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Calcium carbide-induced alterations of some haematological and serum biochemical parameters of wistar rats

Igbinaduwa Patrick .O[1]*, Aikpitanyi-Iduitua Rosemary .O[2]

1 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
2 Department of Physiology, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

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***Corresponding author:**

Email : patrick.igbinaduwa@uniben.edu
Tel.: +234(0)7063806665

ABSTRACT

This study seeks to investigate the possible effect of the consumption of fruits ripened with calcium carbide on some haematological and serum biochemical parameters of wistar rats. Three fruits (banana, apple and oranges) were ripened using calcium carbide.. These were fed to the animals orally and by mixing with their feed for six weeks. Haematological and serum biochemical analyses were carried out on whole blood and plasma respectively. The haematology result shows that there was a very high statistically significant difference ($p < 0.001$) in the total white blood cell count (WBC) of the rats mostly in group C (orange peel), H (orange juice) and F (banana juice); when compared with the control. Significant difference ($p < 0.05$) was also observed in the lymphocyte (LY) and granulocyte (GR) counts. There was a general transient elevation in the levels of creatinine, urea, total bilirubin, direct bilirubin, AST, ALT, Na^+, K^+, $HCO3^-$ and Cl^-. The creatinine levels of the rats fed on banana peel increased significantly ($p<0.004$). Also, chloride levels of rats fed on apple juice and orange (pulp) juice were significantly raised ($p<0.05$). On the contrary, the level of potassium ions in rats fed on whole orange was found to have declined significantly ($p<0.05$). Consumption of fruits or skin of fruit ripened with carbide may cause some inflammatory effect, trigger some allergic reactions and have an adverse effect on the kidneys.

INTRODUCTION

Fruits are one of the best natural food consumed raw. Fruits play a vital role in human nutrition by supplying the necessary growth regulating factors essential for maintaining normal health [1-2]. The rising demand of fruit safety has inspired researchers about the risk related to the use of fruit contaminated by pesticides, heavy metals or toxins [3-4]. In recent years, there has been considerable research in the literature concerning the action of different chemicals on the ripening processes of fruits [5]. The different ripening agents include calcium carbide, acetylene, ethylene, propylene, ethrel (2-chloroethyl phosphonic acid), glycol and ethanol. Calcium carbide is commonly used to induce ripening fruits artificially in many countries because it is cheap and readily available [6]. This enables the farmers to harvest immature fruits and prevent damage of transportation of ripe fruits thereby enhancing the profits of the sellers.

Fruits ripened with calcium carbide are soft and have good peel color development but poor in flavor. Calcium carbide is known to cause cancer, food poisoning, gastric irritation and mouth ulcers, headache, dizziness, mood disturbances, sleepiness, mental confusion, memory loss, cerebral edema and seizures [7].

Treatment of food with carbide is extremely hazardous because it contains traces of arsenic and phosphorous [8]. The use of this chemical in fruit industry is being discouraged worldwide and already banned in some countries due to dangers of explosion and carryover of toxic materials like arsenic and phosphorus to consumers, thus making the healthy fruit poisonous [9-10].

MATERIALS AND METHOD

Mature unripe bananas, apples and oranges were purchased from Uselu market, Egor local government area of Edo State. The fruits were exposed to calcium carbide. The exposure was done by applying the calcium carbide powder (2g/kg weight of fruit) on the surface of the fruits. The fruits were then placed in a sack and the sack was tied up and covered in a container. After a period of twenty four hours, the fruits (now ripe fruits) were exposed by opening the sack. The various fruits were then ground into paste (juice) using an electric blender. These were stored in a

refrigerator for further use.

Fifty four (54) female albino rats (wistar strain) weighing between 150g to 200g were obtained from the animal house of the Department of Anatomy, University of Benin. The animals were kept in clean plastic cages under a 12/12hours light/dark cycle and housed in the animal house of the department of Pharmacology, University of Benin. The rats were allowed to acclimatize to the laboratory environment for a period of two weeks before commencement of the experiment. Standard feed and water were provided ad-libitum. All experimental animals were handled in accordance with the US National Institutes of Health Guidelines for the care and use of laboratory animals.

Table 1. : Showing the average weight of rats before administration of fruits ripened with calcium carbide and weight after six weeks of administration.

Groups	Average Weight of rats before administration (g)	Average Weight of rats After six weeks of administration (g) p value	
A	134.8 ± 1.65	176.2 ± 1.33	p<0.05)***
B	182.5 ± 1.64	156.0 ± 1.91	p<0.05)***
C	158.0 ±1 .44	144.2 ± 1.01	p<0.05)***
D	179.1 ± 1.04	149.1 ± 1.2.72	p<0.05)***
E	170.8 ± 1.45	160.8 ± 1.36	p<0.05)***
F	207.0 ± 1.45	197.4 ± 1.26	p<0.05)***
G	153.6 ± 2.65	137.3 ± 1.35	p<0.05)***
H	187.9 ± 0.98	161.9 ± 0.99	p<0.05)***

(Results are expressed as mean±SEM)

KEY: A=Control, B= Apple juice, C= Orange skin/peel, D= Banana peel/skin, E=Whole banana (peel and pulp), F=Banana juice (pulp only) G= whole orange (skin and pulp), H=Orange (pulp only)

Table 2. : Summary of Biochemical parameters of the rats administered with fruits ripened with calcium carbide

Parameters	Control(A)	B(A.J)	C(O.S)	D(B.S)	E(W.B)	F(B.J)	G(W.O)	H (O.J)
Total protein	8.83±1.1	10.88±0.5	9.70±0.79	10.03±0.71	10.29±0.74	11.64±0.67	8.95±0.31	10.35±0.54
Albumin	2.911±0.23	3.38±0.26	3.36±0.08	2.23±0.54	3.55±1.57	3.34±0.32	3.38±0.43	2.83±0.19
T. Bilirubin	0.16±0.08	0.19± 0.04	0.41±0.31	0.24±0.09	0.78±0.41	0.14±0.07	0.39±0.25	0.88±0.63
Dir. Bil	0.10±0.03	0.25±0.07	0.82±0.41	0.42±0.27	0.18±0.08	0.92±0.56	0.21±0.11	0.09±0.01
Urea	42.94±9.46	21.66±4.03	38.79±12.84	18.69±7.82	27.27±5.36	32.11±4.21	22.73±8.43	20.50±8.32
Creatinine	1.39±0.18	1.59±1.75	1.60±0.15	2.53±0.20	1.75±0.20	1.45±0.29	1.08±0.15	1.81±0.14
AST	0.29±0.01	0.27±0.03	0.30±0.01	0.30±0.01	0.31±0.01	0.27±0.01	0.28±0.01	0.28±0.01
ALT	0.29±0.01	0.29±0.01	0.26v0.01	0.28±0.01	0.30±0.01	0.26±0.01	0.29±0.027	0.26±0.01
K^+	5.97 ±1.75	6.02±0.63	6.02±1.41	5.40±0.34	6.30±0.26	5.35±0.36	5.47±0.47	6.40±0.24
Na^+	147.35±1.33	144.07±3.45	148.05±3.90	144.28±2.15	146.06±3.61	144.04±3.0	138.23±2.33	149.4±2.56
Cl^-	104±1.04	114±3.70	110±2.31	111.6±3.54	109.3±4.04	113±5.71	109±4.80	112.5±2.23
HCO_3^-	23±1	26±1.37	24±1.21	26.4±1.83	25±1.32	25±0.81	23.67±1.66	24.25±0.75

(Results are expressed as mean±SEM)

Table 2. : Summary of Haematology parameters of the rats administered with fruits ripened with calcium carbide

	Para meters	Control(A)	B(A.J)	C(O.S)	D(B.S)	E(W.B)	F(B.J)	G(W.O)	H (O.J)	P value
1	Total WBC	8.25±1.12^{B}	8.83±1.39^{B}	3.980±0.54^{D}	8.10±1.15^{B}	5.42±1.02^{C}	12.26±1.04^{A}	10.06±1.44^{A}	7.46±1.41^{C}	P<0.001**
2	Lym	3.72±1.11^{B}	5.55±1.08^{A}	2.12±0.25^{B}	3.20±1.037^{B}	2.60±0.73^{B}	6.73±0.83^{A}	5.43±0.96^{A}	4.00±0.72^{A}	P<0.05*
3	Mo	0.87±0.23	0.61±0.11	0.26±0.05	0.68±0.166	0.65±0.13	0.96±0.17	0.86±0.08	0.58±0.13	p>0.05
4	Gra	4.22±0.91^{A}	2.66±0.43^{A}	1.56±0.43^{C}	4.20±0.41^{A}	2.20±43^{B}	4.56±1.03^{A}	3.76±0.81^{A}	2.90±0.72^{B}	P<0.05*
5	Lym%	41.97±9.42	60.48±4.84	55.00±4.77	36.22±7.61	47.02±8.16	55.40±6.36	53.76±6.80	54.02±3.59	p>0.05
6	Mon%	9.32±1.52	7.18±0.77	7.46±1.42	7.94±1.18	11.85±2.30	7.66±1.08	9.03±1.41	0.73±0.36	p>0.05
7	GRA%	48.70±9.15	32.33±4.17	37.68±5.46	55.84±8.17	41.12±7.10	36.93±7.39	37.20±5.75	38.32±3.33	p>0.05
8	RBC	6.73±0.16	6.79±0.05	6.73±0.12	6.76±0.08	6.59±0.22	6.56±0.04	6.53±0.25	6.93±0.11	p>0.05
9	Hgb	15.10±0.43	15.36±0.15	14.94±0.41	15.25±0.22	15.25±0.37	14.63±0.26	14.70±0.70	15.50±0.20	P>0.05
10	HCT	44.17±1.25	45.25±0.54	44.46±1.04	44.45±0.53	43.82±0.84	43.30±0.58`	43.23±0.83	45.14±0.81	p>0.05
11	Plat	409.5±61.53	614.66±68.81	545.8±48.89	476.60±68.24	513.50±41.70	553.00±85.45	638.33±48.69	486.40±37.25	p>0.05

Results expressed as mean ± SEM
NOTE: Similar letters indicate means that are not significantly different (P>0.05)
P>0.05 - Not significantly different
P<0.05 - Significantly different
P<0.001 - Very highly significantly different

After the period of acclimatization, the adult albino rats were randomly assigned into the following nine groups A to H. Group A served as the control, while B to H served as the experimental groups. Six rats were assigned to each group. Group A (control) had rats that were fed on standard feed (chow). To the rats in group B (apple juice group), 2 ml of apple juice was administered to each albino rat using orogastric tube, also 20 ml of the juice (paste) was mixed with 80 g of the feed. In group C (orange peel group), the albino rats were administered 2ml of blended orange peel and 20ml blended orange peel was mixed with 80g of feed. Group D (banana peel group) received 2 ml of banana peel juice/paste using orogastric tube, while 20ml of blended banana peel was mixed with 80 g of pelletized feed. Group E (whole banana group) received 2ml of blended whole banana while 20 ml was mixed with 80 g of peel. To the rats in group F (banana juice group), 2 ml of banana juice was administered to each rat using an orogastric tube while 20 ml was mixed with 80 g of their feed. Group G (whole orange group) had their feed mixed with 20 ml of blended whole orange mixed with 80 g of feed while 2 ml of the juice was administered to each albino rat using an orogastric tube. Group H (orange juice group): 2 ml of orange juice was administered to each of the albino rats using an orogastric tube, while 20 ml of the orange juice was mixed with 80 g of their feed.

The administration of fruits to the various groups was done once daily for six weeks and left feed and water were discarded daily. At the end of the six weeks, the albino rats were sacrificed after being anaesthesized using chloroform. 5 ml of blood was collected from the albino rats by cardiac puncture. 1 ml out of the total blood collected was transferred in containers containing EDTA and was used for haematological investigations. The haematology was carried out using ERMA hematology auto analyser. The remaining 4 ml of the blood collected was transferred into containers containing lithium heparin. The blood samples were spun with a bucket centrifuge at 4,000 rpm for 5 minutes so as to separate the plasma from the packed dells. The plasma obtained was kept in plain blood containers and used for the biochemical analysis. This was carried out in the Department of Physiology, University of Benin, Benin City and Chemical pathology laboratory, University of Benin Teaching Hospital. Searchlight 721 G visible spectrophotometer was used for the spectrophotometric analysis while an ion selective electrode was used for the assay of the electrolytes.

Statistical Analysis

Graph Pad Instat version 2.05 software (UK) and SPSS Version 16.0 was used for the analysis. The values were expressed as mean ± Standard Error of Mean. Statistical analysis was performed by one way variance followed by Turkey Kramer multiple comparison tests. P values <0.005 were considered as significant.

RESULTS

Table 1 showing the average weight of rats before administration of fruits ripened with calcium carbide and weight after six weeks of administration.

DISCUSSION

Fruits play a vital role in human nutrition [9]. Fruit vendors in order to meet up with the growing demand; ripen fruits in large quantity by using chemicals such as calcium carbide [11]. In this study, the need to investigate the alterations/changes that may arise in some haematological and serum biochemical parameters following the consumption of calcium carbide ripened fruits came under focus. This is because most of the fruits commonly sold in the locality is believed to be artificially ripened, and the resultant adverse effect on health is of public concern [12].

From the observations made, table 1 shows clearly that there is a significant decline in the weight of all the animals fed with various fruits ripened artificially with calcium carbide whereas in the control group, the weight of the animals increased. This decline can be attributed to the fact that most fruits are rich in fibre which tends to be very filling leading to reduction in food intake and a subsequent loss in weight [13]. This explains the reason some folks engage in consumption of fruits as a strategy for weight loss management. Also, the presence of calcium carbide or its impurities in the fruits may have caused loss of appetite.

The result of the haematological investigations reveal that there were diverse changes in the various parameters. The total WBC was noted to be significantly raised in rats in group F i.e those fed with banana juice (12.26 + 1.04) and in group G (10.06 + 1.44) i.e the group fed with whole orange when compared with the control (8.25 + 1.12). This is probably an immune response of the rat to foreign agent it may have been exposed to. It could also be as a result of the vitamins in the fruits involved which must have aided the boost in immunity hence it was reflected in the WBC by a subsequent rise. However, the reverse was observed in the rats fed on orange skin (group C). The total WBC for this group was significantly lesser (3.98 + 0.54) when compared with the controls (8.25 + 1.12). This low WBC shows that the rats in this group have impaired ability to combat infection which may be occasioned by arsenic toxicity. This is in agreement with a previous report by researchers that arsenic poisoning can lead to leucopenia [14].

Granulocytes (neutrophils) are usually the first responders to microbial infections. Their number was observed to be significantly lower (1.56 + 0.43) from the control (4.22 + 0.91) in the group C rats (orange skin) others also found to be low are the groups E (whole banana) and H (orange juice). Neutropenia (low neutrophil level) can be an indication of susceptibility to bacterial infection . No statistically significantly difference was observed in the RBC, HC and HCT when compared with the control. This finding disagrees with that made by Djembace, 2012, who reported a decrease in these parameters [15]. Platelet counts for all the groups was observed to increase with the rats fed on apple juice having the highest (614.667 + 68.811) when compared with the control (409.500 + 61.531).

From the observations made on biochemical parameters, the results indicated that there was a slight but statistically insignificant increase in the levels of the total protein, total bilirubin, direct bilirubin when compared with the control (Table 2). In the case of the AST and ALT the results obtained showed a varied (though not statistically significant) response of the experimental animals to the treatments when compared to the control group. A slight raise in AST levels was noticed in groups C, D and E (that is, orange peel, banana peel and whole banana) which might be an indication that there may be a disturbance in the activity of the liver. AST and ALT are liver enzymes concerned with amino acid metabolism. They are performed to investigate liver disease and myocardial infarction. Increases in plasma levels of AST and ALT serve as reliable indices of assessment of damage to the parenchymatous cells of the heart and liver, respectively [16]. Thus the observed significant increases in the activities of these enzymes are pointers to calcium carbide-induced lesions in heart and liver tissues. Since the various liver function tests are slightly though not statistically significantly increased, it is possible that prolonged consumption of such calcium carbide ripened fruits may result in findings that are statistically significant.

Creatinine and urea are tests performed to investigate kidney function. The results obtained on the creatinine reveal that the rats given banana peel showed a significant increase (2.53± 0.20) when compared with the control (1.39± 0.18). This suggests impairment in kidney function. Creatinine is removed, or cleared, from the body entirely by the kidneys. If kidney function is abnormal, creatinine level increases in the serum [17]. Higher levels of creatinine indicate a falling glomerular filtration rate and as a result a decreased capability of the kidneys to excrete waste products. Increase in creatinine has been used as important indices for the evaluation of the effects of chemicals on the kidney [18].

The plasma albumin level was observed to significantly increase in rats fed on whole apple. This may be as a result dehydration or probable liver function impairment as the liver is responsible for protein metabolism. Inadequate breakdown of protein by the liver makes the protein inaccessible to the cells, hence resulting in protein malnutrition and subsequent elevated albumin level.

Electrolytes are positively and negatively charged molecules called ions, which are found within the body's cells and extracellular fluids, including blood plasma. These ions are measured to assess renal (kidney), endocrine (glandular), and acid-base function. The various electrolytes analyzed showed that there was no statistically significant difference in sodium, potassium and bicarbonate results in all the treatment groups when compared with the control. However, in the groups administered with apple juice and orange juice, the chloride values increased significantly ($p<0.05$). Also, a reduced sodium level was noticed in the group given whole orange juice. The kidney maintains the acid-base balance of the body. The imbalance in these electrolytes may suggest a deficiency in the kidneys ability to maintain acid-base balance, a condition which can lead to oedema.

CONCLUSION

Consumption of fruits ripened with Calcium carbide possess a health risk. It can cause alterations in some haematological parameters like total WBC, lymphocytes and granulocytes. The liver and kidney may be impaired as well. In the present study, variations were recorded in some of the haematological and biochemical parameters considered. The result of this study suggests that consumption of fruits ripened with carbide can lower the body's ability to resist infection and weaken the immune system. It also suggests that calcium carbide causes alterations in some vital kidney and liver functions. Thus it is indicated that the consumption of calcium carbide ripened fruits is causing harmful

alterations in the haematological and biochemical profile of wistar rats and hence legislations should be strengthened and enforced to prevent the use of this chemicals in the ripening of fruits.

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Author's Statements:

Competing Interest: The authors declare no conflict of interest.

Animal Rights: The institutional and international ethical guidelines for the care and use of laboratory animals were followed.

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