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Effects of Nicotine and Sodium Bicarbonate on Blood Parameters of Albino Rats (*Rattus norvegicus*)

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Abstract: In this study, the effects of nicotine and sodium bicarbonate on some blood parameters of male albino rats were investigated. Seventy five albino rats of 90 days old and average body weight of 190g were used. These animals were randomly selected into five groups, A, B, C, D, and E of five rats each. This experiment was replicated three times. Group A was the control group and they were given normal saline. Group B was treated with only nicotine of 0.5ml of 1g/100ml while other groups, C, D, and E received this same concentration of nicotine but with varying concentration levels of (1ml, 2ml, and 3ml of 2g/100ml) of sodium bicarbonate respectively. The treatments were administered orally using gastrointestinal cannular for four weeks. The result showed elevations of serum level in aspartate aminotransferase (AST), alanin aminotransferase (ALT) and alkaline phosphatase (ALP) in the entire treated groups but there was no significant difference, when compared with the control. There was significant difference ($P < 0.05$) in WBC of group B when compared with the control group, and the value obtained from group A was $10.6 \times 10^3 \pm 333$ cells $mm^{-3,}$ while $4.4 \times 10^3 \pm 333$ cells mm^{-3} was obtained from group B. There was also significant difference in RBC value of group B, ($P < 0.05$) when compared with the control group, and the value obtained from group A, the control group was $10.6x10^6 \pm 620$ cells mm^{-3} while $4.6 \times 10^6 \pm 340$ cells mm^{-3} was obtained from group B. Also, there was significant difference in PCV mean values of group B, C, and D, ($P < 0.05$) when compared with the control group. The value obtained from group A, the control group was 42 ± 0.5 % while 27.3 ± 1.7 %, 35 ± 1.5 %, and 36 ± 2.3 % were obtained from group B, C, and D respectively. Generally, effects of nicotine were much in group B that received 0.5ml of nicotine without sodium bicarbonate, and these effects decreased as concentration of sodium bicarbonate increased in other groups with fixed concentration of nicotine.

Keywords: Albino Rats, Nicotine, Sodium Bicarbonate and Blood Parameters

1. Introduction

Tobacco plant (*Nicotina tobacum)* has been used for several years irrespective of the location of human race. Tobacco is used in different ways, but cigarettes constitute the largest share manufactured tobacco products in the world, accounting for 96% of total sales [1]. Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarettes smoking in many people, particularly, as they derive satisfaction and pleasant sensation from inhaling nicotine [5]. Nicotine is widely consumed through cigarette smoking and tobacco chewing [1]. In Nigeria, nicotine can also be consumed through cow urine concoction, a local panacea for treatment of convulsion among the Yoruba's, because nicotine is an important constituent of cow urine [9].

Available evidence has shown that nicotine affects many biological activities. For instance, it causes bronchitis and interferes with alveoli, causing development of emphysema-like lesion and cancer of the lungs [16]. It also decreases exercise tolerance and causes coronary thrombosis [5; 19]. Cigarette and tobacco have been shown extensively to affect offspring's of mothers who are exposed to smoking, for instance, intrauterine growth retardation, this has been observed in babies of smokers [18]. So also are low birth weight [3], increase incidence of respiratory tract infection [10], asthma [22], prenatal and neonatal death or mortality [20] and reduction in uterine blood flow in both rats and

humans [7, 8]. Men who smoke cigarette have been shown to have immature sperm, low sperm count, sperm with multiple heads [21] and decreased sperm mobility and penetrability through cervix.

Cigarette smoking is known to have effects on visceral tissue in women as epidemiologic studies have clearly mediated that women who smoke suffer a lower fertility [17; 12]. [14] and [21] also reported early menopause among smokers. These toxic effects of cigarette smoking are associated with the nicotine content of cigarette [11].

Sodium bicarbonate or sodium hydrogen carbonate is the chemical compound with the formula $NaHCO_3$. Sodium bicarbonate is a white solid substance that is crystalline but often appeared as a fine powder. It has a slightly salty, alkaline taste resembling that of washing soda (sodium carbonate) [6]. Since it has long been known and widely used, the salt has many related names such as baking soda, bread soda, cooking soda and bicarbonate of soda [6]. Sodium bicarbonate is mainly prepared by the reaction of sodium chloride, ammonia, and carbon (IV) oxide in water. $NaHCO_3$ may also be obtained by the reaction of carbon (IV) oxide with an aqueous solution of sodium hydroxide. The initial reaction produces sodium carbonate. Further addition of carbon (IV) oxide produces sodium bicarbonate [6].

Sodium bicarbonate can be used as a wash to remove any acidic impurities from a crude liquid, producing purer samples [21]. Reaction of sodium bicarbonate and an acid produces salt and carbonic acid, which really decomposes to carbon (IV) oxide and water. $NaHCO_3$ is used for neutralization of acids and bases by many laboratories, because sodium bicarbonate is amphoteric, reacting with acids and bases. Sodium bicarbonate mixed with water can be used to treat acid indigestion and heartburn [13]. It is also used as medical ingredient in gripe water for infants, as treatment for aspirin overdose as aspirin requires acidic environment for proper absorption, and basic environment diminishes aspirin [13].

It has been emphasized that baking soda reduces nicotine cravings by increasing the elimination of nicotine in urine with higher pH levels [6]. With this in mind, this study was carried out to check the efficacy of sodium bicarbonate in reducing nicotine effects when administer together in mammals using albino rats to represent this group.

2. Materials and Methods

2.1. Procurement of Experimental Animal

A total of 75 male albino wistar rats of 90 days old of average body weight of 180g were used for the experiment. The animals were randomly grouped into five treatment groups of five rats in each cage. Each group was replicated three times. The rats were allowed to acclimatize in their new environment for one week before the commencement of the experiment.

2.2. Experimental Treatments

Treatment A contained five rats that were used as a positive control and they received only normal saline. Treatment B contained rats that were used as a negative control and they received 0.5ml of 1g/100ml of nicotine only. Treatment C contained rats that received 0.5ml of 1g/100ml of nicotine and 1ml of 2g/100ml of sodium bicarbonate. Treatment D contained rats that received 0.5ml of 1g/100ml of nicotine and 2ml of 2g/100ml of sodium bicarbonate. Treatment E contained rats that received 0.5ml of 1g/100ml of nicotine and 3ml of 2g/100ml of sodium bicarbonate. The treatments were administered orally using gastrointestinal cannular for four weeks. The experimental animals were given chick mash as their feed and they have free access to clean water.

2.3. Data Analysis

Red blood cell count and White blood cell count were measured by the principle of a calibrated capillary tube for blood sampling described by [2] Serum alanin aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were determined using methods of [5] while the estimation of alkaline phosphate (ALP) was done using the method of [5] The PCV was determined using microhaematocrit centrifuge. The result of the experiment was analyzed using Analysis of variance (ANOVA). The comparison of mean was separated using a post Hoc test (Least Significant Difference), [3]

3. Results

Table 1. *Mean Values of PCV of Albino Rats in different treatments.*

Group	Initial PCV	Wk 1 PCV	Wk 2 PCV	Wk 3 PCV	Wk 4 PCV
A N_i 0.0ml N_a 0.0ml	42±0.5	42.3±6	43±1.0	41.6±0.3	42±0.5
B N_i 0.5ml N_a 0.0ml	42.6±1.4	41.3±0.6	38.6±0.3	35±0.5[b]	27.3±1.7[b]
C N_i 0.5ml N_a 1ml	43±2	42±2	41.6±1.8	39.6±1.4	35±1.5[b]
D N_i 0.5ml N_a 2ml	40±1.2	40.6±1.2	40±1.1	39±1.5	36.6±2.3[b]
E N_i 0.5ml N_a 3ml	41.6±0.5	40±1.1	39.6±1.4	40±1.5	38.6±1.8
P_{values} (ANOVA)	0.225	0.350	0.188	0.016	.001

Values are means ± SE. ANOVA, analysis of variance, [b] significant different ($P<0.05$).
Ni=nicotine, Na = sodium bicarbonate, trmt = treatment, PCV = packed cell volume and wk =week

There was reduction in the PCV of all the treatment groups with little fluctuations in the mean of the control group as shown in table 1. The reduction was much in the group B that

received 0.5ml of 1g/100ml of nicotine without sodium bicarbonate and less in group E that received the highest dose of $NaCO_3$ concentration (3ml of 2g/100ml) and the same concentration of nicotine (0.5ml of 1g/100ml). The entire treatment mean except treatment E were significantly different, ($p < 0.05$) when compared with the control group as seen in table 1. The value obtained from group A, the control group was 4.2 ± 0.5% while 27.3 ± 1.7%, 35 ± 1.5% and 36.6 ± 2.3% were obtained from group B, C, and D respectively. And all the mean PCV were significantly different, ($p < 0.05$) when compared with their control and initial mean values.

Table 2. *The mean values of RBC count (× 10^6 mm^{-3}) of Albino rate in different treatments of Nicotine and Sodium Bicarbonate.*

Treatment Groups	Week 0	Week 1	Week 2	Week 3	Week 4
A Ni 0.0ml Na 0.0ml	10.2x10^6±435	10.4x10^6±440	10.6x10^6±440	10.6x10^6±450	10.6x10^6±620
B Ni 0.5ml Na 0.0ml	10.4x10^6±435	10 x10^6±340	8.6x10^6±440	6.8x10^6±440 [b]	4.6x10^6±340[b]
C Ni 0.5ml Na 1ml	9.8 x10^6±440	9.8 x10^6±445	9.2x10^6±446	7.8 x10^6±448	6 x10^6±450
D Ni 0.5ml Na 2ml	9.8 x10^6±440	9.8 x10^6±447	9.8x10^6±449	9.2 x10^6±450	8.0 x10^6±455
E Ni 0.5ml Na 3ml	10 x10^6±440	10.2x10^6±440	10.2x10^6±440	10.2x10^6±450	9.8 x10^6±500
P_{values}(ANOVA)	0.975	0.975	0.109	0.002	<0.01

Values are means ± SE. ANOVA, analysis of variance. [b] Significant different ($P < 0.05$).
Ni=nicotine, Na = sodium bicarbonate, trmt = treatment, RBC = red blood cell and wk =week

Table 2 shows the effects of nicotine and sodium bicarbonate on the red blood cell count, (RBC) (×10^6 cells mm^{-3}) of the albino rats, there was reduction in the mean values of red blood count of treatment groups when compared with the control group. Statistically, only group B was significantly different, ($p < 0.05$). However, the red blood cell count of the control group was 10.6x10^6 ± 620 cells mm^{-3} while 4.6x10^6 ±340 cells mm^{-3} was obtained from group B. The Values obtained from mean of red blood cell count of group A was 0.4 x 10^6 ± 460 cells mm^{-3}, while 0.4 x 10^6 ± 420 cells mm^{-3} and -3.5 ± 350 mm^{-3} were obtained from group B and C respectively table 2.

Table 3. *Mean values of white blood cell count (WBC) (×10^3 cells mm^{-3}) of albino rats in different treatments of nicotine (Ni) and sodium bicarbonate (Na).*

Treatment Groups	Week 0	Week 1	Week 2	Week 3	Week 4
A Ni 0.0ml Na 0.0ml	10.2x10^3±333	10.4x10^3±333	10.4x10^3±333	10.4x10^3±333	10.6x10^3±333
B Ni 0.5ml Na 0.0ml	10.4x10^3±333	10.2x10^3±333	8.4x10^3±333[b]	6.0x10^3±333[b]	4.4x10^3±333[b]
C Ni 0.5ml Na 1ml	10 x10^3±333	10 x10^3±333	9.2 x10^3±333	7.2 x10^3±333	6.4 x10^3±333
D Ni 0.5ml Na 2ml	9.6 x10^3±333	9.6 x10^3±333	9.4 x10^3±333	8.4 x10^3±333	7.4 x10^3±333
E Ni 0.5ml Na 3ml	9.8 x10^3±333	9.8 x10^3±333	9.8 x10^3±333	9.4 x10^3±333	8.6 x10^3±333
$P_{values\ (ANOVA)}$	0.294	0.084	0.001	0.001	0.001

Values are means ± SE. ANOVA, Analysis of Variance [b] Significant different

Table 3 shows the effects of nicotine and sodium bicarbonate on white blood cell (×10^3 cells mm^{-3}) count of albino rats, the WBC count decreased in all the treatment groups. But the reduction was much in group B that did not receive sodium bicarbonate but only 0.5 ml of nicotine, and this effect decreases as concentration of sodium bicarbonate increases with fixed concentration of nicotine in other groups, and it was less in group E that received highest dose of sodium bicarbonate (3ml) and 0.5 mg of nicotine. But only mean of treatment B was significantly different ($p < 0.05$) when compared with the control group statistically as shown in table 3.

Table 4. *Mean values of AST, ALT & ALP (µmm³) of albino rats in different treatment of nicotine and sodium bicarbonate.*

Treatment Groups	Aspartate	Alanine	Alkaline
A Ni 0.0ml Na 0.0mL	62.00 ± 3.786	25 ± 0.5	64.00 ± 2.309
B Ni 0.5ml Na 0.0ml	80 ± 6.429	49.67 ± 7.53	93.67 ± 12.863
C Ni 0.5ml Na 1ml	74 ± 3.786	40.67 ± 7.42	91.33 ± 11.724
D Ni 0.5ml Na 2ml	72.67 ± 3.712	37.00 ± 5.686	78.33 ± 5.783
E Ni 0.5ml Na 3ml	66.0 ± 1.0	34.33 ± 4.410	67.33 ± 1.764
P_{values} (ANOVA)	0.072	0.066	0.141

Values are means ± SE. ANOVA, Analysis of Variance
[b]Significant different, (P = < 0.05).
Ni=nicotine, Na = sodium bicarbonate, trmt = treatment, AST = aspartate aminotransferase,ALT =alanin aminotransferase and ALP =alkaline phosphatase

Table 4 shows the effects of nicotine and sodium bicarbonate on blood serum levels aspartate aminotransferase (AST), alanin aminotransferase (ALT), and alkaline amino phosphatase (ALP) (µmm³) of albino rats. There were slightly elevations in AST, ALT, and ALP in the entire treated groups when compared with the control group, but it was much in the group B that received 0.5ml of 1g/100ml of nicotine without sodium bicarbonate and less in group E that received highest concentration of sodium bicarbonate (3ml of 2g/100ml) with the same concentration of nicotine (0.5ml of 1g/100ml). But when compared with their control group statistically, the elevations were not significantly different, ($p > 0.05$) as seen in table 4.

4. Discussion

From the result obtained above, in the haematology aspect, the results showed that nicotine significantly decreased the level of blood PCV, RBC, and WBC. These decreases were observed more in the group B that received only nicotine and less in the group E that received the highest dose of sodium bicarbonate. These significant decrease in packed cell volume (PCV) and Red blood cell (RBC) may be attributed to erythrocyte lyses caused by the nicotine as reported by [22] that nicotine reduces Packed cell volume (PCV), Red blood cell count (RBC) and Hemoglobin concentration. This significant decrease in WBC is an indication that nicotine reduces WBC and immune level and thereby exposes the body to various infections. And this finding is in line with the previous report that nicotine reduces the level of immunity if taken without sodium bicarbonate [4]. Nicotine has been reported by [4] to cause direct bone marrow stem toxicity which can also contribute to decreased white blood cell observed in this study.

Again, from the results obtained in this study, nicotine elevated the serum levels of AST, ALP, and ALT which is highly indicative of hepatic toxicity induced by nicotine as the liver try to detoxify the effects of the nicotine. But the elevation was much in the group B that received only nicotine and less in the group E that received the highest dose of sodium bicarbonate when compared with the control group, which is an indication that sodium bicarbonate reduces the effects of nicotine. This finding is in line with the previous report that nicotine causes elevation in serum levels of the hepatic enzymes but sodium bicarbonate can reduce the toxicity of nicotine on the hepatic enzymes [24] It is clear from the result of this study that administration of nicotine alone induced more toxic effects on the liver than when it was administered with sodium bicarbonate. These toxic effects may be due to increased oxidative stress induced by the nicotine and also explain, the increase observed in ALT, AST, and ALP in this study. Hepatic toxicity of nicotine has been reported by [4]. The use of sodium bicarbonate to control the effects of nicotine was also reported by [4].

5. Conclusion

In this study, nicotine administration affected the entire blood parameters checked; however, the use of sodium bicarbonate to control these effects was effective as these effects of nicotine on the treated animals were observed more in the group that received only nicotine without sodium bicarbonate and also, the effects reduce as concentration of sodium bicarbonate increases with fixed concentration of nicotine. Therefore, sodium bicarbonate is effective in the treatment of adverse effect of nicotine.

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