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ORIGINAL ARTICLE

Scientific basis of the combination of Coca-Cola drink and tomato paste in the management of anaemia.

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Abstract

Anaemia is a global health problem affecting people of all works of life. Anaemia has a lot of adverse effects hence the need for a cost-effective and better treatment strategy. Ethnopharmacological information indicates the use of the combination of Coca-Cola drink and tomato paste to manage anaemia in Ghana. The study investigated the effect of the combination of Coca-Cola drink and tomato paste on haemoglobin levels using animal models. 20 male Wistar rats were grouped as normal, 2,4-DNPH only, Coca-Cola drink only (0.5 ml Coca-Cola drink/100 gm b. wt./day), tomato paste only (20 mg tomato paste/100 gm b. wt./day) and combination of Coca-Cola drink and tomato paste (0.5 ml Coca-Cola drink/100 gm b. wt./day and 20 mg tomato paste/100 gm b. wt./day) with 4 animals in each group. Animals in other groups apart from the normal group were injected with 2,4-DNPH consecutively for 7 days (2 mg/100 gm w.bt/day i.p,). Animals in 2,4-DNPH only group were sacrificed on the 8th day. All animals in the remaining groups were maintained till the 21st day. Body weight was significantly reduced ($p<0.05$) in all groups with 2,4-DNPH injection in the first 7 days. From the 8th-21st day, body weight was significantly gained ($p<0.05$) in the combination of Coca-Cola drink and tomato paste group. Haemoglobin concentration, red blood cell count and packed cell volume were not significantly reduced in the 2,4-DNPH only group. However, haemoglobin concentration, red blood cell count and packed cell volume were significantly increased ($p<0.05$) in the tomato paste only group and in the combination of Coca-Cola drink and tomato paste group. Conclusively, a combination of Coca-Cola drink and tomato paste had a significant increase on the haemoglobin levels indicating some haematinic and anti-anaemic potential.

Keywords: Haemolytic anaemia, Haemoglobin concentration, red blood cell count, packed cell volume, Coca-Cola drink, Tomato paste.

Introduction

Anaemia is a global health problem that affects people of all races, ages and ethnicity. Anaemia is a condition characterized by a significant reduction in the total body red blood cells (erythrocyte) mass or haemoglobin level (McLean et al., 2009). WHO estimates the number of people suffering from anaemia worldwide to be a staggering two billion.

Anaemia is common among pre-school children, pregnant women and school-aged children with worldwide prevalence of 47.4%, 41.8% and 30.2% respectively. According to Balarajan et al. (2012), anaemia is estimated to have 9% and 43% prevalence in highly developed and developing countries respectively (Goodhand et al., 2012). The differences in the prevalence rates are mostly attributed to social and economic differences in standards of living (Balarajan et al., 2012; Jelkmann and Jelkmann, 2013).

In Ghana, anaemia is common among children under 5years, pregnant women, women of reproductive age (15-49years) and

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non-pregnant women (15-49years) with prevalence of 76.10%, 62.40%, 56.40% and 55.90% respectively as of 1995-2011 (Stevens et al., 2013). To manage anaemia, there is the need to know the type, cause and severity of the condition. Some general management practices are dietary changes and use of drugs or supplements. These management procedures are aimed at increasing total red blood cell count or haemoglobin level (Gisbert and Gomollón, 2008; Hayden et al., 2012).

Ethnopharmacological information indicates the use of the combination of Coca-Cola drink and tomato paste to manage anaemia in Ghana. Coca-Cola drink is made from Cola leaf from the family *Erythroxylaceae* (Bohm et al., 1982; Hastorf, 1987). Cola leaf contains cocaine, benzeoylecgonine and ecgonine as its active ingredients (Johnson et al., 2002). Coca-Cola drink is rich in nutrients such as ascorbic acid, vitamin D, Riboflavin 5 phosphate and thiamine mononitrate (Dignass et al., 2015; Johnson et al., 2005).

According to Haytowitz and Bhagwat (2010), the USDA National Nutrient Database and US Department of Agriculture defines a standard tomato paste as one which contains nutrients such as vitamin A, vitamin K, vitamin C, folate, copper, iron and vitamin B_{12} (Barrett et al., 2007; Di Tomo et al., 2012). Efforts to reduce the worldwide prevalence of anaemia to its minimum have not been easy over time due factors such as economical differences in standard of living (Ayogu et al., 2016; Bekele et al., 2016). In Ghana, most indigenous people have resorted to the use of the combination of Coca-Cola drink and tomato paste as an alternative method in managing anaemia due to the high cost of effective drugs and the adverse side effects of the drugs to combat this problem (Pierce and Nester, 2011). Therefore, there is the need to investigate the effect of the combination of Coca-Cola drink and tomato paste, on the haemoglobin levels, using animal model.

The study will educate the general populace on the effects of the various constituent samples on the general health of an individual by emphasizing some of the scientific basis of the effects of combining Coca-Cola drink and tomato paste as a substitute for effective drugs.

Methodology

Study design and data collection

A stepwise multiple comparison study was conducted between April, 2017 and June, 2017 at the Kwame Nkrumah University of Science and Technology, 20 Wistar rats were purchased from the Pharmacy Department of KNUST and acclimatized for 2 weeks before the study.

Animal grouping and treatment

From the start of the study, the animals were placed in individual metabolic cages in an environmentally controlled room with a constant temperature (20-22ºc), a 12-hour light-dark cycle and 55-60 % humidity. The weight of the study animals was measured throughout the study period with a mass balance. Treatment of the rats in each of the groups was similar to the study by Das et al., (2015) (Table 1).

Determination of haematological parameters

About 5-10ml of blood sample was obtained from rats in each of the study groups and sent to the Clinical Analysis Laboratory (CAnLAB) of the Department of Biochemistry for analysis. Haemoglobin concentration, red blood cell (RBC), packed cell volume (PCV) of the blood sample was measured using an automated haematology analyser (Ashour, 2014)

Table 1. Treatment in experimental periods.

GROUPS (4 EACH)	TREATMENT
Group 1 (control group)	Given 0.1 ml distilled water/100 gm b.wt./day for first 7 days through intraperitoneal (i.p.) and from 8th-21st day through oral feeding.
Group 2 (2,4-DNPH group)	Given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p (SACRIFICE ON 8th DAY)
Group 3 (TP only group)	Given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p. and 20 mg tomato paste/100 gm b.wt./day in 0.3ml distilled water/100gm from 8th -21st day through oral feeding.
Group 4 (CC only group)	Given 2 mg 2,4-DNPH/100 gmb.wt./day for first 7 days through i.p. and 0.5 ml coca-cola drink/100 gmb.wt./day from 8th – 21st day through oral feeding.
Group 5 (CC-TP group)	Given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p. and 0.5 ml coca-cola drink/100 gm b.wt./day + 20 mg tomato paste/100 gm b.wt./day from 8th – 21st day through oral feeding

Statistical analysis

Data entry and analysis was done using SPSS version 20.0 and Microsoft Excel 2013. One Way ANOVA with a significant F test ($p < 0.05$) was used to determine significant differences in means among groups. The post hoc Turkey's test was also used to compare haematological and growth parameters among groups. In all comparisons, group 1 served as the control.

Ethical Approval

The study was approved by the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST and Komfo Anokye Teaching Hospital

Results

Body Growth Rate

Body weight measurements in the study was considered in two phases, 1st- 7th day and 8th- 21st day of treatment. In comparison to group (I), there was a statistically observed significant reduction ($p<0.05$) (Table 2) in the body weight for the first phase (1st- 7th day) where there was consecutive treatment of 2,4-DNHP (in group II, III, IV, V) at a dose of 2 mg/100 gm b.wt./day (Table 1). In the next 14 days, though animals in group III and IV gained an observed body weight, it was not statistically significant ($p>0.05$) when compared to the control group (group (I)) (Table 2). However, animals in group V after the second phase (8th-21st day) gained a significant ($p<0.05$) increase in body weight compared to group I. (Table 2).

Haemoglobin concentration, red blood cell count and packed cell volume

There was an observed decrease in the haemoglobin concentration, red blood cell count and packed cell volume of group (II) which was not statistically significant ($p>0.05$) compared to group (I) (Table 3). There was only an observed increase in the haemoglobin concentration, red blood cell count and packed cell volume of group (IV) which was also not statistically significant when compared to group (I) and group (II). However, the observed increase in the haemoglobin concentration, red blood cell count and packed cell volume of group (III, V) was statistically significant ($p<0.05$) (Table 3) in a post hoc test when it was compared to group (I) and group (II).

Table. 2. Mean Weight Changes among study Groups

GROUPS	Day 0 (g)	1st day -7th day(g)	8th day -21st day (g)	P value
Group I (Normal)	132.35± 6.69	133.61 ± 3.00	132.59 ± 3.11	0.17
Group II (2,4-DNPH only)	135.37 ± 14.13	128.48 ± 4.09*	Sacrificed on 8th day	0.021
Group III (Tomato paste after 2,4-DNPH withdrawal)	135.17 ± 11.07	129.95 ± 3.99*	135.49 ± 3.90	0.03
Group IV (Coca-Cola drink after 2,4-DNPH withdrawal)	140.27 ± 5.94	131.67 ± 2.34*	137.65 ± 2.16	0.01
Group V (Coca-Cola drink and tomato paste after 2,4-DNPH withdrawal)	162.97 ± 7.53	155.25 ± 2.74*	169.1 ± 1.94*	0.015

*Values are expressed as mean ± SEM, n=4. P<0.05 is considered as significant. For the turkey post hoc test, values with superscript * shows a significance compared to the group I(control)*

Table 3. Changes in Haemoglobin concentration, red blood cell count and packed cell volume among the Groups

STUDY GROUPS	Hb (g/dL)	Red Blood Cells ($\mu L \times 10^6$)	PCV (%)
Group I (Normal)	10.92 ± 0.377	6.58 ± 0.210	43.07 ± 2.657 #
Group II (2,4-DNPH only)	9.42 ± 0.335	6.22 ± 0.427	35.59 ± 1.790
Group III (Tomato paste after 2,4-DNPH withdrawal)	13.82 ± 0.593 *#	7.99 ± 0.140 *#	54.40 ± 2.48 *#
Group IV (Coca-Cola drink after 2,4-DNPH withdrawal)	12.00 ± 0.070	6.897 ± 0.037	45.77 ± 1.506 #
Group V(Coca-Cola drink and tomato paste after 2,4-DNPH withdrawal)	15.07 ± 0.381 *#	8.1800 ± 0.101 *#	56.60 ± 1.683 *#
P value	*0.023*	*0.015*	*0.02*

*Values are expressed as mean ± SEM, n=4. P<0.05 is considered as significant. For the turkey post hoc test, Values with superscript * shows a significance in comparison to group I and values with superscript # shows a significance in comparison to group I.*

Discussion

From table 2, a significant reduction in body weight was observed with reference to group (I) in the first phase (day 1-7) where 2, 4-DNPH was used to cause oxidative stress induced haemolytic anaemia. This observation agrees with previous work done by Das et al. (2015), on the haematinic potential of *jussiaea.* In his work, there was an observed significant decrease in body growth rate after consecutive administration of 2, 4-DNPH for 7 days (2 mg 2, 4-DNPH/100 gmb.wt./day). With reference to work done by Sies (1997) on oxidative stress, 2,4-DNPH caused a significant reduction in glutathione levels in the blood leading to oxidative stress because reduced glutathione levels cannot participate in the elimination of reactive intermediates by conjugation or direct free radical quenching. According to Gudmand-Høyer and Skovbjerg (1996), the oxidative stress induced by 2, 4-DNPH has been linked to reduced disaccharidase (enzyme involved carbohydrate metabolism) activity in anaemic rats. Hence, the reduction in the body weight may be due to the fact that there was inadequate carbohydrate metabolism due to the reduced disaccharidase activity as a result of 2, 4-DNPH induced oxidative stress.

In the second phase (day 8-21), the general observed increase in body weight could be due to the presence of some constituents having the ability to scavenge some of the free radicals produced by 2,4-DNPH. Mopping up free radicals is a well-known characteristic of ascorbic acid which is also a constituent in Coca-Cola drink and tomato paste (Ndem et al., 2013).

According to Bartlett et al. (2010), some chemicals can influence appetite by regulating the synthesis of serotonin, norepinephrine and dopamine. All these neurotransmitters are involved in the control of appetite and prevent overeating. By reducing the synthesis of the neurotransmitters, there is reduction in appetite and vice versa. According to Patil et al. (2000), 2,4-DNPH has side effects such as fever, dehydration ,nausea, vomiting, dizziness and loss of appetite. The general increase in the body weight can be attributed to the fact that the animals had regained appetite or the side effect of the drug in causing the loss of appetite was reducing since there was no administration of 2, 4-DNPH in the second phase of the experiment. The possible regain of appetite might have caused the animals to have the appetite to feed well hence contributing to the general observed weight gain.

There was an observed general increase in the haemoglobin concentration, red blood cell count and packed cell volume exhibited by the ingested fluids (Coca-Cola drink alone, tomato paste alone and the combination of Coca-Cola and tomato paste). The observed increase shows each of the fluids has the haematinic potential of causing the formation and development of red blood cells. Earlier studies done by Maduka et al. (2002), revealed the presence of minerals and vitamins as the major haematopoietic factors responsible for the haematinic potential of an anti-anaemic fluid. According to Johnson *et al.* (2002), the USDA National Nutrient Database for Standard Reference estimates the major nutritional constituents of Coca-Cola drink and tomato paste to be vitamins and minerals such as ascorbic acid, folate, vitamin B_{12}, magnesium and vitamin D. Hence, the ability of the ingested fluids (Coca-Cola drink alone, tomato paste alone and the combination of Coca-Cola and tomato paste) to cause an increase in the haematolgical parameters indicates the presence of some essential vitamins and minerals required for effective erythropoiesis.

From table 3, there was an observed increase in the haemtological parameters of group (IV) which was not statistically significant compared to group (II), the observed increase could be due to the fact that Coca-Cola drink has the potential of causing an increase in red blood cell production due to the presence of some vitamins and/or minerals. The insignificant increase in the haemoglobin concentration, red blood cell count and packed cell volume of group (IV) can also be due to the fact that, there were not enough or more minerals and vitamins to cause a significant increase in the haemoglobin concentration, red blood cell count and packed cell volume by effective erythropoiesis. Also, there can be a possibility where the mineral or vitamin constituents of the Coca-Cola drink work best in synergy because there was an observed significance in group (V) where Coca-Cola drink was combined with tomato paste. Hence, there may be a possibility where Coca-Cola drink may require another medium in order to release a full complement of its constituents to the maximum effect.

The varying increases in the haemoglobin concentration, red blood cell count and packed cell volume by the ingested fluids (Coca-Cola drink only, tomato paste only and combination of Coca-Cola drink and tomato paste) can be attributed to the differences in their haematinic potential conferred by the differences in the amount of vitamins and minerals. According to Ologundudu et al. (2006), vitamins and minerals are capable of stimulating effective erythropoiesis and haemoglobin synthesis. Hence, the more vitamins and minerals present, the more effective erythropoiesis and haemoglobin synthesis (Sinclair, 2013; Smith et al., 2016).

Conclusion

Coca-Cola drink alone had no significant effect on the haemoglobin levels. However, tomato paste alone and the combination of Coca-Cola drink and tomato paste had a significant effect by causing a significant increase in the haemoglobin levels.

Further studies should be done to determine whether the significance in the combination of Coca-Cola drink and tomato paste is due to the presence of the tomato paste alone or there is a synergy since Coca-Cola drink alone did not show any significant change and a group should be kept throughout the experimental period upon 2,4-DNPH withdrawal to determine whether there is a contribution by natural homeostasis in causing the restoration of haemoglobin concentration, red blood cell count and packed cell volume. Other studies should also focus on identifying effective concentrations or amounts that are required to show positive results. Due to the different varieties of coca cola and tomato paste available in Ghana, studies should also focus on using specific coca cola types and tomato pastes to validate these findings.

Conflict of Interest:

Authors declare no conflict of interest.

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