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EFFECT OF KHAT ON THE METABOLISM OF ERYTHROCYTES

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Abstract—The plant khat "Catha Edulis Forsk" is widely distributed among most East African countries, Yemen and many other areas of the world.

Administration of khat extract by the intragastric route in rabbits affected the metabolism of erythrocytes. There is a significant decrease in pyruvate kinase and the level of reduced glutathione ($P < 0.001$), and a highly significant increase in both glucose-6-phosphate dehydrogenase and glutathione reductase activities ($P < 0.001$) in khat-fed rabbits as compared to controls. On the other hand the activity of uridyl transferase as well as the concentration of 2,3-diphosphoglycerate were not significantly changed in experimental khat-fed rabbits ($P > 0.5$).

"Khat" is a common name for Catha Edulis Forsk, a shrub widely found in East Africa and the Arabian Peninsula [1]. The cultivation and consumption of khat has profound social and economic consequences for the areas concerned. In the rural areas of Yemen, the habit of chewing khat is acquired within the family, usually at an age of between 10 and 14 years, whereas in urban areas khat use is usually the result of peer group influence, and abstention can lead to social isolation [2].

Mainly because of the social and economic problems associated with khat use, international organizations became concerned with this issue as early as 1935.

The growing use of khat has motivated an interest in further knowledge of its active ingredients and their pharmacological effects. A number of studies have therefore been made in an attempt to throw light on the khat problems. Tash *et al.* [3–6] studied the effect of khat and its active principles on glycogen metabolism, liver functions, proteolytic activity and blood coagulation. Farag *et al.* [7, 8] reported the effects of these components on lipid metabolism and composition of human milk.

This work, however, is concerned with some enzymatic activities as well as some metabolites in erythrocytes in khat-consuming animals to show if there are any abnormalities associated with khat chewing. Pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G-6-P-DH), glutathione reductase (GR), uridyl transferase (UT), reduced glutathione (GSH) and 2,3-diphosphoglycerate (2,3-DPG) concentration were considered in this study.

MATERIALS AND METHODS

Khat extract. A hundred grams of fresh top leaves of the plant were subjected to water extraction according to the method described by Kamel *et al.* [9]. The extract indicated a measure of 3.27 mg active ingredients (cathine and cathinone)/ml. The extract was stored in 10 ml aliquots/vial at −20°.

Experimental animals. Twenty healthy adult rabbits, weighing 1500–2000 g each, were kept in the animal house for 10 days before starting the experiment. The animals were divided into two groups, 10 rabbits each. One group received 10 ml saline daily by the intragastric route using a fine tubing and served as a control. The second group received 10 ml of the crude khat extract daily also by the intragastric route followed by a small amount of water and was considered as test animals for 28 successive days. On day 29 the animals were sacrificed and blood samples were collected in heparinized tubes, centrifuged at 2500 *g* for 10 min, after which plasma was removed and erythrocytes were washed twice with physiological saline (0.9% g NaCl). The packed cells were resuspended in an equal volume of saline and kept at 0° for enzymatic assay. For determination of 2,3-DPG, the blood was collected, kept at 20–25° and deproteinization was carried out as follows:

A 1 ml blood sample was pipetted slowly into 2 ml perchloric acid, 0.75 mol/L, and mixed thoroughly. The tube was kept in an ice-bath for 5–10 min and then rapidly spun down the protein precipitate at 2500 *g* for 10 min. The whole supernatant perchloric acid extract was pipetted into another tube containing 100 mg $KHCO_3$. The pH was adjusted using pH paper and more hydrogen carbonate was added if the pH was below 5. The perchlorate precipitated in the cold, centrifuged at 2500 *g* for 15 min and the supernatant was used for 2,3-DPG analysis.

Reagents and chemicals were purchased as kit-reagents from Sigma Chemical Co. (St Louis, MO).

Pyruvate kinase activity was determined according to the method proposed by the International Committee for Standardization in Haematology (1977). The amount of phosphoenol pyruvate converted to pyruvate per unit time as shown by the decrease in absorbance at 340 nm, using an SP 6-550 UV/VIS Pye UNICUM spectrophotometer,

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Table 1. Effect of khat on the activities of PK, G-6-P-DH, GR and UT in erythrocytes

	PK (U/g Hb)		G-6-P-DH (U/g HB)		GR (U/g HB)		UT (U/g Hb)	
	Control (10)*	Khat-treated (10)*	Control (10)*	Khat-treated (10)*	Control (10)*	Khat-treated (10)*	Control (10)*	Khat-treated (10)*
Mean	9.62	6.03	11.93	18.47	8.92	18.23	0.62	0.63
SEM	0.334	0.34	0.53	0.86	0.39	0.76	0.03	0.04
t	6.58		5.64		9.44		0.21	
P	<0.001†		<0.001†		<0.001*		>0.5‡	

* The number of cases.
† Highly significant.
‡ Non-significant.

due to oxidation of NADH + H, is a measure of the PK activity, U/g Hb.

A standard procedure for estimation of G-6-P-DH, based on the method of Zinkham *et al.* [10] was used. The rate of increase in absorbance at 340 nm of NADPH +H is a measure of the enzymatic activity (U/g Hb). NADPH +H (reduced form) is produced from conversion of G-6-P to 6-phosphogluconate utilizing NADP (oxidized form) as a coenzyme.

Glutathione reductase activity was assayed according to the method of Beutler *et al.* [11], which is based upon following the decrease in absorbance at 340 nm of NADPH+H due to its oxidation in the reaction converting oxidized glutathione (G-S-S-G) to the reduced form (G-SH). The enzymatic activity was expressed as U/g Hb.

The concentration of the reduced glutathione was measured in the whole blood by a fluorimetric method as described by Hissin and Hill [12] in μmol/L.

UDP-glucose: α-D-galactose-1-phosphate uridyl transferase, EC 2.7.7.12 (UT) was estimated in erythrocytes (U/g Hb) according to the method described by Isselbacher [13]. The method is based on measurement of G-1-P which is formed with phosphogluconate and G-6-P-DH at 340 nm.

Estimation of 2,3-DPG was carried out according to the method described by Keitt [14], which is based upon following the glycolytic pathway of 2,3-DPG (as a primary substrate). Phosphoglycerate mutase acts as a phosphatase in the presence of effector, glycolate-2-P, and liberates 3-phosphoglycerate. Dihydroxyacetone phosphate is formed using the opposite direction of the Embden–Meyerhof pathway which consumed 1 mol of NADH+H to form 3-phosphoglycerate. So two moles of NAD^+ are liberated per mol of 2,3-DPG. The decrease in absorbance of NADH+H at 340 nm was taken as a measure of the reaction rate. The content of 2,3-DPG was calculated to be mmol per litre of erythrocytes at 20–25°.

RESULTS AND DISCUSSION

It is well known that red blood cells maintain their function and integrity by the energy produced through the anaerobic oxidation of glucose. This is clearly shown in our results obtained in normal samples (Tables 1 and 2). Administration of khat has its altering effect on the metabolism of erythrocytes as indicated by the marked inhibition of PK enzyme activity. In human erythrocytes, PK is one of the three postulated rate-controlling enzymes of glycolysis, deficiency of which, or its marked inhibition, is the most common enzymatic cause of hereditary haemolytic anaemia [15]. Lack of this enzyme reduces the utilization of glucose for production of ATP in the red cell and consequently leads to interruption in the integrity of its membrane.

In previous work in our laboratory, it was demonstrated that fasting blood glucose levels in khat-fed rabbits were significantly reduced as compared to their respective controls. In addition, Al-Safadi and Al-Qirbi [16] proved that administration of khat in rabbits effected structural and functional changes in the chief, parietal, enteroendocrine and mucous neck cells of the stomach associated with a decrease in its secretory granules. They also showed that khat administration for long periods (over 1 month) produced an inhibitory effect on goblet and enteroendocrine cells of the ileum and an increase in the degeneration of the absorptive cells in the khat-treated rabbits which may lead to depression in the blood glucose levels in these animals. Hers and Hue [17] stated that under conditions of glucose shortage, gluconeogenesis is stimulated by glucagon hormone to raise glucose level for further utilization through the production of c-AMP which in turn activates c-AMP-dependent protein kinase, the latter may, consequently, inactivate pyruvate kinase enzyme. This may explain the decreased activity of PK in khat-fed rabbits.

On the other hand, it is known that the red cell maintains its integrity through oxidation of one-tenth of glucose (G-6-P) via hexose monophosphate shunt (HMP-shunt). This is achieved by the activity of G-6-P-DH in order to give a continuous supply of NADPH+H, which is required to keep the intracellular concentrations of the reduced glutathione [18, 19].

Glutathione reductase, a flavoprotein enzyme, has the primary role in maintenance of GSH concentration in erythrocytes which in turn is available for the direct reduction of oxidized protein thiol groups [20]. Our results showed that both G-6-P-DH and GR were significantly increased in khat-fed rabbits with a marked decrease in GSH level. Reduction of GSH indicated that there was

Table 2. Effect of khat on the levels of GSH and 2,3-DPG in erythrocytes at 20–25°

	GSH (μmol/L)		2,3-DPG (mmol/L)	
	Control (10)*	Khat-treated (10)*	Control (10)*	Khat-treated (10)*
Mean	320	190	3.89	3.94
SEM	6.33	3.41	0.64	0.14
t	18.08		0.29	
P	<0.001†		>0.5‡	

* The number of cases.
† Highly significant.
‡ Non-significant.

not sufficient NADPH+H to keep the reduced glutathione level, which indicated that both G-6-P-DH and GR were compensated within the cell due to shortage in G-6-P and NADPH+H respectively. This assumption agrees with the findings obtained by Eldgran and Bremer [21] and Beutler and Teeple [22], who found that the increased levels of oxidized glutathione, G-S-S-G (as a result of the reduction in GSH), inhibited hexokinase activity; the first key enzyme in anaerobic oxidation of glucose.

Unfortunately the susceptibility of the red blood cell to haemolysis as a result of khat administration was not tested in this study to confirm the role of reduced glutathione on the maintenance of the cell membrane, which is now under investigation in our laboratory.

In addition, our results confirmed a non-significant change in the UT and 2,3-DPG in erythrocytes. The enzyme is responsible for transformation of Gal-1-P to G-1-P in the presence of UDPG. Interconversion of these hexoses in other tissues, e.g. liver, may be used as a measure of hepatic function in the galactose tolerance test [23]. Several different genetic defects have been described that cause reduced rather than total transferase deficiency. As the enzyme is normally present in excess, a reduction in activity to 50% or even less does not cause clinical manifestation of galactosemias [23].

2,3-DPG has a special function in most mammalian erythrocytes as a regulator for the oxygen affinity of haemoglobin [24]. Its concentration in erythrocytes is influenced by protons, inorganic phosphate and circulatory conditions (the mean transit time of the erythrocyte on the venous side of the circulation) as reported by Rapoport [25].

Although the effects of khat chewing can be satisfactorily explained by the presence of (−)cathinone and (+)norpseudoephedrine in the leaves, it cannot be excluded that other khat constituents might be involved in khat syndrome.

It might be granted, however, that the khat habit has certain positive aspects, since it furthers social interaction and structures social life. This is the predominant reason for its long tradition and wide social acceptance, especially in Yemen where the khat session is, for most males, the main recreational activity.

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