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Full-Length Research Article

Short-term Acute Constipation, Not Short-term Acute Diarrhea, Altered Cardiovascular Variables in Wistar Rats

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Summary: Gastrointestinal dysmotility is a substantial public health challenge globally. Based on previous findings in developed countries, it has been observed that there is an association between diarrhea, constipation, and some cardiovascular variables. This study investigated the effects of experimentally-induced short-term acute constipation and short-term acute diarrhea on certain cardiovascular variables in rats. Thirty (30) male Wistar rats were divided into three groups; Control, Diarrhoea, and Constipation. The experiment was carried out in 2 phases, the period after induction and the recovery period, and 5 animals per group were used for each phase. The control group received an equivalent amount of distilled water while Diarrhoea and the Constipation group were induced by oral administration of 2ml Castor oil and administration of Loperamide (3mg/kg, b.d, orally x 3 days), respectively. Cardiovascular variables were assessed using the Edan Scientific® Electrocardiography and Heart Rate Variability machine. Recovery was allowed for 4 days after the onset of the procedure and cardiovascular parameters were reassessed. Post-induction Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP) and Heart Rate (HR) significantly increased in constipated rats (153.2 ± 2.9 mmHg; 109.0 ± 3.7 mmHg; 123.7 ± 3.2 mmHg; 123.4±5.6 bpm) when compared with the control values (95.5±4.8 mmHg; 61.2 ± 3.5 mmHg; 72.6 ± 3.6 mmHg; 72.3 ± 5.2 bpm), respectively. The recovery SBP, DBP, MAP, and Heart Rate in the constipated group remained significantly higher compared to the control. Diarrhea had no significant effect on the parameters determined in both post-induction and recovery phases. The electrical activities did not change in both experimental groups compared to the control. This study revealed increased SBP, DBP, MAP, and HR in short-term acute constipated rats but not so with short-term acute experimental diarrhea.

Keywords*: Blood pressure, heart rates, constipation, diarrhea, electrical activities, rats*

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INTRODUCTION

The fundamental functions of the gastrointestinal system include motility, sensation, absorption, digestion, secretion, and intestinal barrier function. The symptoms of functional gastrointestinal dysmotility are mostly triggered by meal intake and abnormalities in the physiological processes are responsible for the generation of symptoms. Irritable Bowel Syndrome (IBS) is a form of gastrointestinal disorder with symptoms subcategorized into painful sensations and altered intestinal motility patterns (Li *et al*., 2020), with constipation and diarrhea presenting in an alternate pattern. Constipation is a major gastrointestinal disorder common at presentations at the primary health centres, with a prevalence of about 30% of the entire human race in their time(Bharucha *et al*., 2013). However, constipation is often regarded as benign and may not be so dangerous (Dennison *et al*., 2005), when it becomes chronic, the quality of life of patients becomes compromised socially and economic-wise (Guerin *et al*., 2014). It is one of the commonest disorders of gastrointestinal motility and secretions which result in infrequent and difficulty in stool passage with pains and stiffness. This often is due to the very slow movement of food residues through the colon (slow transit constipation) or difficulty moving bowels after reaching the rectum (outlet dysfunction) (Martins *et al*., 2015). Acute constipation causes closure of the intestine in which surgery may be required. The common symptoms of constipation are hard or lumpy stools with consequential straining to defecate; less than three bowel movements per week; and blocked rectum (Mugie *et al*., 2011).

Diarrhea, on the other hand, is categorized as a gastrointestinal dysmotility that occurs as a symptomatic secretory response of the gastrointestinal mucosa to a wide range of stimuli (Berthoud *et al*., 2004). The increase in water content and fluidity of stool is due to the increased secretion of the gastrointestinal mucosa with a possibility of an underlying infection or disease. The underlying disease could cause inflammation or ulceration of the intestinal mucosa, especially in the large intestine or the rectum, then there is the tendency for blood and mucus to be seen in the faeces (Grover *et al*., 2012). Diarrhoeal diseases are related to about 1.3 million annual reported deaths (Troeger *et al*., 2017). The majority of these deaths occur in low-income countries (Kotloff *et al*., 2013; Fiedoruk *et al*., 2015; Troeger *et al*., 2017). Stools with excess loss of water and electrolytes can result in dehydration, hyponatremia, hypokalemia, and loss of other essential electrolytes which

are critical for survival. All of these could predispose individuals to the barrage of complications emanating from the alterations in the epithelial barrier from inflammation, ulceration, and altered circulation (Bern *et al*., 1992).

It is well-known that 10% of deaths in the United States have gastrointestinal disorders as the root cause(Peery *et al*., 2015), while some patients present with cardiovascular complaints as the primary symptoms which have made the relationship between cardiovascular symptoms and gastrointestinal disorders an interesting area of focus(Martins *et al*., 2015). Constipation is associated with the risk of cardiovascular events when treated with laxatives (Sumida *et al*., 2019). In some cases, the associated risk of atherosclerosis is increased blood pressure, and other cardiovascular events are due to changes in altered intestinal microbiota (Attaluri *et al*., 2010; Kim *et al*., 2015; Ishiyama *et al*., 2019). The prevalence of constipation increases with the increasing age of an individual(Ishiyama *et al*., 2019). Other reports implicated arteriosclerotic cardiovascular disease in constipation (Salmoirago-Blotcher *et al*., 2011; Sumida *et al*., 2019), and increased risk of coronary heart disease and ischaemic stroke (Ishiyama *et al*., 2019). In the case of diarrheal diseases, it was suggested with the associated dehydration and electrolytes derangement the possibility of deranged blood pressure in childhood, but that assertion was debunked in the cohort study in children where there was no blood pressure change, no risk of coronary heart disease (Batty *et al*., 2007a). Other reports explored the association between diarrhea in pediatrics or childhood and its effect on blood pressure later in life and also its relationship with coronary heart disease(Batty *et al*., 2007b; Pearce *et al*., 2008).

Considering the enormous suffering from gastrointestinal dysmotility by patients any observed relationship with cardiovascular events or diseases would be of utmost interest to public health and in the management of the associated cardiovascular risk if it ensued. This study was designed to further characterize the relationship between short-term acute constipation and short-term acute diarrhea in rats' cardiovascular events with the hope to relate the outcome to the previous findings on these experimentally induced gastrointestinal derangements.

MATERIALS AND METHODS

Materials: Plastic cages, castor oil, loperamide, gloves, 5ml, and 2ml syringe, cotton wool, methylated spirit, surgical instrument, distilled water, Edan Scientific® Electrocardiography Heart Rate Variability Machine, Kent Scientific® Blood Pressure machine, USA, were used.

Animal Care and Grouping: Thirty male Wistar rats with a bodyweight range of 155-178g were used. The rats were bred and acclimatized for 14 days in the Animal House of the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were randomly distributed into cages based on the groupings, with beddings that were replaced every other day. The rats were grouped into 3 (n=10 per group). The 10 rats in diarrhea and constipation groups were induced, and 5 rats each from each group were examined for post-induction changes and the recovery phase. The rats were monitored for cardiovascular changes in both phases of the experiment. Group 1 (Control): Rats in this group were allowed free access to water and given an equivalent amount of distilled water as a sham. Group 2 (Diarrhea)**:** rats in this group were orally administered 2ml of castor oil per rat to induce diarrhea. Group 3 (Constipation): rats in this group were administered loperamide (3 mg/kg) twice per day for 3 days to induce constipation.

Animal ethics: Experimental protocols and procedures used in animal handling were strictly in adherence to the ethical guidelines of the University of Ibadan, Animal Care and Use for Research Ethics Committee (UI-ACUREC/046-0521/20).

Diarrhea and Constipation Induction procedures: The rats in group 2 were orally administered with castor oil -2ml per rat to induce diarrhea and waited for 2 hours after the onset of diarrhea before determining the cardiovascular changes. The rats were observed for soft, smooth, or mucus-coated and confirmed diarrheic clinically. Group 3 rats were orally administered loperamide (3 mg/kg) twice per day for 3 days to induce constipation (Bustos *et al*., 1991; Wintola *et al*., 2010). The rats were examined for constipation with evidence of a distended abdomen and were used for the experiment.

After the initial assessment, the experimental rats were kept in the animal house and total recovery was noticed after 4 days. Constipated rats were examined and discovered to no longer have distended abdomen and diarrhea was resolved evidenced by the passage of normal stool.

Assessment of Arterial Blood Pressure (BP): Blood pressure assessment of rats was determined non-invasively with a rat tail-cuff connected to a volume pressure sensor of the Kent Scientific® Blood Pressure Machine. Each rat was restrained in a cone restrainer and placed on a manually-controlled warming plate with its tail inserted into the V and O cuff of the machine's transducer. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were monitored through the transducer connected to a computer via a data acquisition system. The blood pressure variables were recorded in triplicate and the mean values of the assessed parameters were determined per session.

Determination of Mean Arterial Pressure (MAP): The MAP was determined using the formula below (Vedel *et al*., 2016);

$$\text{MAP} = DBP + \frac{Pulse\ Pressure}{3}$$

Where:
Pulse pressure = SBP – DBP;
MAP = Mean Arterial Pressure;
DBP = Diastolic Blood Pressure

Assessment of Heart Rate Variability (HRV): The HRV of each rat was monitored through the connection to the Edan Scientific® Electrocardiography Heart Rate Variability machine for 5 minutes, and the mean of recorded HRV values was determined as previously reported by Reed *et al*., (Reed *et al*., 2005)

Determination of Electrical Activities of the Heart: Rats from each group were anesthetized intra-peritoneally with 0.1 ml/100 g of xylazine and 25 mg/100g of ketamine

hydrochloride. The ECG lead electrodes were placed in their respective positions (chest, right arms, right legs, left arms, and left legs) on the animals as previously described (Farraj *et al*., 2011; Ahmad *et al*., 2016).

Statistical Analysis: The results of the experiment were expressed as Mean ± Standard Error Mean (SEM) and analyzed using Analysis of Variance (ANOVA). The Bonferroni test (GraphPad Prism Version 7.0® Software) was used to analyze all results where significant. Data were considered significant at P≤0.05.

RESULTS

Effect of Experimentally Induced Diarrhea and Constipation on Systolic Blood Pressure (SBP): Table 1 describes the SBP (mmHg) significantly increased in constipated rats (153.2±2.9) compared with the control rats (95.5±4.8). The SBP significantly increased in constipated rats (124.5±2.3) compared with the SBP of control rats (113.4±2.1) after recovery. There was no significant change when the diarrhea group was compared to the control in both the induction and recovery phases.

Effect of Experimentally Induced Diarrhea and Constipation on Diastolic Blood Pressure (DBP): The DBP (mmHg) increased significantly in constipated rats (109.0±3.7) compared with the control rats (61.2±3.5), table 2. The significant increase in DBP was sustained during the recovery phase in constipated rats (89.3±2.0) when compared with the control rats (78.7±1.4). Again, there was no significant change in DBP of the diarrhea group compared to the control in both the induction and recovery phases (Table 2).

Table 1:
Systolic Blood Pressure of Rats After the Induction and Recovery from Diarrhoea and Constipation Compared with the Control Group

Groups	Systolic Blood Pressure (mmHg)	
	Induction Phase	Recovery Phase
Control	95.5±4.8	113.4±2.1
Diarrhoea	100.0±5.4^{ns}	108.0±3.7^{ns}
Constipation	153.2±2.9*	124.5±2.3*

** = Significant Increase at P≤0.05 compared with control, ns = no significant difference compared to control*

Table 2:
Diastolic Blood Pressure of Rats After the Induction and Recovery from Diarrhoea and Constipation Compared with the Control Group

Groups	Diastolic Blood Pressure (mmHg)	
	Induction Phase	Recovery Phase
Control	61.2±3.5	78.7±1.4
Diarrhoea	69.9±3.9^{ns}	72.0±3.0^{ns}
Constipation	109.0±3.7*	89.3±2.0*

** = Significant Increase at P≤0.05 compared with control, ns = no significant difference compared to control*

Table 3:
Mean Arterial Pressure of Rats After the Induction and Recovery from Diarrhea and Constipation

	Groups		
Phases	**Control**	**Diarrhea**	**Constipation**
Induction	72.6±3.6	79.9±3.9^{ns}	123.7±3.2*
Recovery	90.3±0.9	84.2±3.1^{ns}	101.0±2.0*

** = Significant Increase at P≤0.05 compared with control, ns = no significant difference*

Effect of Experimentally Induced Diarrhea and Constipation on Mean Arterial Pressure (MAP): The MAP (mmHg) significantly increased in constipated rats (123.7±3.2) compared with the MAP of control rats (72.6±3.6). A significant increase in MAP in constipated rats (101.0±2.0) was sustained after the recovery phase compared to control rats (90.3±0.9), (Table 3). The diarrhea values obtained were not significantly different from control rats in both the induction and recovery phases.

Effect of Experimentally Induced Diarrhea and Constipation on Heart Rate Variability (HRV): Heart rate variability (HRV) (m/s) increased significantly in constipated rats (123.4±3.2) compared with the control rats (72.3±3.7), Figure 1. The significant increase was persistent in the recovery phase in HRV of constipated rats (100.8±1.9) when compared with control rats (89.9±1.0) (Figure 1).

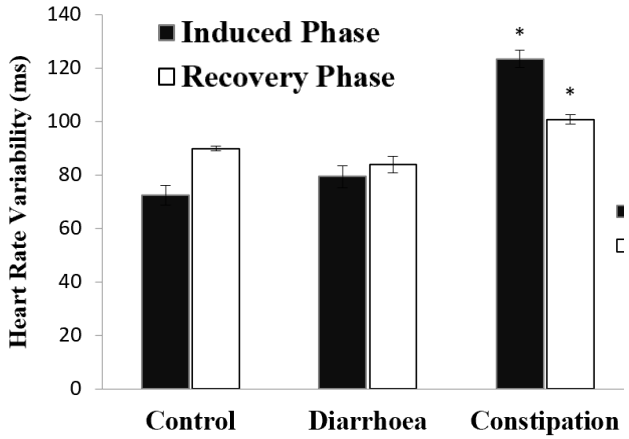

Figure 1:
The Heart Rate Variability After the Induction and Recovery from Diarrhea and Constipation
*Significant increase at P≤0.05 compared to control.

Effect of Experimentally Induced Diarrhoea and Constipation on ECG Tracing: Based on the statistical analysis of both the induced phase of gastrointestinal dysmotility and after recovery, there was no significant change in the ECG tracing of both diarrhea and constipated rats when compared to the control rats (Plate 1 and Table 4).

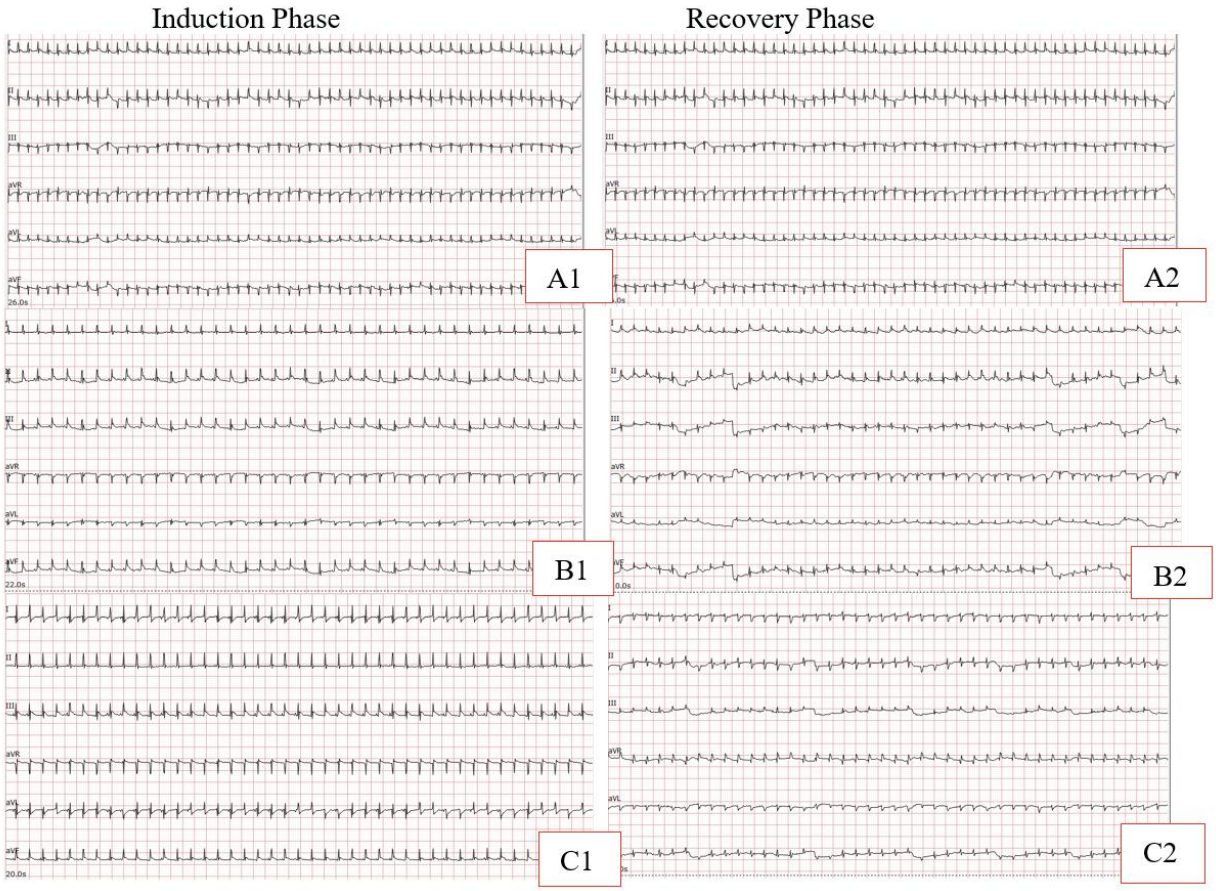

Plate 1:
Representative ECG Tracings After the Induction and Recovery from Diarrhea and Constipation. A = Control, B= Diarrhea, C= Constipation, 1= induction Phase, 2=recovery phase

Table 4:
ECG Tracing in Rats After the Induction and Recovery from Diarrhea and Constipation

	Groups					
ECG Tracing	**Induction Phase**			**Recovery Phase**		
	Control	**Diarrhea**	**Constipation**	**Control**	**Diarrhea**	**Constipation**
P (ms)	29.5±5.0	32.0±6.0^{ns}	35.5±2.0^{ns}	39.0±3.7	26.0±5.4^{ns}	32.5±3.1^{ns}
PR (ms)	43.5±8.9	53.5±1.3^{ns}	46.0±1.1^{ns}	49.3±4.9	37.8±6.1^{ns}	42.8±1.5^{ns}
QRS(ms)	19.0±2.7	22.0±1.3^{ns}	21.75±2.3^{ns}	28.0±5.7	24.8±2.8^{ns}	24.0±3.2^{ns}
QT (ms)	76.3±5.2	96.3±2.6^{ns}	111.0±11.2^{ns}	101.8±19.3	89.0±15.2^{ns}	129.3±10.9^{ns}
QTC(ms)	159.8±12.7	189.8±8.9^{ns}	238.5±21.6^{ns}	207.0±40.8	183.3±33.1^{ns}	280.3±33.4^{ns}
Ramv	0.3±0.0	0.5±0.1^{ns}	0.4±0.1^{ns}	0.3±0.1	0.2±0.1^{ns}	0.4±0.0^{ns}

ns = no significant difference compared to the control

DISCUSSION

Gastrointestinal dysmotility is a cause of significant morbidity worldwide, and it is one of the most prevalent conditions presented to medical practitioners, specialists, and surgeons across sub-specialties (Peery *et al*., 2015). Few studies have reported the association of gastrointestinal dysmotility, especially regarding diarrhea and constipation with increased cardiovascular risk (Hunter *et al*., 2001; Batty *et al*., 2007b; Ishiyama *et al*., 2019; Salmoirago-Blotcher *et al*., 2011; Sumida *et al*., 2019) but none have been able to describe the relationship in the line of the basic blood pressure variables, mean arterial pressure, heart rate variableness, and the electrical activities of the events during short-term acute constipation and short-term acute diarrhea. In this study, we observed the aforementioned changes during the induction phases of diarrhea and constipation and determined further if these changes were sustained after the recovery period.

Blood pressure (SBP and DBP) was increased in the constipated group when compared with the control rats. This increase in BP was sustained during the recovery period as well. Rats suffering from hypertension during the onset of constipation did not recuperate completely from high blood pressure after the period of recovery. It has been previously explained that the distention of the smooth muscle walls during straining to defecate and the similarity of the smooth muscle components to the blood vessels directly affect the normal functioning of the vascular system (Martins *et al*., 2015). More so, the smooth muscles of the blood vessels and the gut walls have similar fibers. They rely on the same principle even though their numbers differ considerably.

These similarities aid in lower gastrointestinal dysmotility being accompanied by BP fluctuation (Martins *et al*., 2015). Thus, the increase in BP following straining to defecate by a constipated person allows the flow of a large volume of blood to the brain, which then increases pressure in blood vessels and arteries (Salmoirago-Blotcher *et al*., 2011).

In the current study, constipation was observed to affect MAP. The reported increase in MAP in the constipated rats during both the induction and recovery phases corroborates the findings on the SBP and DBP in the constipated group. It further gave an insight into the reported increase in blood pressure as reported from our findings. Previous studies revealed that disturbances in the microbial gut are most common in patients with constipation and have been associated with stiffness of the arteries (Mancabelli *et al*., 2017; Menni *et al*., 2018), and increased blood pressure (Yang *et al*., 2015). Other effects of constipation have to include atherosclerotic cardiovascular diseases(Wang *et al*., 2011), and heart failure (Tang *et al*., 2019). Although the lipid profile and the microbiomes were not considered in this study, it is important to consider these as major limitations of our study. The heart rate variability was observed to be increased compared with the HRV of control rats and as well during the recovery phase. Abdominal cramps and straining can stimulate the vagus nerve thereby increasing the blood pressure via increased heart rate.

Diarrhea had no significant effect on both SBP and DBP from our study. This is in line with observations from previous studies that show no association between diarrheal disease and blood pressure or mortality in later life (Batty *et al*., 2007a; Batty *et al*., 2008), but infant diarrhea that results in severe dehydration in early infancy has been linked to increased blood pressure later in life (Davey Smith *et al*., 2006; Lawlor *et al*., 2006). Eventhough the rats used were adult rats, the associated prevalence of the microbiomes and arteriosclerosis as earlier discussed in constipation could not be ascertained in diarrhea disease. The physiological mechanism following the promptness and duration of diarrhea before assessment in our study could also have reduced the expected effect in terms of volume and electrolyte loss.

The MAP and HRV did not change significantly in the diarrhea group compared to the control. Further buttressing its docile effect on blood pressure and invariably on the measured cardiovascular variables in this study. Also, diarrhea and constipation had no significant effect on ECG tracing. This is particularly strange with diarrhea which is often associated with fluid and electrolyte loss. These elements are important for electrical activities and are usually deranged in diarrhea. However, the short-term acute diarrhea nature of our study might not be adequate to elicit any significant change in the ECG tracing, particularly for diarrhea.

In conclusion, constipation has been observed to have a significant aftermath effect on SBP, DBP, MAP, and HRV whereas diarrhea from our study did not show any effect on the cardiovascular variables determined. The short duration following the diarrhea induction and determination of cardiovascular variables measured was different from the usual presentation of diarrhea and constipation in terms of duration. Despite the limitation, it is however important for health practitioners to pay more attention to the cardiovascular variables that might accompany patients who present with constipation.

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