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Research Article

Effect of Oral Amodiaquine administration on the urinary electrolytes, protein and bilirubin in Caffeinated products users

Fagbohun A.B.*, Aje A.A. and Olaitan O.J.

Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State Nigeria.

email: ayodelefagbohun@yahoo.com Tel No: +2348033469738.

ABSTRACT

Background: Amodiaquine (AQ) is a popular 4-aminoquinoline antimalarial drug. It is a pro-drug which is metabolized to the active drugs, desethylAQ, bisdesethylAQ and 2-hydroxydesethylAQ. AQ is administered mainly orally as well as by intramuscular route. It is rapidly and almost completely absorbed from the gastrointestinal tract. It is excreted mainly in the urine and saliva and only about 6.0% or less is excreted unchanged in urine.

Methodology: The effect of oral Amodiaquine (AQ) administration on some urine electrolytes and protein in sixteen (16) healthy male subjects aged between 24 and 28years was reported. The subjects were divided into four (4) groups with four (4) subjects per group. 24hour urine samples were collected as the control, labeled and kept in the refrigerator at temperature of 4^{o}C. The subjects in the groupsI-IV were given 20g of kolanut, 20g of bitterkola, 350mls of coffee drink and 350mls of soft drink respectively.24hour urine samples were also collected, labeledand kept as the control samples. All the subjects were then given a single dose of AQ Hydrochloride (600mg) tablets.24hour urine samples were collected, labeled and kept the other samples collected. Colorimetric and photometric methods were used for the determination.

Result: The results showed that in group I, the level (mean±S.D) of urine sodium and potassium decreased slightly from 208.0mEq/L ± 38.89 to 195.0mEq/L±21.68 and 55.87mEq/L±28.90 to55.07mEq/L±21.38 respectively. However, in group II, urine sodiumand potassium increased from 193.3mEq/L±45.0 to 209.25mEq/L±49.08 and 42.20mEq/L±11.98 to 69.0mEq/L±47.87respectively. Similarly, a marked increase from 159.75mEq/L±58.07 to207mEq/L±73.05 of urine sodium was observed in group III while the urine potassium decreased from 53.9mEq/L±13.0 to 37.38mEq/L±18.46. In group IV, urine sodium and potassium increased from 194.25mEq/L±1.97 to 24.5mEq/L±17.9 and 46.15mEq/L±19.1 to 55.65mEq/L±33.16 respectively. Urine protein increased generally in all the four groups. Urine bilirubin was not detected in any of the sixteen (16) volunteers.

Discussion and Conclusion: The result from the research showed that oral AQ administration enhanced an increase in the excretion of sodium and potassium electrolytes in the users of bitterkola and soft drink; a slight decrease in kolanut users but increase and decrease in sodium and potassium electrolytes respectively in the case of volunteers who took coffee while protein level increased in all. Urine bilirubin was not detected.

Keywords: Amodiaquine, urinary, sodium, potassium, protein, bilirubin, caffeinated products.

INTRODUCTION

Malaria is a mosquito-borne infectious disease of human and other animals caused by parasitic protozoans of the Plasmodium type[1]. It's a major problem of tropical countries in the world and several

antimalarial drugs have been used for either prophylaxis or therapeutic purposes. Amodiaquine (AQ) is a popular 4-aminoquinoline antimalarial drug. It is a pro-drug which is metabolized to the active drugs, desethylAQ, bisdesethylAQ and 2-hydroxydesethylAQ[2]. Its use is probably more practicable in long term visitors and persons who will reside in Africa and also on the WHO list of essential medicines needed a basic health system[3]. AQ is administered mainly orally as well as by intramuscular route[4]. It is rapidly and almost completely absorbed from the gastrointestinal tract. It is excreted mainly in the urine and saliva and only about 6.0% or less is excreted unchanged in urine[5].

The body consists of the intracellular and the extracellular compartments. The amount of fluid in the extracellular compartment is 15litres in a 70kg adult[6]. The normal potassium concentration in a 24hour urine sample is between 40-80mEq[7]. Potassium moves from the extracellular fluid to the intracellular fluid either when protein is being deposited of glucose is being taken in and metabolized during extracellular alkalosis[8]. About 20% per day is lost even if there is no intake [8]. The normal concentration of sodium in a 24hour urine sample is between 80-180mEq[7]. In normal renal function, sodium is reabsorbed from the urine in order to forestall its loss. The protein molecules may be regarded as a very elaborate polypeptide consisting of a large number of amino acid residues[9]. The loss of most plasma protein through glomeruli is restricted by the size of the pores in, and by the negative charge on the basement membrane that repel negatively charged protein molecules. Normal subjects excrete up to 0.08g of protein a day in the urine[10]. Proteinurea may be due to renal disease, or more rarely, may occur because large amount of low molecular weight protein are circulating and therefore being filtered[11]. Bilirubin, a breakdown product of hemoglobin, is produced by reticulo endothelial cells scattered throughout the body[11]. The liver conjugates bilirubin with glucuronide to form conjugated bilirubin which is water soluble and hence easily removed from the body via renal excretion. In the intestine, bilirubin is degraded by colonic bacteria into urobilinogen[12]. Plasma urobilinogen enters the urine and the urine urobilinogen level reflects the amount of urobilinogen formed from the intestine bilirubin freely[12]. In normal individual, bilirubin should not be contained in the urine[13].

Previous works on the effect of AQ on the blood glucose and urine electrolytes have shown that there was a decrease in the blood glucose and urine electrolytes[14].

However, this investigation is therefore an attempt to verify the effect of caffeine, a diuretic, on the urine electrolytes, protein and bilirubin.

INSTRUMENTS AND CHEMICALS

Corning 410 Flame Photometer and Jenway 6051Colorimeter were used for the analysis. The reagents include Barium chloride (J.T. Baker), $CuSO_4.5H_2O$ (BDH), KI (BDH), NaOH (East Anglia Chemicals), H_2SO_4(BDH)while Alkaline Tartrate, Trichloroacetic acid (20%) and distilled water were collected from the Department of Pharmaceutical & Medicinal Chemistry Laboratory, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

The sodium and potassium working standards, urine protein (Biuret reagent), blank reagent (protein) and protein standards were prepared by the reference methods used in the Chemical Pathology laboratory, Olabisi Onabanjo University Teaching Hospital (O.O.U.T.H.), Sagamu, Ogun State, Nigeria

METHOD

Sample collection

Sixteen (16) healthy male volunteers aged between 24 and 28years were used for the study. None of the subjects was on regular medication and none had taken any form of antimalarial drug three months prior to the study. None of them also had any clinical or laboratory features of malaria during the study. Evaluation of the subjects was performed to ascertain their health status. The tests included blood film for malaria parasites, serum electrolyte and urea and blood pressure measurement. They were all normal. The subjects were divided into four (4) groups with four (4) subjects per group. 24hr urine samples were collected into plastic bottles (1.5L capacity) containing 5.0ml 0.1M H_2SO_4 as the control, labeled and kept in the refrigerator at temperature of 4^0C. The subjects in the groups I-IV were given 20g of kolanut, 20g of bitter kola, 350mls of coffee drink and 350mlsof soft drink respectively.24hr urine samples were also collected into another plastic bottles (1.5L capacity) containing 5.0ml 0.1M H_2SO_4, labeled and kept in the refrigerator at 4^0C. All the subjects were then given a single dose of AQ Hydrochloride (600mg) tablets (Parke-Davis & Co Detroit, USA) and 24hr urine samples were collected into another plastic bottles (1.5L capacity) containing 5.0ml 0.1M H_2SO_4, labeled and kept like the others, 24hrs after the administration of AQ.

Urine analysis

Sodium standard working solution was prepared by adding 7.0ml/150mEq of sodium stock solution to 2.0ml/150mEq of potassium stock solution and 1.0ml of deionized was later added. The potassium standard solution was also prepared by adding 5.0ml/5mEq of potassium stock solution to 4.0ml/5mEq of sodium stock solution and 1.0ml of deionized water was also added. For the urine bilirubin, Fouchet's Reagent was used while the Biuret Reagent was used for the protein analysis. After the samples were allowed to thaw at room temperature, the volume of each 24hr urine sample was carefully measured and aliquots of 10mls each were taken from the urine samples. 0.1ml of the urine samples was withdrawn using a gradated pipette and added to 20.0ml of deionized water in a labeled universal bottle and well shaken to effect proper mixing. The urine Sodium and Potassium were determined by flame emission spectrometric method as described by Christian[15] using the Corning 410 Flame photometer. For the protein determination, 1.0ml of the filtered urine samples was placed in centrifuge tubes and 1.0ml 20% Trichloroacetic acid was added. This was mixed and allowed to stand in a refrigerator overnight. The samples were then centrifuged at 3000rev/min for 5mins. The supernatant was discarded and the precipitation drained. 0.5ml of N-NaOH was added to the precipitate, stirred until they dissolve and 1.5ml of deionized water was added. 2.0ml of Biuret Reagent was added to all the solution gotten above. The Jenway 6051Colorimeter set at 540nm wavelength was validated with the blank reagent. The absorbance of the protein standard and other tests samples were measured. The calibration was revalidated after every four readings with the blank and standard solutions. For the bilirubin, 5.0ml of $BaCl_2$ was pipetted into forty-eight (48) test-tubes and 5.0ml of urine samples pipetted into each of the 48 test-tubes. The sediment was filtered and the residue was allowed to drain. Drops of Fouchet's reagent were applied to the residue on the filter paper to detect any colour change from yellow to green which indicates the presence of bilirubin.

DISCUSSION

The Sodium, Potassium and Protein levels in the urine excretion of subjects in group I, II, III and IV are shown in tables 1, 2, 3 and 4 subjects

Although some of the subjects complained of dizziness and a slight headache, they were relieved without any medical treatment. This is due to some of the side effects of the drug.

Figure A showed that was a significant increase in the urinary sodium concentration after the oral administration of AQ. This is quite different from the results in figure B and C. The normal urinary sodium concentration is between 80-180mEq/24hr[7].Hence, it can be said that there was an excessive urinary loss of sodium above the normal daily loss following oral administration of AQ. Previous work showed that AQ caused a reduced urinary excretion of sodium, potassium and chloride[14].Since the volunteers were given caffeinated products 24hour prior to the administration of the drug, then the observed trend could have been directly or indirectly connected with the caffeine content taken being a diuretic. One could also submit that the effect of caffeine on the urinary excretion of sodium must have over ridden the known effect of AQ on the excretion of sodium or that in the presence of caffeine. AQ causes increased excretion of sodium whereas in the absence of caffeine, the reverse is observed. In case of the urinary potassium, the highest concentration of urine potassium recorded being 69.0mEq/L and lowest, 37.38mEq/L following the administration of AQ the increase observed was still within the limit of the normal urine potassium which is between 40-80mEq/24hr[7]. In the case of urinary protein, there was a slight increase following AQ administration. After the administration of the caffeinated substance and the drug, the urine protein increased from 0.9g/100ml to 1.023g/100ml which was far above the normal value urine protein (about 0.5g per day)[16]. With this concentration of protein in the urine, the volunteers may be said to have exhibited moderate proteinuria[16]. Presence of bilirubin was not found in the urine excreted by the volunteers.

Table 1
Sodium, Potassium and Protein levels in the urine excretion of group I subjects

	Sodium (mEq/L)	Potassium (mEq/L)	Protein (g/100ml)
Control	183.25± 49.82	42.90 ± 21.00	0.91± 0.12
Kolanut	208.00 ± 33.89	55.88 ± 28.01	0.90 ± 0.08
Kolanut + Drug	195.00 ± 21.68	55.08 ± 21.39	0.97 ± 0.13

Values in mean ± standard deviation (SD)

Table 2
Sodium, Potassium and Protein levels in the urine excretion of group II subjects

	Sodium (mEq/L)	Potassium (mEq/L)	Protein (g/100ml)
Control	162.25 ± 69.89	51.72 ± 38.74	0.92 ± 0.11
Bitterkola	193.50 ± 45.60	42.00 ± 11.99	0.95 ± 0.14
Bitterkola + Drug	209.25 ± 49.09	69.00 ± 47.85	1.06 ± 0.17

Values in mean ± standard deviation (SD)

Table 3
Sodium, Potassium and Protein levels in the urine excretion of group III subjects

	Sodium (mEq/L)	Potassium (mEq/L)	Protein (g/100ml)
Control	157.25 ± 34.80	42.48 ± 14.76	0.91 ± 0.06
Coffee	159.75 ± 58.07	53.90 ± 13.00	0.94 ± 0.05
Coffee + Drug	207.25 ± 73.05	37.38 ± 18.46	1.06 ± 0.15

Values in mean ± standard deviation (SD)

Table 4
Sodium,Potassium and Protein levels in the urine excretion of group IV subjects

	Sodium(mEq/L)	Potassium (mEq/L)	Protein (g/100ml)
Control	204.25 ± 35.33	47.15 ± 10.17	0.90 ± 0.05
Soft drink	194.25 ± 19.97	46.15 ± 19.11	0.84 ± 0.03
Soft drink + Drug	224.50 ± 17.90	59.65 ± 33.16	1.01 ±0.09

Values in mean ± standard deviation (SD)

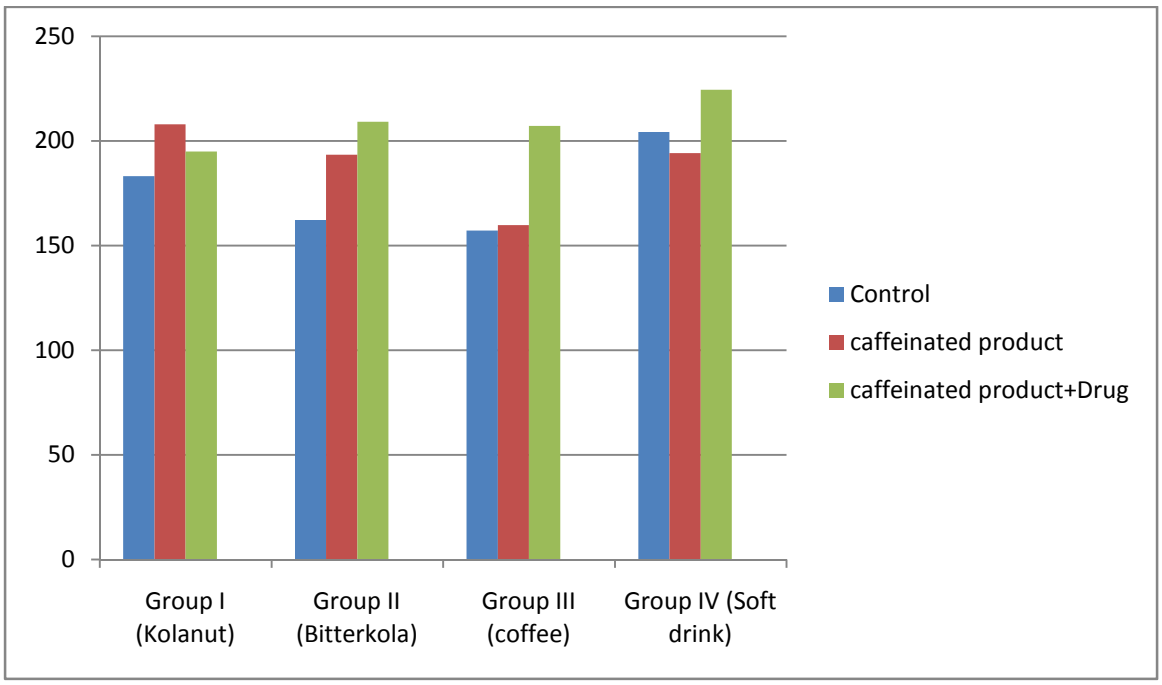

Figure A
The effect of AQ on the urinary sodium of group I-IV

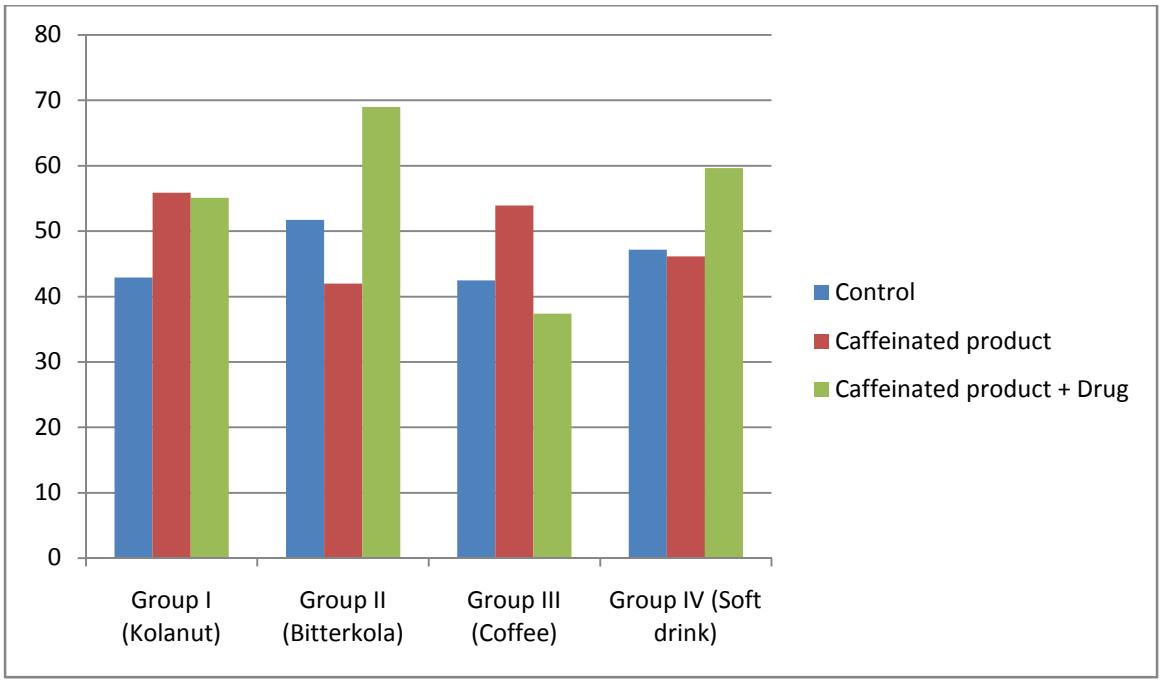

Figure B
The effect of AQ on the urinary potassium of group I-IV

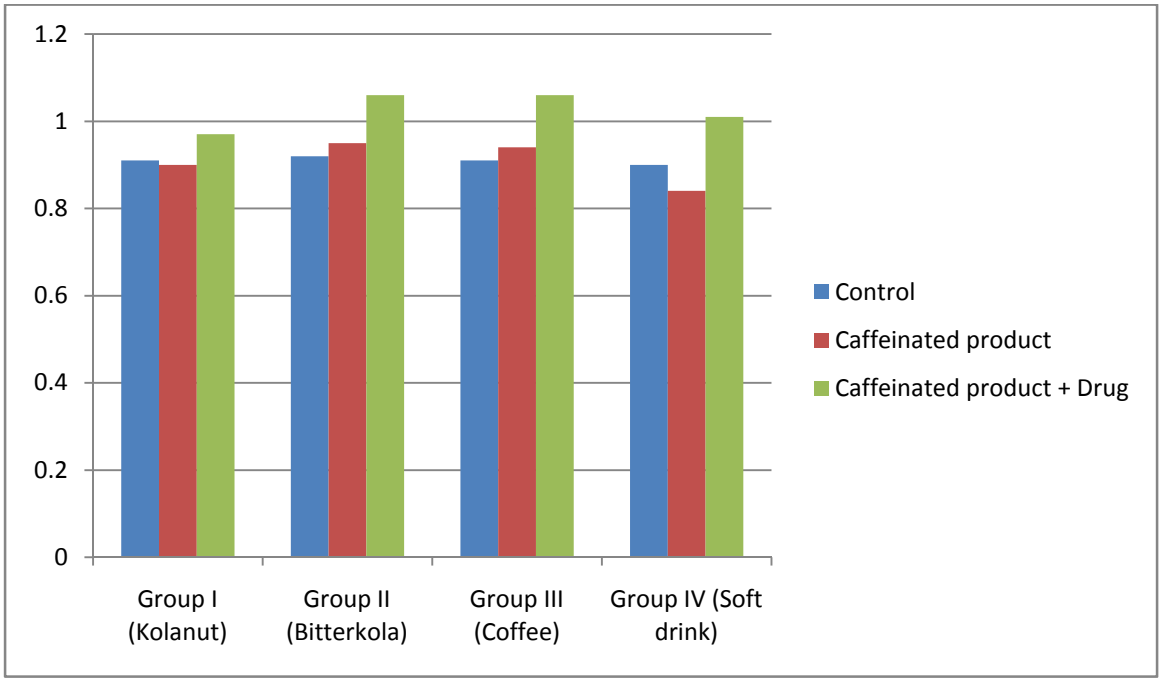

Figure C
The effect of AQ on the urinary protein of group I-IV

Previous researches have shown that the intake of Amodiaquine and Artesunate with a high fat meal resulted in a statistically significant increase in blood level of Amodiaquine and Diethylamodiaquine which

may affect the safety and tolerability of the studied drug. It also showed a decrease in Artesunate and Dihydro-artemisinin blood levels which may affect efficacy. These results suggest that the fixed-dose combination should not be administered with a high-fat meal[17,18]. Likewise, according to Parrot et al 2009, in the fed state, longer gastric residence allow more time for drug to dissolve and the Cmax is higher[19]. This food effect for controlling release formulation in man was similar to that seen in dog[19].

CONCLUSION

From the results obtained, it is observed that oral administration of AQ to users of caffeinated products caused an increase in the urinary level of sodium, potassium and protein. However, the increased urinary potassium level fell within the specified safe limit. These findings suggest that administration of AQ to users of caffeine may precipitate hyponatremia which may lead to several disease states including congestive heart failure [20]. As the drug is found to increase the urinary protein excretion of users of caffeinated products, it may lead to proteinuria.

Hence, the drug should be administered with caution to individual that are users of caffeinated products.

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