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Research Article

Hematological Changes in $_{L}$-Arginine Co-Treated Paracetamol-Intoxicated Wistar Rats

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Abstract

Paracetamol, a commonly used over the counter analgesic and antipyretic could be abused with concomitant adversity evidenced in hematological changes. $_{L}$-Arginine that is common in nuts could offer wide range of bio-benefits. This study used standard protocols to investigate hematological changes in $_{L}$-arginine co-treated paracetamol-intoxicated male Wistar rats. The rats comprising twenty-five were randomly allocated into five groups (n = 5) and fed with feed and water (Group A), $_{L}$-arginine, 60 mg/kg body weight, bwt, (Group B), Paracetamol, 1000 mg/kg bwt (Group C), $_{L}$-arginine, 60 mg/kg bwt and Paracetamol, 1000 mg/kg bwt, (Group D) and high dose $_{L}$-arginine, 120 mg/kg bwt and Paracetamol, 1000 mg/kg bwt, (Group E). Treatment was daily and *per oral* for 14 consecutive days. The result showed that the significantly ($p < 0.05$) lowered red blood cell (RBC) count in all treated groups when compared to the control was marked in paracetamol-intoxicated rats (group C) and least in $_{L}$-arginine co-treated groups (group D and group E). However, the significantly ($p < 0.05$) higher white blood cell (WBC) count in $_{L}$-arginine mono-treated group ($12.40 \pm 0.13 \times 10^{9}$/L) and paracetamol-intoxicated group ($10.50 \pm 0.13 \times 10^{9}$/L) compared to control ($9.15 \pm 0.07 \times 10^{9}$/L) was lowered ($p < 0.05$) in $_{L}$-arginine co-treated groups (group D ($8.35 \pm 0.07 \times 10^{9}$/L) and group E ($8.30 \pm 0.10 \times 10^{9}$/L)). The lowered hemoglobin (Hb) and packed cell volume (PCV) counts in the group of rats concomitantly exposed to $_{L}$-arginine and intoxicating dose of paracetamol (group E) was significant ($p < 0.05$) unlike the changes in the HB and PCV counts of rats in the other groups (B, C and D) compared to the control. Thus, $_{L}$-arginine co-administration significantly and dose dependently ameliorated paracetamol-intoxication-related alterations in particularly RBC and WBC counts of rats. The implication of the present study on the physiological integrity of the rats may be significant warranting follow up studies perhaps in higher primates including humans.

Keywords: *Red Blood Cell; Haemoglobin; Erythropoietin; Packed Cell Volume; White Blood Cell*

Abbreviations

RBC: Red Blood Cell; WBC: White Blood Cell; Hb: Hemoglobin; PCV: Packed Cell Volume; WHO: World Health Organization; SPSS: Statistical Package for Social Sciences; LSD: Least Significant Difference; SEM: Standard Error of the Mean

Introduction

Paracetamol or acetaminophen with the chemical name N-acetyl-p-aminophenol is the active metabolite of acetanilide and phenacetin [1] and a widely used over-the-counter analgesic and antipyretic [2]. Paracetamol is available in tablets and injection forms [3]. The commonly available paracetamol tablet is an odourless white crystalline solid with a bitter taste [4,5]. As a mild analgesic, it is commonly used for the relief of headaches and other minor aches, pain and is a major ingredient in numerous cold and flu remedies [6]. However,

acute paracetamol toxicity caused destruction of RBC and anemia leading to reduction in erythropoiesis and inhibition of erythropoietin release from the kidney [2]. Thus, possibility of acute paracetamol overdose exists with potentially fatal physiological dysfunctions including that related to functional integrity of the kidney [4,6] and liver [7].

$_{L}$-Arginine, a semi (conditionally) essential amino acid [8] plays a number of significant beneficial bio-functions, including protein synthesis [9], secretion of human growth hormone (HGH) [10,11] and the production of nitric oxide (NO) [12,13]. Thus, $_{L}$-arginine could improve many health dysfunctions including various heart conditions [14]. In particular, $_{L}$-arginine through its major metabolite and an important bio-molecule, NO, could improve endothelial function by increasing vasodilation and elevating blood flow [15,16]. $_{L}$-Arginine is present in common natural foods including nuts and seeds [17], thus possible inadvertent physiological elevation in $_{L}$-arginine during paracetamol intoxication exits.

Hematological changes are common bio-indicators of physiological status [18-21]. In particular, red blood cell is of importance in transporting oxygen and carbon dioxide in the body and a reduced red blood cell count implies a reduction in the level of oxygen but build up of the level of carbon dioxide [19,22-24]. On the other hand, white blood cells fight infections, defend the body by phagocytosis against invasion by foreign organisms and produce (or transport) and distribute antibodies in immune response [23,25]. Thus, changes in the WBC could adversely affect these important bio-functions. Packed cell volume, PCV, the percentage (%) of red blood cells in blood, is involved in the transport of oxygen and absorption of nutrients [24]. Thus, increased PCV indicates improved oxygen transportation and nutrients absorption. These warranted the present study aimed at determining the hematological changes in $_{L}$-arginine co-treated paracetamol-intoxicated Wistar rats.

Materials and Methods

Chemicals and reagents

Paracetamol (tablet form) was procured from Orchad Pharmacy, Nigeria, $_{L}$-arginine (AnalaR grade) manufactured by BDG Chemical Ltd, Poole England, was purchased from Fecotex Chemical Ltd Aba, Nigeria. All other chemicals used in the study were of analytical grade.

Concentration determination/justification

$_{L}$-arginine concentration (60 mg/kg b.wt) used in this study was based on the concentrations calculated from the WHO reported daily oral intake of arginine as used in earlier studies [12,17,26-28]. Intoxicating concentration of paracetamol used in this study (1000 mg/kg bwt) was based on the intoxicating concentrations used in earlier studies [2,29-31].

Animals and treatments

Animals used in this study were male Wistar rats. They were procured from the animal house of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA, for ethical treatment of laboratory animals as adapted by Ethical Committee of Michael Okpara University of Agriculture. Twenty-five male Wistar rats with mean body weight of 88.5g were acclimatized for one week before they were randomly assigned to five groups of five rats each. Group A rats served as control group and were administered feed and distilled water for 2 weeks. Group B rats were administered 60 mg/kg body weight of $_{L}$-arginine dissolved in 0.2 ml of distilled water. Group C rats were administered 1000 mg/kg body weight of paracetamol dissolved in 1.0 ml of distilled water. Group D rats were administered 60 mg/kg (low dose) of $_{L}$-arginine and 1000 mg/kg of paracetamol dissolved in 1.2 ml of distilled water. Group E rats were administered 120 mg/kg (high dose) of $_{L}$-arginine and 1000 mg/kg of paracetamol dissolved in 1.4 ml of distilled water. Treatment was by daily oral intubation for 14 days. The rats in the respective groups were housed in stainless steel cages at room temperature (28 ± 2^{0}C) and exposed to a normal daylight/dark cycle under humid tropical condition with free access to feed and water. The rats were sacrificed on the fifteenth day after an overnight fast.

Sample collection and preparation

The rats were sacrificed 24 hrs following overnight fast. The blood sample of respective rat was collected through cardiac puncture under mild ether anesthesia into anti-coagulated containers to obtain whole blood used for hematological determination.

Determination of hematological parameters

Red blood cell, white blood cell, packed cell volume and hemoglobin content respectively in the rats' whole blood was determined by respective method in Ochei and Kolhatkar [32] as respectively described previously [33].

Statistical analysis

Statistical analysis of the data was achieved using Statistical Package for Social Sciences (SPSS) version 20. The differences between the groups were tested using the least significant difference (LSD) and p-values < 0.05 were considered statistically significant. The results obtained were presented as the Mean ± SEM (Standard error of the mean).

Results

The result showed that the significantly ($p < 0.05$) lowered red blood cell (RBC) count in all treated groups when compared to the control was marked in paracetamol-intoxicated rats (group C) and least in $_{L}$-arginine co-treated groups (group D and group E) (Table 1).

Groups	RBC $\times 10^{12}$/L	Change relative to A (%)	Change relative to C (%)	Change relative to B (%)	Change relative to D (%)	Change relative to E (%)
A (Control)	1.50 ± 0.03	0.00	+25.00	+20.00	+11.11	+11.11
B (Arginine 60 mg/kg bw)	1.25 ± 0.02*	-16.66	+4.16	0.00	-7.40	-7.40
C (Paracetamol 1000 mg/kg bw)	1.20 ± 0.03*	-20.00	+0.00	-4.00	-11.11	-11.11
D (Paracetamol 1000 mg/kg bw + Arginine 60 mg/kg bw)	1.35 ± 0.02*	-10.00	+12.50	+8.00	0.00	0.00
E (Paracetamol 1000 mg/kg bw + Arginine 120 mg/kg bw)	1.35 ± 0.02*	-10.00	+12.50	+8.00	0.00	0.00

Table 1: *RBC count changes in $_{L}$-arginine co-treated paracetamol-intoxicated Wistar rats.*
*Result expressed as Mean ± SEM. *Significantly different from the control at P < 0.05.*

The result showed that the significantly ($p < 0.05$) higher white blood cell (WBC) count in $_{L}$-arginine mono-treated group ($12.40 \pm 0.13 \times 10^{9}$) and paracetamol-intoxicated group ($10.50 \pm 0.13 \times 10^{9}$) compared to control ($9.15 \pm 0.07 \times 10^{9}$) was lowered ($p < 0.05$) in $_{L}$-arginine co-treated groups (group D ($8.35 \pm 0.07 \times 10^{9}$) and group E ($8.30 \pm 0.10 \times 10^{9}$)) (Table 2).

Groups	WBC $\times 10^{9}$/L	Change relative to A (%)	Change relative to C (%)	Change relative to B (%)	Change relative to D (%)	Change relative to E (%)
A (Control)	9.15 ± 0.07	0.00	-12.85	-26.20	+9.58	+10.24
B (Arginine 60 mg/kg bw)	12.40 ± 0.13*	+35.51	+18.09	0.00	+48.50	+49.39
C (Paracetamol 1000 mg/kg bw)	10.50 ± 0.13*	+14.75	0.00	-15.32	+25.74	+26.50
D (Paracetamol 1000 mg/kg bw + Arginine 60 mg/kg bw)	8.35 ± 0.07*	-8.74	-20.47	-32.66	0.00	+0.60
E (Paracetamol 1000 mg/kg bw + Arginine 120 mg/kg bw)	8.30 ± 0.10*	-9.28	-20.95	-33.06	-0.59	0.00

Table 2: *WBC count changes in L-arginine co-treated paracetamol-intoxicated Wistar rats.*

*Result expressed as Mean ± SEM of n = 5 rats. *Significantly different from the control at P < 0.05.*

The lowered hemoglobin (Hb) and packed cell volume (PCV) counts in the group of rats concomitantly exposed to $_{L}$-arginine and intoxicating dose of paracetamol (group E) was significant ($p < 0.05$) unlike the changes in the HB and PCV counts of rats in the other groups (B, C and D) compared to the control (Table 3 and 4).

Groups	HB g/dl	Change relative to A (%)	Change relative to C (%)	Change relative to B (%)	Change relative to D (%)	Change relative to E (%)
A (Control)	12.63 ± 0.12	0.00	-2.32	-4.53	-3.44	+7.67
B (Arginine 60 mg/kg bw)	13.23 ± 0.12	+4.75	+2.32	0.00	+1.14	+12.78
C (Paracetamol 1000 mg/kg bw)	12.93 ± 0.12	+2.37	0.00	-2.26	-1.14	+10.23
D (Paracetamol 1000 mg/kg bw + Arginine 60 mg/kg bw)	13.08 ± 0.12	+3.56	+1.16	-1.13	0.00	+11.50
E (Paracetamol 1000 mg/kg bw + Arginine 120 mg/kg bw)	11.73 ± 0.21*	-7.12	-9.28	-11.33	-0.10	0.00

Table 3: *HB count changes in L-arginine co-treated paracetamol-intoxicated Wistar rats.*

*Result expressed as Mean ± SEM of n = 5 rats. *Significantly different from the control at P < 0.05.*

Groups	PCV %	Change relative to A (%)	Change relative to C (%)	Change relative to B (%)	Change relative to D (%)	Change relative to E (%)
A (Control)	38.75 ± 0.86	0.00	+1.30	-2.51	-2.51	+16.54
B (Arginine 60 mg/kg bw)	39.75 ± 0.49	+2.58	+3.92	0.00	0.00	+19.54
C (Paracetamol 1000 mg/kg bw)	38.25 ± 0.92	-1.29	0.00	-3.77	-3.77	+15.03
D (Paracetamol 1000 mg/kg bw + Arginine 60 mg/kg bw)	39.75 ± 0.49	+2.58	+3.92	-2.51	0.00	+16.54
E (Paracetamol 1000 mg/kg bw + Arginine 120 mg/kg bw)	33.25 ± 1.46*	-14.19	-13.07	-13.07	-16.35	0.00

Table 4: *PCV count changes in L-arginine co-treated paracetamol-intoxicated Wistar rats.*

*Result expressed as Mean ± SEM of n = 5 rats. *Significantly different from the control at P < 0.05.*

Discussion

Changes in hematological indices provided useful information on physiological status [18-21]. The result showed that the significantly ($p < 0.05$) lowered red blood cell (RBC) count in all treated groups when compared to the control was marked in paracetamol-intoxicated rats (group C) and least in $_{L}$-arginine co-treated groups (group D and group E) (Table 1). $_{L}$-arginine co-administration could elicit dose-dependent amelioration of paracetamol-intoxication-related reduction in RBC count of rats. The reduction of RBC as observed indicated impeded or insufficient flow of blood to the tissues and apparent unavailability of oxygen at tissue level [22,24] due perhaps to destruction of matured RBC. A significant reduction in RBC due to matured RBC destruction following paracetamol intoxication was also reported [2]. The lowered ($p < 0.05$) RBC count in rats could be indicating impeded physiological transport of oxygen and carbon dioxide and anemic state, perhaps following diminished erythropoiesis (RBC synthesis) or enhanced RBC destruction in the rats [19,22-24,34-36]. And, the result herein demonstrated the ameliorating capacity of $_{L}$-arginine against these possible consequent dysfunctions following paracetamol-intoxication-related RBC count reduction.

White blood cells spike immunity, hence an important bio-indicator of immune response status [23,25,37]. The result showed that the significantly ($p < 0.05$) higher white blood cell (WBC) count in $_{L}$-arginine mono-treated group ($12.40 \pm 0.13 \times 10^{9}$) and paracetamol-intoxicated group ($10.50 \pm 0.13 \times 10^{9}$) compared to control ($9.15 \pm 0.07 \times 10^{9}$) was lowered ($p < 0.05$) in $_{L}$-arginine co-treated groups

(group D (8.35 ± 0.07 × 10^9) and group E (8.30 ± 0.10 × 10^9)) (Table 2). Thus, $_{L}$-arginine co-administration in paracetamol-intoxicated rats elicited dose-dependent and marked reversal in higher WBC count of rats mono-treated with either $_{L}$-arginine (60 mg/kg bwt) or intoxicating dose of paracetamol. This is indicative of apparent beneficial synergistic interactive effect of $_{L}$-arginine and intoxicating dose of paracetamol on WBC count and related bio-functions in the rats. WBC counts are usually increased during immune response and the observation herein of reduced WBC could be indicating absence of immune response need. Nevertheless, reduced white blood cells could also be due to increased defense mechanism activity in response to toxic assault in the rats, warranting further confirmatory studies on the present observation.

Packed Cell Volume, PCV, and hemoglobin, HB, function to transport oxygen [24]. In a previous study, Nabila [38] reported a significant increase in HB and PCV values following administration of $_{L}$-arginine. In the present study, the seemingly similar observation was not significant ($P > 0.05$) and could be attributed to acute design-type of this study. However, the lowered hemoglobin (Hb) and packed cell volume (PCV) counts in the group of rats concomitantly exposed to $_{L}$-arginine and intoxicating dose of paracetamol (group E) was significant ($p < 0.05$) unlike the changes in the HB and PCV counts of rats in the other groups (B, C and D) compared to the control (Tables 3 and 4). Thus, it could be that $_{L}$-arginine co-administration elicited a negligible and dose independent alteration in the HB and PCV counts in paracetamol-intoxicated rats. This is indicative of apparent non-definitive influence of $_{L}$-arginine and intoxicating dose of paracetamol either alone or together in rats. The observation is worrisome and deserves follow up as concomitant reduction in RBC counts and PCV value of animals indicated reduction in erythropoiesis, possible inhibition of erythropoietin release from the kidneys [2] and anaemic conditions [21].

Conclusion

Thus, $_{L}$-arginine co-administration significantly and dose dependently ameliorated paracetamol-intoxication related alterations in particularly RBC and WBC counts of rats. The implication of the present study on the physiological integrity of the rats may be significant warranting follow up studies perhaps in higher primates including humans.

Conflict of Interest

None exists.

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