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Research Article

**EFFECT OF CONSUMPTION ENERGY DRINKS AND KHAT  
(CATHA EDULIS) ON THE ANTIOXIDANT SYSTEM OF  
PLASMA AND KIDNEY OF RABBITS**

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Running title: Energy drinks and *Catha edulis*

Abbreviations used: CAT, Catalase; ED, Energy Drink; GSH, Reduced glutathione; P-SH, Protein thiols; T-SH, Total thiols; UA, Uric acid.

**Abstract:**

**Background and objective:** Premixing energy drinks (EDs) with other stimulants becomes a habit widespread among adolescent and young adult worldwide, the present study has been designed to investigate the short term effect of consumption of both EDs and Khat (*Catha edulis*) the main constituent is cathinone in the plasma antioxidants of rabbits.

**Methods:** The animals were segregated randomly into four groups; control, Khat (5ml extract/kg), ED (5ml/kg) and Khat + ED groups. After 35 days of administration, the plasma was collected from all animals and their kidneys were removed, perfused and different biochemical assays were performed along with histopathological observations.

**Results:** We have seen that catalase activity and albumin levels were reduced in the Khat + ED group as compared with control, whereas, uric acid level was increased in Khat + ED animals compared with control. Significant changes between other groups were also seen in thiol contents, protein, albumin, cholesterol and UA levels. No significant changes were reported in the plasma glucose and urea levels of rabbits exposed to Khat + ED. However, the hepatocytes morphology has been affected in all treated animals compared with those of normal control.

**Conclusion:** It is concluded that mixing ED with *Catha edulis* during chewing session might lead to alter the plasma antioxidant status after short term administration which was also seen in the kidney antioxidants and histology.

**Key words:** Antioxidant; Caffeine; *Catha edulis*; Energy Drinks; Khat.

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## INTRODUCTION:

The use of stimulants (sometimes combination of two or more stimulants) among adolescents has become one of the major concerns worldwide. One of those combinations includes consumption of Energy Drinks (EDs) along with Khat (*Catha edulis*). The American Academy of Pediatrics raised a significant concern regarding the consumption of EDs by youth. They issued that caffeine and other stimulant substances contained in EDs have no place in the diet of children and adolescents [1]. The worldwide significant growth of EDs raised health concerns as this sector worth expected to reach USD 61.7075 billion by 2021 compared to USD 39.7608 billion in 2013 [2]. The major functional constituents in EDs include caffeine that provides a stimulating effect, hence, enhancing physical and mental performances [3, 4]. Consumption of higher concentrations (500-600 mg per day) of caffeine can cause caffeine intoxication which includes; restlessness, headaches, nervousness, irritability, anxiety, nausea, vomiting and in severe cases cardiovascular symptoms [5]. In addition to caffeine presents as major constituent of EDs, other constituent like taurine, guarana, carnitine and vitamin B complex must be considered [6]. Biochemical changes were reported in the plasma following consumption of ED [7].

Premixing EDs with other stimulants and drinks becomes a habit widespread among adolescent and young adult worldwide [8]. One of the stimulants that widely used in East horn of Africa and Yemen is Khat (*Catha edulis*) which has an amphetamine like action and the main constituent of Khat is cathinone along with cathine [9]. The habit of chewing Khat becomes of major concern in countries where the plant is cultivated (East African Horn and Yemen) or in western countries where the immigrants from those countries are still having the habit of chewing Khat [10]. Unfourtantly, the Khat chewers used to consume different EDs during the Khat gathering and this may raise concern about the health effect of these substances.

As consuming EDs has adverse effect in health [7] also Khat alone is a harmful substance to human body. The presence of free radicals and hence oxidative stress in the serum of Khat abusers has been reported [11]. Where, overproduction of free radicals and reactive oxygen species (ROS) causes progressive cell damage and death due to the harmful actions of these ROS because of their higher affinity to disrupt cell molecules by oxidation of lipid, proteins and DNA and responsible for wide range of diseases like cancer and neurodegenerative diseases.

ROS are produced during normal metabolic processes or from external sources including environmental or industrial toxins [12]. Our lab works focused on the Khat toxicity in different tissues; we have reported that Khat consumption responsible for reduction in antioxidant capacities in red blood cells [13] plasma [14] and saliva [15]. We also reported that chewing Khat might affect liver and kidney functions among female Khat chewers [13]. As many adults in Yemen have the habit of chewing Khat for many hours a day and consume ED during the Khat session, here and for the first time we designed this study to investigate the short term effect of consumption of both EDs and Khat present in Khat leaves in the plasma and kidney antioxidants of rabbits.

## MATERIALS AND METHODS:

### Experimental animals

Twenty four local male rabbits weighing between 700 - 1350 grams were purchased and housed in cages in the facility of Faculty of Applied Science, Tamar University, Yemen with a 12:12 hour light-dark cycle and temperature was maintained at  $22\pm 2^{\circ}\text{C}$  with humidity levels of  $55\pm 5\%$  and fed food and water *ad libitum*. They were divided into four groups each having 6 animals as follows:

1. Control group: animals received food and water *ad libitum* only.
2. Khat group: animals given 5ml of Khat juice/Kg orally for 35 days.
3. Energy drink (ED) group: animals received 5ml energy drink/kg for 35 days (each ml contains 0.22 mg of caffeine).
4. Khat-Energy drink (Khat + ED) group: animals received both Khat and ED as in groups 2 and 3.

The above doses were selected based on a survey distributed to adult Khat chewers in Tamar City. In the survey, the body weight, approximate Khat consumption period and quantity and ED consumed during the Khat session have been questioned to choose approximate consumption per Kg. The study was approved by ethical Committee at Chemistry department, Tamar University and followed the guidance of animal use and care. The doses were given between 9-11 AM daily for 35 days to check the short term effect of this combination on the plasma antioxidant. In Khat +ED group the doses were given separately one after the other and never given together (the Khat juice was given first followed by ED). At the end of the study, after 24 hours of last dose blood samples were withdrawn from rabbits and plasma were separated and used for measuring biochemical assays.

**Khat juice preparation**

The leaves of local Khat was purchased fresh and was grinded on daily basis, then the juice was separated and 5 ml/kg body weight was given to each animal in the Khat groups.

**Plasma separation**

At the end of the study, blood samples were collected from rabbit's heart into an EDTA-containing tube, then the plasma was separated by centrifugation at 1200 rpm for 5 minutes. The plasma supernatant was used directly for measurement of biochemical assays.

**Kidney homogenate and histopathology preparation**

The kidneys were perfused and homogenized in phosphate buffered saline (10% w/v, pH 7.4) and were used for measuring biochemical assays. Some parts of the kidneys of all groups were kept in formalin (40%) and used for histopathological studies with normal eosin staining.

**Biochemical Assays**

All chemicals and kits used in this study were of highest commercial grade. DTNB purchased from HiMedia, India.

**Catalase activity**

Catalase (CAT) was measured according to Luck method [16], the absorbance of a reaction mixture containing of 12.5 mM H<sub>2</sub>O<sub>2</sub> in 0.067M phosphate buffer (pH 7.0) and plasma/or kidney supernatant was read at 240 nm for 2 minutes. Results were expressed as  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> decomposed/min/g protein (molar extinction coefficient of H<sub>2</sub>O<sub>2</sub> is 71 M<sup>-1</sup> cm<sup>-1</sup>).

**Total thiol (T-SH) content**

T-SH levels were quantified in the plasma according to the method of Ellman [17], as modified by Sedlak and Lindsay [18]. The reaction mixture of plasma/or kidney homogenate sample with phosphate buffer 0.02 M and EDTA (pH 8.2) together with 100 $\mu$ l of 0.01 M DTNB was incubated for 15 minutes at room temperature and then centrifuged at 1,200 rpm for 5 minutes. The absorbance was measured at 412 nm and results were expressed as nmoles of T-SH/g protein (molar extension coefficient of DTNB is 13,600 M<sup>-1</sup> cm<sup>-1</sup>).

**Glutathione (GSH) levels**

GSH level was measured in the in the plasma according to the method of Ellman [17]. Trichloroacetic acid was added to the plasma/or kidney homogenate sample and centrifuged at 3000 rpm for 5 minutes. The supernatant was added to DTNB in 0.1 M phosphate buffer (pH 8.0) and the absorbance was read spectrophotometrically at 412 nm after 2 minutes. Results were expressed as nmoles of GSH /g protein (molar extension coefficient of DTNB is 13,600 M<sup>-1</sup> cm<sup>-1</sup>).

**Protein thiol contents (P-SH)**

P-SH contents were measured by subtracting GSH from T-SH and the results were expressed as nmoles of P-SH /g protein (molar extension coefficient of DTNB is 13,600 M<sup>-1</sup> cm<sup>-1</sup>).

**Other Biochemical assays**

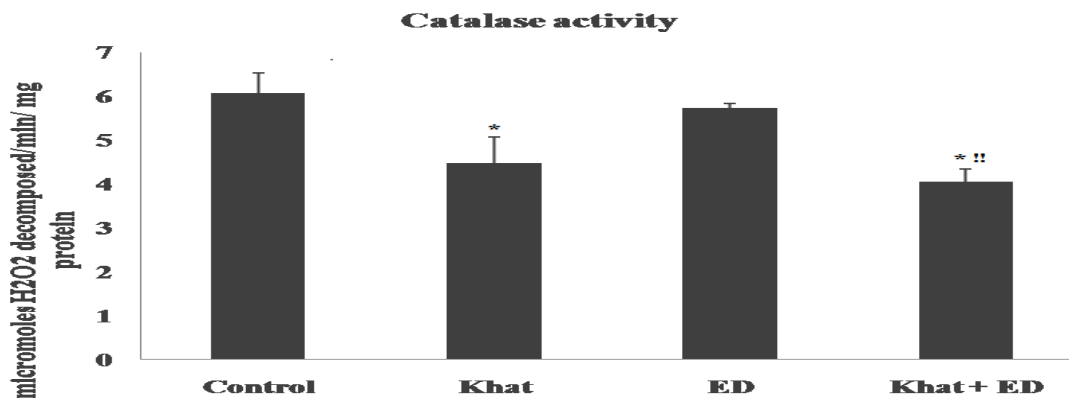
Measurement of uric acid, urea, total protein, albumin and glucose concentrations were done using commercial kits (Spinreact, Spain), whereas, cholesterol assay was measured using the kit purchased from Enzopak (India), the CVs% range between 0.21 to 1.64.

**Statistical analysis**

All measurements were performed in for all groups (N=6 each group) and all values are presented as mean  $\pm$  standard deviation using one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple-comparison post hoc test. Differences between groups were considered significant when  $p < 0.05$ . All analyses were performed using the sigma-stat software (version 3.5).

**RESULTS:****Plasma results****CAT activity**

Short term administration of both Khat and EDs resulted in a significant decrease in the enzyme activity in Khat + ED group as compared to control animals; however, Khat alone produced significant decrease in the CAT activity as compared to control (Fig. 1,  $p < 0.05$ ). The changes among other groups were statistically non-significant.



**Fig. 1:** CAT activity in the rabbit plasma of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D.,\* are significantly different from control group  $p < 0.05$ . !! are significantly different from ED group  $p < 0.05$ ,  $n = 6$  each group.

### Thiol Contents

The changes in the T-SH group were non-significant as compared to control following short term administration of Khat and ED, the only significant decrease was seen in the ED group as compared to Khat + ED (Table 1). On the other hand, GSH levels also shown non-significant changes among all groups. However, P-SH level was reduced in ED group as compared to both control and Khat + ED groups (Table 1).

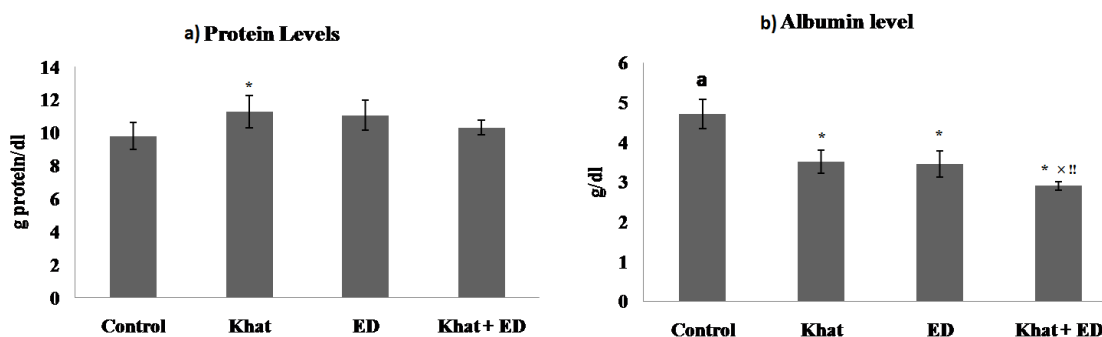
**Table 1:** Thiol contents in the plasma of control, Khat, ED, and Khat + ED in rabbits (nmoles/g protein)

Group/Test	GSH	P-SH	T-SH
Control	1.98 $\pm$ 0.42	9.12 $\pm$ 2.51	11.10 $\pm$ 2.93
Khat	1.91 $\pm$ 0.49	7.29 $\pm$ 0.4	9.20 $\pm$ 0.9
ED	2.4 $\pm$ 0.23	6 $\pm$ 0.34*	8.40 $\pm$ 0.6
Khat+ ED	2.4 $\pm$ 0.38	9.65 $\pm$ 1.4!!	12.05 $\pm$ 1.78 !!

Results are expressed as mean  $\pm$  S.D.;  $n = 6$ . \* are significantly different from control group  $p < 0.05$ . !! are significantly different from ED group  $p < 0.05$ .

### Total Protein and Albumin levels

A significant increase in the total protein level (total protein includes gluobulin, albumin and other measurable proteins in the plasma) was observed in the Khat group compared with that seen in control animals (Fig. 2a), whereas, albumin levels were decreased in all treated groups as compared to control (Fig. 2b,  $p < 0.05$ ), the less level of albumin was seen in the Khat + ED as compared to other groups which was statistically significant at  $p < 0.05$ .



**Fig. 2:** a) Total protein level and b) albumin level in the rabbit plasma of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., \* are significantly different from control group  $p < 0.05$ . x are significantly different from Khat group  $p < 0.05$ . !! Are significantly different from ED group  $p < 0.05$ ,  $n = 6$  each group.

### Uric acid and Urea

Khat + ED group showed increase in the level of uric acid (UA) as compared with control and other two groups (Fig. 3a,  $p < 0.05$ ). No significant changes were reported in urea levels in the plasma of all groups compared to control following short term administration of Khat and/or ED (Fig. 3b).

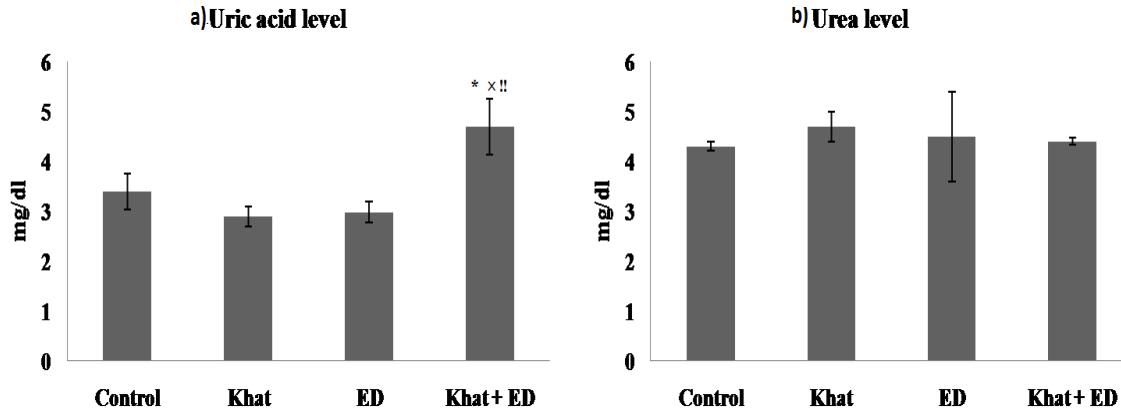


Fig. 3: a) UA level and b) urea level in the rabbit plasma of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., \* are significantly different from control group  $p < 0.05$ . x are significantly different from Khat group  $p < 0.05$ . !! are significantly different from ED group  $p < 0.05$ ,  $n = 6$  each group.

### Glucose and Cholesterol levels

Administrations of Khat and/or ED did not change glucose levels in all treated animals compared with control (Fig. 4a), however, cholesterol level was reduced in the Khat group as compared to control or ED group (Fig. 4b,  $p < 0.05$ ).

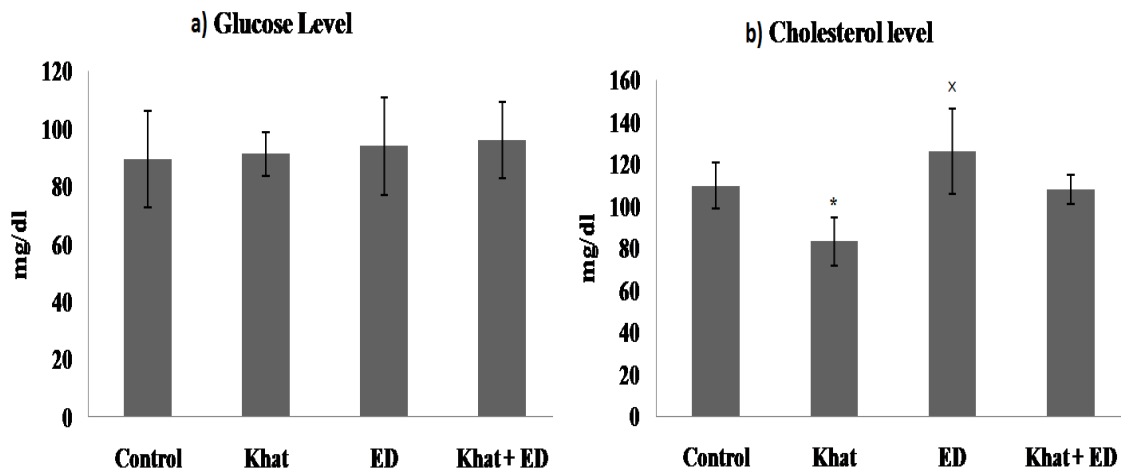
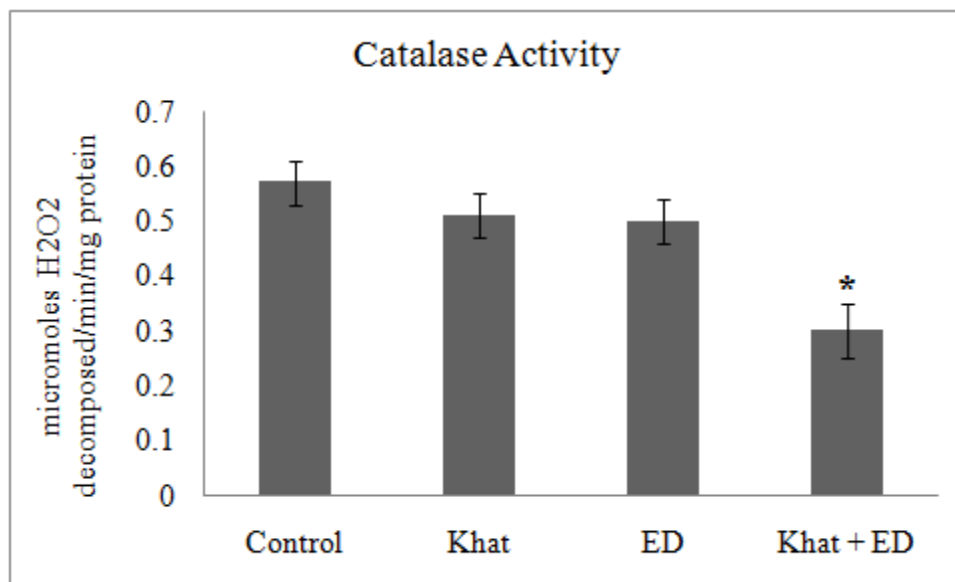


Fig. 4: a) glucose level and b) cholesterol level in the rabbit plasma of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., \* are significantly different from control group  $p < 0.05$ . x are significantly different from Khat group  $p < 0.05$ .  $n = 6$  each group.

### Kidney results

#### Catalase activity:

Mixing Khat and ED results in the reduction in CAT activity in the kidney as compared with control animals ( $p < 0.05$ ), neither Khat administration or ED alone was able to significantly change kidney CAT activity compared with controls Fig 5.



**Fig. 5: CAT activity in the rabbit kidney of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., Superscript alphabets are significantly different from their corresponding group  $p < 0.05$  considered significant compared to control,  $n=6$ .**

#### Thiol Contents

Significant reductions in the kidney levels of T-SH, GSH and P-SH were seen in response to exposure to Khat and ED as compared to control animals (Table 2,  $p < 0.05$ ). Exposure to these drugs alone also resulted in reduction of thiol contents in all groups as compared to control. T-SH and P-SH levels were not significantly reduced in all treated groups compared with each other. However, GSH contents of Khat group was the lowest and significantly different from other treated animals (Table 2,  $p < 0.05$ ).

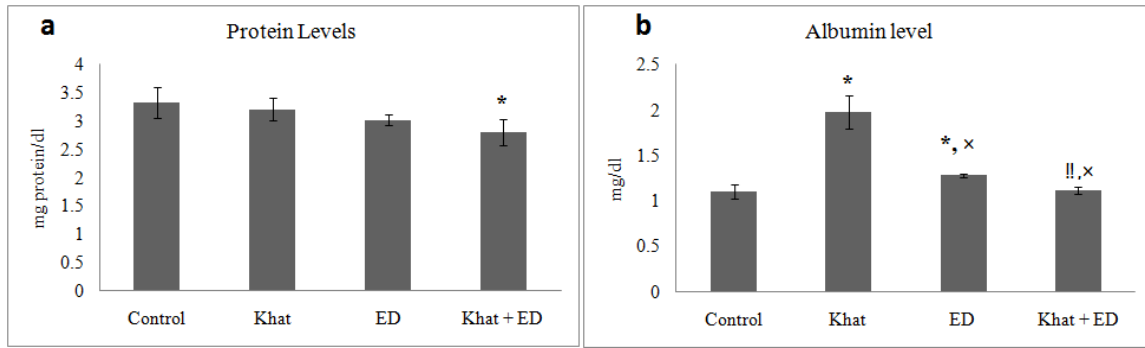
**Table 2: Thiol contents in the kidney of control, Khat, ED, and Khat + ED in rabbits (nmoles/mg protein)**

Group/Test	GSH	P-SH	T-SH
Control	$3.54 \pm 0.34$	$10.86 \pm 1.23$	$14.4 \pm 1.75$
Khat	$1.14 \pm 0.12^*$	$5.6 \pm 0.62^*$	$6.74 \pm 0.24^*$
ED	$1.84 \pm 0.2^*$	$4.6 \pm 0.09^*$	$6.44 \pm 0.29^*$
Khat+ ED	$1.89 \pm 0.31^*$	$4.79 \pm 0.49^*$	$6.68 \pm 0.8^*$

Results are expressed as mean  $\pm$  S.D.;  $n=6$ . \* are significantly different from control group  $p < 0.05$ . !! are significantly different from ED group  $p < 0.05$ .

#### Protein and Albumin levels

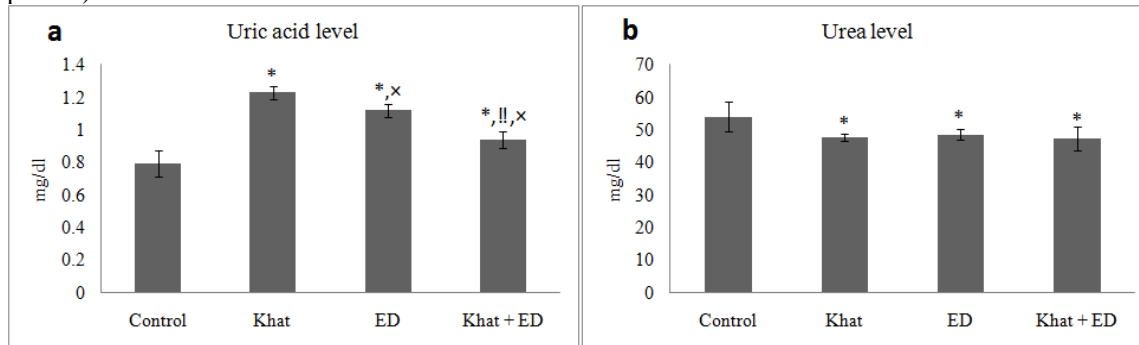
Kidney protein levels were significantly reduced only following mixing Khat with ED as compared with control animals or with other treated groups (Fig. 6a,  $p < 0.05$ ). Higher increase in the albumin levels was observed in the Khat group as compared with other groups, ED alone also showed a significant increase in the albumin levels as compared with control but no changes in the kidney albumin were seen when Khat mixed with ED as compared with controls (Fig. 6b,  $p < 0.05$ ). The results shown here indicate that administration of ED or Khat increases albumin level if they are administered alone and not when mixed.



**Fig. 6:** a) Protein level and b) albumin level in the rabbit kidney of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., Superscript alphabets are significantly different from their corresponding group  $p < 0.05$  considered significant compared to control,  $n = 6$ .

#### Uric acid and Urea

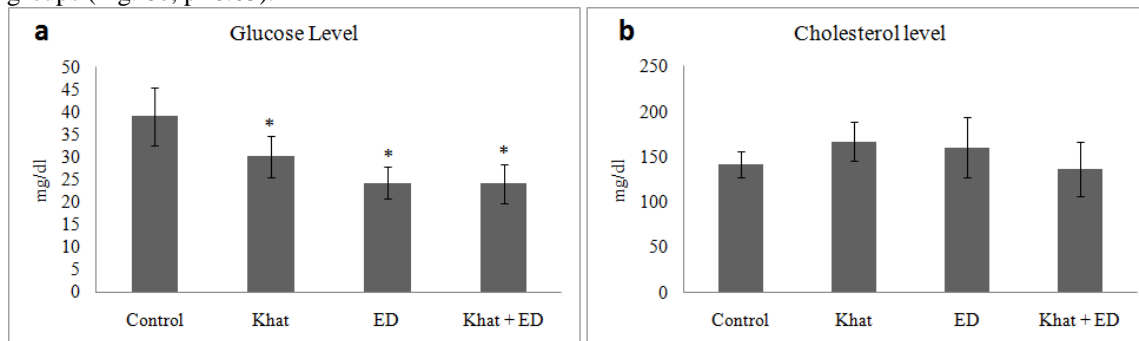
UA levels were significantly changed among all treated groups compared with control (Fig. 7a,  $p < 0.05$ ). It seems that the highest level was seen in the kidney of animals treated with Khat as compared with the other three groups, also the changes among the treated animals were statistically significant. Urea in the kidney of all treated animals was reduced as compared with control, but no significant changes were seen among the treated groups (Fig. 7b,  $p < 0.05$ ).



**Fig. 7:** a) UA level and b) urea level in the rabbit kidney of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., Superscript alphabets are significantly different from their corresponding group  $p < 0.05$  considered significant compared to control,  $n = 6$ .

#### Glucose and Cholesterol levels

Kidney Glucose levels were decreased in all treated animals when compared with controls and no significant changes seen between the treated groups (Fig. 8a). The changes in cholesterol levels were non-significant in all groups (Fig. 8b,  $p < 0.05$ ).

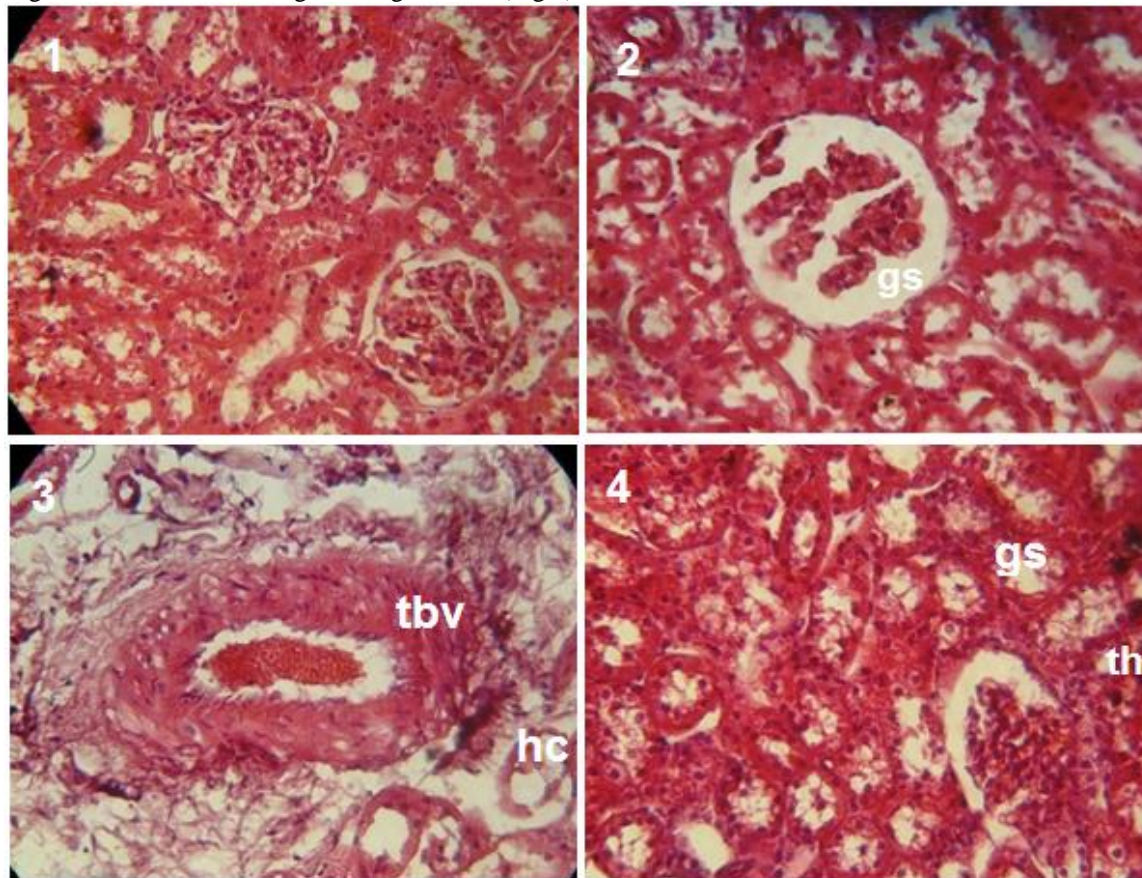


**Fig. 8:** a) glucose level and b) cholesterol level in the rabbit kidney of control, Khat, ED and Khat + ED groups. Results expressed as mean  $\pm$  S.D., Superscript alphabets are significantly different from their corresponding group  $p < 0.05$  considered significant compared to control,  $n = 6$ .



### Histopathological Changes

The changes in kidney of control revealed normal cell morphology. Glomerular shrinkage and degeneration, tubular hydropic degeneration, Hyaline casts, Thickened B.V and Tubular hydropic degeneration have been observed in the kidney of ED. Meanwhile Khat ingestion was responsible for glomerular shrinkage and degeneration and tubular hydropic degeneration. Both Khat and ED showed glomerular shrinkage and degeneration, tubular hydropic degeneration and decreasing size of glomeruli (Fig 9).



**Fig. 9: Histopathological changes in the liver of (1) control, (2) Khat, (3) ED and (4) Khat + ED groups (gs; glomerular shrinkage, tbv; thickened blood vein, th; tubular hydropic degeneration and hc; hyaline casts). (1) Normal cell morphology, (2) Glomerular shrinkage (gs) and degeneration and tubular hydropic degeneration. (3) Glomerular shrinkage and degeneration, tubular hydropic degeneration, Hyaline casts (hc), Thickened B.V (tbv) and Tubular hydropic degeneration. (4) Glomerular shrinkage and degeneration, tubular hydropic degeneration (th) and decreasing size of glomeruli**

### DISCUSSION:

Many bad habits spread widely among young and adolescent, one of those is premixing EDs with other stimulants raising concerns regarding their safety and potential role in adverse health effects and death. Here we report for the first time the changes in the plasma antioxidant levels following a short term of consumption both ED with Khat, another widely used stimulant in many countries which has cathinone as a main component. After 35 days of administration of Khat and/or ED we have found that CAT activity and albumin levels were reduced in the Khat + ED group as compared with control, whereas, UA level was increased in Khat + ED animals compared with

control. We also observed high levels of T-SH and P-SH in Khat + ED group as compared to ED. Significant changes between other groups were also seen in thiol contents, total protein, albumin, cholesterol and UA levels. No significant changes were seen in the plasma glucose and urea levels of rabbits exposed to Khat and/or ED.

These changes in the three antioxidant parameters of the present study (decrease CAT, increase UA and decrease albumin levels) of the Khat + ED group could give a positive sign of the oxidative action of using the combination of those two substances. However, the literature reported changes in the



biochemical parameters including antioxidant following consumption of Khat or ED separately. It has been reported that consumption of caffeine (2mg/ml) reduced superoxide dismutase (SOD) and CAT activities in a concentration-dependent manner in human neuronal SH-SY5Y cells [8]. Moreover, consumption of higher concentrations (500-600 mg per day) of caffeine can cause caffeine intoxication which includes; restlessness, headaches, nervousness, irritability, anxiety, nausea, vomiting and in severe cases cardiovascular symptoms [5]. The presence of caffeine in the plasma can be sufficiently high in young adolescents after caffeinated ED's ingestion, leading to cause serious caffeine intoxication [19]. We only observed a reduction in the albumin level and P-SH in the ED group compared to control and not in other parameters which could be attributed to the low dose of caffeine found in the ED we used in the present study, however, this reduction in albumin and P-SH levels could be a sign of production of ROS as both albumin and P-SH have an antioxidant capacity and hence can react with ROS [20].

The low dose of caffeine in the present study (0.22mg/ml) and short term of this study was not sufficient enough to produce changes in the parameters studied, keeping in mind the potential antioxidant effect of caffeine [6, 21]. In reverse to our observation it has been reported that the consumption of ED increased glucose levels in young healthy adults [22]. and this also could be in Khat group as compared to control, these results are in consistent with our previous findings where CAT and other non-enzymatic antioxidants were significantly reduced in red blood cells, [13], plasma [14] and saliva [15]. Chewing Khat also induces changes in total protein levels, albumin and cholesterol as a result of oxidative damage caused by Khat. No changes in the levels of UA, urea and glucose have been observed in Khat group compared with control which could be attributed to the short period of Khat administration.

### CONCLUSION:

From the data obtained here it is concluded that consumption ED with/without Khat during chewing session might lead to alter the plasma antioxidant status, with a recommendation to emphasize this effect in a long term and high concentrations study.

### Conflict of Interest

The authors declare no conflict of interest.

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