

At the end of the 30 days period, all animals were sacrificed under chloroform anaesthesia. Blood samples were collected through cardiac puncture using 5 ml syringes with 21G needles into sample

bottles and EDTA vials for measurement of serum electrolytes concentration and haematological parameters respectively.

2.5 Assessment of Haematological Parameters

Haematological parameters were measured using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer. Parameters measured were: red blood cells (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (P-LCR).

2.6 Assessment of Serum Electrolyte Concentration

The blood samples collected were allowed 1h to clot and retract. The serum was obtained after centrifuging blood in the sample bottles at 300rpm at room temperature for 15 minutes using a bucket centrifuge machine (B-Bran Scientific and Instrument Company, England). The serum was used to determine serum Na^+, K^+, Cl^- and HCO_3^- levels using ion-selective electrolyte analyser (Biolyte 2000/ BioCare Corporation, Hsinchu 300, Taiwan).

2.7 Assessment of Serum Concentration of Creatinine and Urea

Assay for creatinine was carried out using the Reflotron Dry Chemistry Analyzer as described by Estridge *et al*. [28]. Blood urea concentrations were determined using Berthelot's reaction as described by Kaplan and Teng [29].

2.8 Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Data were analysed using One way analysis of variance (ANOVA) along with post hoc multiple comparison test (Tukey test) using Statistical Package for Social Science (SPSS) (version 17.0). $p<0.05$ was considered statistically significant.

3. RESULTS

3.1 Haematological parameters among the different experimental groups

Red blood cell indices

Table 1 shows the RBC count ($x10^6$ cell/µL), Hb concentration (g/dL), PCV (%), MCV, MCH and MCHC for control, chilli pepper and capsaicin groups. RBC count and Hb concentration were significantly ($p<0.01$) reduced in chilli pepper and capsaicin groups compared with control. PCV was also significantly ($p<0.01$) lower in chilli pepper and capsaicin groups compared with the control group. There was no significant difference in MCV and MCH in the different experimental groups. MCHC was significantly ($p<0.01$) increased in chilli pepper group compared with control and significantly ($p<0.05$) lower in capsaicin group compared with chilli pepper group.

Table 1: Comparison of red blood cell indices between the different experimental groups

Parameters	Control	Chilli Pepper	Capsaicin
RBC ($x10^6$ cell/µL)	10.17 ± 0.36	8.22 ± 0.39 ^{b}	8.16 ± 0.27 ^{b}
Hb (g/dL)	16.33 ± 0.23	14.26 ± 0.72 ^{b}	13.76 ± 0.25 ^{b}
PCV (%)	59.46 ± 1.10	51.96 ± 2.17 ^{b}	47.28 ± 0.64 ^{b,x}
MCV	58.74 ± 1.48	63.40 ± 1.59 ^{NS}	58.20 ± 1.53 ^{NS}
MCH	16.15 ± 0.51	17.35 ± 0.39 ^{NS}	16.91 ± 0.29 ^{NS}
MCHC	27.48 ± 0.32	40.00 ± 0.42 ^{b}	29.10 ± 0.35 ^{x}

Values are expressed as mean ± SEM, n = 5.
NS = not significant vs control;
b = $p<0.01$ vs control;
x = $p<0.05$ vs chilli pepper.

- ***Platelet indices***

Table 2 shows platelet count (x10^3 cell/µL), PDW (fL), MPV (fL) and P-LCR (%) for control, chilli pepper and capsaicin groups. Platelet count was significantly ($p<0.01$) lower in capsaicin group compared with control and chilli pepper groups. PDW was not significantly changed in the different experimental groups. MPV was significantly ($p<0.05$) increased in chilli pepper group compared with control. P-LCR was significantly ($p<0.01$; $p<0.001$ respectively) reduced in capsaicin group compared with control and chilli pepper groups.

Table 2: Comparison of platelet indices between the different groups

Parameters	Control	Chilli Pepper	Capsaicin
Platelet Count (x10^3 cell/µL)	972.86 ± 50.82	1115.80 ± 52.29 ^{NS}	787.00 ± 78.25 $^{b, y}$
PDW (fL)	8.45 ± 0.24	8.32 ± 0.20 ^{NS}	7.41 ± 0.63 ^{NS}
MPV (fL)	10.05 ± 0.70	13.44 ± 1.36 ^{a}	11.92 ± 0.51 ^{NS}
P-LCR (%)	8.60 ± 0.23	12.42 ± 0.72 ^{NS}	6.54 ± 0.14 $^{b, z}$

Values are expressed as mean ± SEM, n = 5.
NS = not significant vs control;
a = $p<0.05$, b = $p<0.01$, vs control;
y = $p<0.01$, z = $p<0.001$ vs chilli pepper.

3.2 Comparison of serum concentration of electrolytes in the different experimental groups following 30 days of treatment with chilli pepper and capsaicin

Results for serum concentrations of Na^+ (mmol/L), K^+ (mmol/L), Cl^- (mmol/L) and HCO_3^- (mmol/L) for control, chilli pepper and capsaicin groups are shown in table 3. Na^+ and Cl^- did not differ significantly among the different experimental groups. K^+ was significantly increased ($p<0.001$) in chilli pepper group and reduced ($p<0.01$) in capsaicin group compared with control. K^+ was also significantly ($p<0.001$) reduced in capsaicin group compared with chilli pepper group. HCO_3^- was significantly ($p<0.05$) increased in capsaicin group compared with control but did not change significantly between control and chilli pepper groups. (Table 3).

Table 3: Comparison of serum concentration of electrolytes among the different experimental groups

Parameters	Control	Chilli Pepper	Capsaicin
Na^+ (mmol/L)	147.14 ± 0.34	147.60 ± 0.43 ^{NS}	145.60 ± 1.02 ^{NS}
K^+ (mmol/L)	8.60 ± 0.23	12.42 ± 0.72 ^{c}	6.54 ± 0.14 $^{b, z}$
Cl^- (mmol/L)	103.14 ± 0.56	103.20 ± 1.05 ^{NS}	98.00 ± 0.46 ^{NS}
HCO_3^- (mmol/L)	19.43 ± 0.48	22.40 ± 0.43 ^{NS}	28.00 ± 0.27 ^{a}

Values are expressed as mean ± SEM, n = 5.
NS = not significant vs control;
a = $p<0.05$, b = $p<0.01$, c = $p<0.001$ vs control;
z = $p<0.001$ vs chilli pepper.

3.3 Comparison of serum creatinine and urea concentrations among the different experimental groups

Results for serum concentrations of creatinine (µmol/L) and urea (µmol/L) for control, chilli pepper and capsaicin groups are shown in table 4. Creatinine concentration was significantly reduced in chilli pepper ($p<0.01$) and capsaicin ($p<0.05$) groups compared with control. Urea concentration did not differ significantly in the different experimental groups. (Table 4).

Table 4: Comparison of serum creatinine and urea concentrations among the different experimental groups

Parameters	Control	Chilli Pepper	Capsaicin
Creatinine (µmol/L)	82.71 ± 1.98	70.40 ± 3.69 [b]	71.20 ± 2.19 [a]
Urea (µmol/L)	2.70 ± 0.07	2.34 ± 0.06 [NS]	2.54 ± 0.07 [NS]

Values are expressed as mean ± SEM, n = 5.
NS = not significant vs control;
a = p<0.05, b = p<0.01 vs control.

4. DISCUSSION

Chilli pepper is a spice that is widely grown worldwide and used as a basic ingredient in most global cuisine. Its effects are elicited mainly by its active substance, capsaicin, which also gives chilli pepper its pungent sensation. Chilli pepper and capsaicin have been reported to affect the physiology of the human body in different ways. This study investigated and compared the effect of ethanolic extract of chilli pepper and capsaicin on blood parameters and serum electrolytes concentration in female Wistar rats.

Red blood cell count, Hb concentration and PCV were significantly decreased in all treated groups compared with the control. PCV was significantly decreased in capsaicin group compared with chilli pepper group. These results contradict the report of Dougnon *et al.* [9], which showed that chilli pepper fed to Hubbard broilers did not significantly change haematological parameters but corroborate that of Shahverdi *et al.* [10] which showed decreased PCV and Hb concentration following treatment with chilli pepper on broilers. Our results suggest that both chilli pepper and capsaicin may have inhibitory effect on erythropoiesis which is marked by the decreased RBC count, but the effect is greater with capsaicin. PCV was decreased probably because of the decreased RBC count. It is also likely that heme biosynthesis was impaired along with erythropoiesis which led to decreased Hb concentration. Capsaicin has been reported to reduce the levels of haemoglobin despite taking high iron diet [13]. From our study, it is also likely that chilli pepper and capsaicin may have suppressed the synthesis of iron which may have led to the presence of microcytic erythrocytes resulting in the reduced Hb concentration observed. We therefore suggest that chilli pepper and capsaicin have similar effects on RBC count and Hb concentration, but capsaicin has a more reducing effect on PCV than chilli pepper. MCV and MCH were not significantly changed in the treated groups compared with the control. MCHC of capsaicin group was not significantly changed compared with control. But MCHC was significantly increased in chilli pepper group compared with control, and significantly reduced in capsaicin group compared with chilli pepper group. Further work is needed to give a clearer understanding of the effect of capsaicin and chilli pepper on Hb concentration.

The non-significant change in MCV indicates that chilli pepper and capsaicin did not alter the Na^+ transport across cell membrane since Na^+ transport into a cell is accompanied by water to increase the intracellular volume. This is evident in our result which shows no significant change in Na^+ levels in all experimental groups. This may also mean that both capsaicin and chilli pepper at the doses used in this study, do not affect the renin angiotensin aldosterone system.

Alterations in some serum electrolytes were observed. Increased K^+ elicits antihypertensive effect [30]. From our results, chilli pepper increased K^+ levels while capsaicin decreased K^+ levels compared with control. This shows that chilli pepper exhibit antihypertensive effect unlike capsaicin. But this antihypertensive effect of chilli pepper must be very mild since Na^+ was not significantly affected. Na^+ is the major extracellular electrolyte implicated in hypertension. High levels of Na^+ causes contraction of blood vessels to increase such that a great force is required to pump blood with a consequent hypertension [31]. HCO_3^- was significantly increased in capsaicin group compared with the control, but, it was not significantly different between chilli pepper and control group. HCO_3^- is a marker for measuring the pH of blood. It acts as a buffer to maintain the pH of blood and body fluids. The results of this study show that capsaicin has the capacity to increase the pH of blood, while chilli pepper has no significant effect on blood pH.

Serum creatinine levels were significantly decreased in chilli pepper and capsaicin groups compared with control group, but was not significantly different between the treated groups. Serum urea levels were not significantly changed in all experimental groups. Our result for decreased creatinine levels is consistent

with that of Ogbonnaya and Muritala [7], who reported that crude ethanolic extract of chilli pepper decreased serum creatinine and urea levels. But our result for urea does not show any significant difference in all groups unlike that of Ogbonnaya and Muritala [7] which showed a significant decrease. Our results show that the reduction of RBC count, Hb concentration and PCV observed is not due to destruction of RBC since erythrocytes destruction may be accompanied by increased blood urea levels. Also, Chilli pepper and capsaicin, administered at the dosage used, may not cause any damage to functional nephron since decreased levels of creatinine were observed in our study. An increase in serum creatinine levels is basically observed if there is a marked damage to functional nephrons [32, 33]. From our study, it is clear that chilli pepper and capsaicin have similar effects on creatinine concentration since creatinine was not significantly different between the treated groups. Both chilli pepper and capsaicin did not significantly alter serum urea concentration.

Platelet count was significantly reduced in capsaicin group compared with control and chilli pepper groups. Platelet count in chilli pepper group was not significantly ($p>0.05$) different from that of control although an increase was observed. These results show that chilli pepper does not have any significant effect on platelet count unlike capsaicin which has significant effect. The results indicate that capsaicin may have an adverse effect on the clotting mechanism of the body unlike chilli pepper. However, chilli pepper and capsaicin may not have any effect on platelet size as indicated by the non-significant changes in PDW (Table 3). The decreased P-LCR observed in capsaicin group in comparison with control and chilli pepper groups shows that capsaicin affects platelet aggregation while chilli pepper has no significant effect on platelet aggregation. From the results of our study, the effect of chilli pepper on P-LCR is opposing to that of capsaicin which reduced P-LCR.

5. CONCLUSION

We therefore conclude that both chilli pepper and capsaicin do not cause serious electrolyte imbalance, but inhibit erythropoiesis, with capsaicin having a greater effect. However, their effects are neither haematoxic nor nephrotoxic. Capsaicin decreases platelet indices while chilli pepper does not have significant effect on platelet indices. Patients with blood coagulation disorders and/or bleeding disorders should use chilli pepper instead of capsaicin as they may worsen their condition with intake of capsaicin.

CONSENT

Not applicable.

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ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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