

Original Research Article

EFFECT OF ETHANOL LEAF EXTRACT OF KHAT (*Catha edulis* (FORSK) ON PREFRONTAL CORTEX OXIDATIVE MARKERS AND HEMATOLOGICAL PROFILE OF WISTAR RATS

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ABSTRACT

Aim: This study evaluated the effect of ethanol leaf extract of *Catha edulis* on the prefrontal cortex oxidative markers and hematological profile of Wistar rats.

Study design: The study design was experimental.

Place and Duration of study: Department of Biochemistry, Kampala International University, Uganda and Faculty of Medicine, Department of Pharmacy, Mbarara University of Science and Technology, Mbarara, Uganda. Between January and May, 2021.

Methodology: Fresh *C. edulis* leaves were collected after on-spot identification, and authenticated before being chopped into small pieces and pulverized mechanically. The powdered material was extracted through cold maceration using 70% ethanol for 72 h. The extract was concentrated *in vacuo* to dryness at 40°C using rotary evaporator. The lethal dose (LD₅₀) of the crude extract was determined using acute toxicity test. The sub-acute toxicity of the crude extract was evaluated by orally administering daily doses of 250, 500, and 1000 mg/kg to the male Wistar rats for 28 days. The animals were monitored for mortality, behavioral and physiological signs of toxicity. Superoxide dismutase (SOD), Catalase (CAT) activities and Malondialdehyde (MDA) levels were estimated using standard methods. The blood samples were analyzed using the automated hematology analyzer: Mindray BC-6000. Shenzhen, China.

Result: The median lethal dose (LD₅₀) of the *C. edulis* leaf extract was greater than 5000mg/kg b w. The SOD and CAT activities of *C. edulis* treated groups compared to the control group were significantly ($p < 0.05$) reduced, the malondialdehyde (MDA) levels were increased in *C. edulis* treated groups compared to the control group. There was no significant change in the hematological parameters, however, there were slight variations in the WBC and RBC counts in some *C. edulis* treated groups.

Conclusion: Long term chewing of *C. edulis* could result in oxidative stress and anemia. It is therefore important to for chronic *C. edulis* leaf chewers to take caution.

Keywords: *Catha edulis*, Catalase, Khat, Lipid peroxidation, Malondialdehyde, Superoxide dismutase

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1. INTRODUCTION

Catha edulis (*C. edulis*) commonly known as khat is a widely cultivated and consumed psychoactive herb in East Africa and the Arabian Peninsula [6, 51]. The chewing of young *C. edulis* leaves has become a common practice among predominant cultivators mainly due to the stimulating and psychoactive effects they derive from them [6]. Plant of *C. edulis* has gradually become the socio-cultural norm among the populations of some Africa and Asia countries as users continuously engage in chewing sessions even during ceremonies [6]. Currently *C. edulis* is currently regarded as the most widely grown, traded and abused illegal drug in the world [45] and about 147 million people now consume it annually worldwide. In Africa, over 34 million people now use *C. edulis* as the main narcotic drug [56]. Cathinone, the potent psychostimulant alkaloid which is reported to have amphetamine-like properties and contributes to the pharmacological effect of *C. edulis* is usually released 15-45 minutes during chewing [25]. On drying or exposure to air, cathinone is hydrolyzed to (+) nor pseudoephedrine (cathine) and (-)-norephedrine which are more stable and less potent molecules. *C. edulis* users therefore obtain and immediately chew the freshly harvested leaves in order to derive adequate psychoactive effects [15, 33]. The stimulating effects of *C. edulis* has continue to induce repeated chewing among the users which could lead to tolerance and subsequent development of psychic or behavioural disorders or both on the users [4]

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Evidence from previous studies using *in vitro* and *in vivo* models [9, 44] have linked prolong consumption of *C. edulis* to many adverse health effects including acute myocardial infarction, ischemia and heart failure in the cardiovascular system [6], euphoria, mild excitement, anorexia, insomnia, and anxiety in the CNS [12], stomatitis, esophagitis, and gastritis in the GIT [3]. Besides the adverse health effects of prolong use, *C. edulis* have also been reported to generate free radicals [8, 10]. Free radicals (FR) are part of normal physiological processes, their abnormal levels can result in the damage of cellular macromolecules notably, proteins, lipids, nucleic acids and carbohydrates through protein peroxidation, lipid peroxidation, DNA or RNA oxidation and glycosylation respectively [11, 57, 23, 36]. The maintenance of cellular homeostasis to prevent the adverse effects of reactive oxygen species (ROS) is therefore an essential process in all aerobic organisms and this is achieved through the body's endogenous antioxidants defense systems which comprise of three main types of antioxidant enzymes namely, superoxide dismutases (SOD), catalases (CAT) and glutathione peroxidases (GPX)[44, 35, 58, 16].

The brain is one of the most targeted organs by the constituents of stimulant drugs or herbs. Unlike other tissues, the brain has weak endogenous antioxidant defense system which makes it vulnerable to imbalance in redox homeostasis [31]. Metabolism in the brain generates high amounts of H₂O₂ whereas, low catalase levels in most regions of the brain have been reported [27, 31]. Hematological parameters especially red blood cells (RBCs) are also susceptible to oxidative insults due to the high cell amount of oxygen and hemoglobin they contain. Under compromised conditions therefore, the RBCs are the first

target cells [12]. The response of the body to stress, puncture, injury and imbalance is therefore usually obtain through the blood profile [14]. Hence, to ascertain the effect of substances and plant extract like *C. edulis*, the evaluation of hematological profile is important [53].

Oxidative stress (OS) which results from the physiological imbalance between the levels of oxidants and antioxidants in the cells [29] has continued to attract investigation by researchers particularly on the possible causes. Interestingly, most studies have investigated the effect of *C. edulis* on oxidative markers in a bit to understand the causes of OS in in serum [8, 10], liver and kidney tissues [22] and the brain [42], but investigations into the effect chronic *C. edulis* use on prefrontal cortex oxidative markers and hematological profile is still missing. This present study therefore evaluated the effect of ethanol leaf extract of *C. edulis* on the prefrontal cortex oxidative markers and hematological profile in Wistar rats.

2. MATERIALS AND METHODS

2.1 CHEMICALS, REAGENTS AND INSTRUMENTS

The chemicals and reagents used in the current study were obtained from Griffchem fine, and LOBA chemicals, India. RADWAG Wagi (MYA.21.4Y.P) weighing balance was used for weighing reagents and Jenway® UV-visible (Model: 6715) spectrophotometer was used for all the spectrophotometric measurements.

2.1.1 EXPERIMENTAL DESIGN

Fresh leaves of *C. edulis* were collected identified and authenticated by a taxonomist. The experimental animals were obtained from the Animal unit of Kampala International University, Western campus. The animals were allowed to acclimatize for two (2) weeks before the start of the study. The Fresh leaves of Khat were extracted using 70% (v/v) ethanol. Part of the extract was used for phytochemical screening, acute toxicity study and sub-acute study. The results of the LD₅₀ of the ethanol plant extract informed the dose selection for sub-acute oral animal administration for twenty-eight (28) days. After 28 days, the animals anaesthetized, sacrificed and blood samples and the brain was carefully removed to analyze the effect of *C. edulis* extract on hematological parameters and oxidative markers activities respectively (Figure 1).

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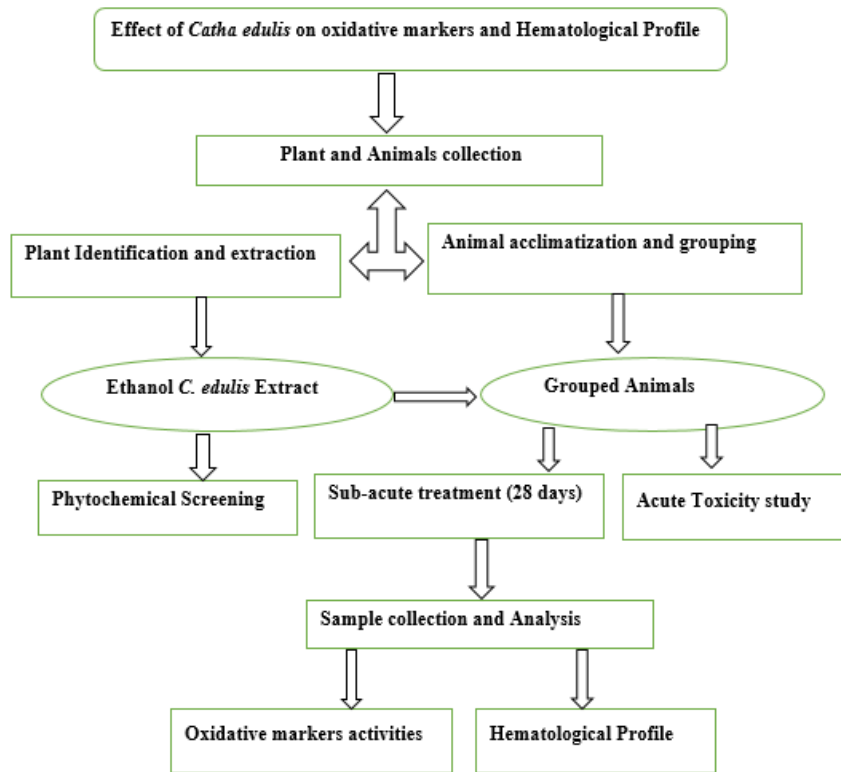


Figure 1: Schematic outline of the experimental design and procedures.

2.1.2 PLANT COLLECTION AND IDENTIFICATION

Young fresh leaves of *C. edulis* were collected after on-spot identification, and authenticated by Olivia Wanyana (taxonomist) before its voucher specimen was deposited at the Makerere University Herbarium, Kampala, Uganda, and was given a voucher number: SA001. Sample of the leaves used in this study is shown in figure 2.



Figure 2 Freshly harvested on-spot identified *Catha edulis* leaves used for the study

2.1.4 EXPERIMENTAL ANIMALS AND CARE

Twenty-eight (28) adult male Wistar rats of 8 to 10 weeks, weighing between 140 – 160 g were used for this study. The rats were obtained from the Animal House Unit of Kampala International University (KIU), Western Campus, Uganda. The rats were acclimatized for 2 weeks before the start of the study and they were separately caged under standard conditions of 12 hours light/dark, temperature; $25\pm 2^{\circ}\text{C}$, relative humidity of $60\%\pm 10\%$, and were fed on a standard rodent diet purchased from NOVITA, Uganda, and water *ad libitum*.

2.2 PREPARATION OF ETHANOL LEAF EXTRACT OF *C. edulis*

Five kilograms (5kg) of the fresh *C. edulis* leaf samples were washed properly under running water to remove dirt and other particles. The leaves were chopped on glass plates and pulverized mechanically. The ground leaves were extracted using modified methods of [19, 36]. In the maceration method of extraction, the plant material was immersed in conical flask containing 70% ethanol in ratio 1:10 and agitated on a shaker for 6 hours and allowed to stand for another 66 hours with frequent agitation. The extract was then filtered using gauze roll to separate the larger particles and thereafter using Whatman No.1 filter paper. The filtered extract was later concentrated *in vacuo* at 40°C to remove the ethanol.

2.2.1 DETERMINATION OF LETHAL DOSE

The acute toxicity study of the *C. edulis* leaf extract was determined using the biphasic method described by Lorke, (1983) [37]. Twelve male Wistar rats were used for this study, nine were used in the first phase and three in the second phase. All the rats in each phase were fasted for 12 hours before administration of the *C. edulis* leaf extract and allowed unrestricted access to standard feed and water *ad libitum* 4h post-administration. Observation for toxic signs was made after 1, 2 and 4 hr intervals after treatment, then 24 hr period. All animals in both phases were observed for signs of toxicity daily for 14 days after treatment including mortality, cognitive, behavioural symptoms and body weight changes during this period [49]. From the study, the median lethal dose (LD₅₀) of *C. edulis* was > 5000mg/kg.

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2.2.2 SUB-ACUTE TOXICITY STUDY OF ETHANOL LEAF EXTRACT OF *C. edulis*

Sixteen male Wistar rats were placed randomly into four groups of four rats each. Considering the potential cumulative effects that may result from repeated administration of the *C. edulis* leaf extract, the procedures and guidance of doses for administration for the sub-acute toxicity study was adapted from Organization for Economic Co-operation and Development (OECD) [49] and the Hodge and Sterner toxicity scale [28]. The groups labeled low dose (LD), medium dose (MD) and high dose (HD) were administered the 250, 500 and 1000mg/kg body weight doses of the *C. edulis* leaf extract which represent a Two to four fold intervals of the LD₅₀ of 5000mg/kg [49] while the control(C) group was given 10ml of distilled water. The extract was administered daily between 9 and 10 am by oral gavage for 28 days and rats were uniquely marked on the tail for proper identification during treatment and measurement of physical and clinical parameters. The animals were weighed before treatment commenced, then, once every week throughout the duration of treatment. At the end of day 28, all rats were sacrificed 24 hours after the last treatment. The rats were anaesthetized using halo ethane while blood samples were obtained by cardiac puncture and the brain was carefully harvested for oxidative markers assay.

2.2.4 EVALUATION OF BODY AND RELATIVE ORGAN WEIGHTS

The body weights (in grams) of the rats were taken on the first day before treatment, then once weekly throughout the experimental period. After 28 days of treatment, the rats were sacrificed and the individual rat brain was carefully removed and weighed.

The percentage change in weight for each rat at was calculated using:

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$$\% \text{ change in weight} = \frac{\text{Difference between interval body weight and initial body weight}}{\text{Initial weight}} \times 100$$

The brain weight was estimated as a percentage ratio using:

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$$\text{The relative organ (brain) weight (ROW)} = \frac{\text{Organ/ tissue weight}}{\text{Total body weight}} \times 100$$

2.3 PREPARATION OF TISSUE HOMOGENATES FOR OXIDATIVE MARKERS ASSAY

The prefrontal cortex was carefully selected from the rat brain and placed into ice cold beaker. The selected prefrontal cortex (1g) was homogenized in 10 ml homogenization buffer (1 mL 0.05 M phosphate buffer) at pH 7.4. The tissue was chopped into small pieces using scissors and poured into a 15 mL homogenizing tube and homogenized. The mixture was centrifuged at 3000rpm for 10 min at 4 °C to remove the unbroken cells and cell debris. The supernatant obtained was placed in refrigerator at -20°C till it was used for estimation of oxidative marker activities.

2.3.1 DETERMINATION OF SOD ACTIVITY

The SOD activity was estimated using the method described by Misra and Fridovich (1972) [42]. The supernatant (0.10 ml) was added to 2.5 ml 0.05 M carbonate buffer (pH 10.2) then, 0.3 mL of freshly prepared 0.003M adrenaline was added to the mixture and mixed thoroughly. The absorbance was read at 480 nm and monitored for 30 sec. then 150 sec. One (1) unit of SOD activity was determined as the quantity of SOD required to cause 50% inhibition of adrenaline oxidation to adrenochrome during 1 min.

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2.3.2 DETERMINATION OF CAT ACTIVITY

The CAT activity was determined using the method described by Cohen et al., (1970) [18]. The supernatant (500ul) was added 5 ml of 0.03M H₂O₂ and mixed by inversion for 3 minutes. After 3 min, 1 mL of 6M sulphuric acid was added to stop the reaction and 7 mL of 0.01 M potassium permanganate was immediately added and absorbance read at 480 nm within 30-60 seconds.

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2.3.3 DETERMINATION OF MALONDIALDEHYDE (MDA)

Lipid peroxidation was determined using the method described by Yagi, (1987) [60] by estimating the concentration of MDA which reacts with thiobarbituric acid reactive substances (TBARS) to form coloured compound whose absorbance was measured at 532nm.

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2.3.4 ANALYSIS OF HEMATOLOGICAL PROFILE

The blood samples were obtained by cardiac puncture and carefully transferred into EDTA-containing tubes. The hematological parameters analyzed from the blood samples were white blood cell (WBC) counts, red blood cell (RBC) counts, platelet (PLT) counts, hemoglobin (Hb) counts, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The blood samples were analyzed using the automated hematology analyzer: Mindray BC-6000 (Bio-Medical Electronics Co., Ltd, Shenzhen, China).

2.3.5 STATISTICAL ANALYSIS

The quantitative data were analyzed using Microsoft Excel 2016. Data was presented as mean \pm standard error of mean (SEM). The variation in a set of data was analyzed through the one-way analysis of variance (ANOVA). The difference among the means was considered at $P < 0.05$ using post-hoc method of Students-Newman Keuls through GraphPad Prism8.0.1.

3. RESULTS

3.1 DETERMINATION OF LETHAL DOSE

There was no toxic signs of lethality or mortality up to 5,000 mg/kg dose of *C. edulis* extract. Corner sitting, diarrhea and restlessness were the early behavioural patterns observed among the treated groups. Therefore, the lethal dose (LD₅₀) of *C. edulis* was $> 5000\text{mg/kg}$.

3.1.1 SUB-ACUTE TOXICITY STUDY OF ETHANOL LEAF EXTRACT OF *C. edulis*

The daily administration of the *C. edulis* extract for 28 days did not cause any physical or marked behavioural changes in the treated groups compared to the control group. No death was recorded among the groups throughout the study period.

3.1.2 BODY AND RELATIVE ORGAN WEIGHTS

The body weight of the *C. edulis* treated groups compared to the control group did not result in significant difference. However, rats dosed 1000 mg/kg of the extract showed a reduction in weight at week 3 of the treatment with a sharp increase at week 4 (Figure 3).

The relative brain to body weight of the *C. edulis* treated groups compared to the control group showed no significant difference. A slight increase in brain weight was however recorded in the group dosed at 500 mg/kg (Figure 4).

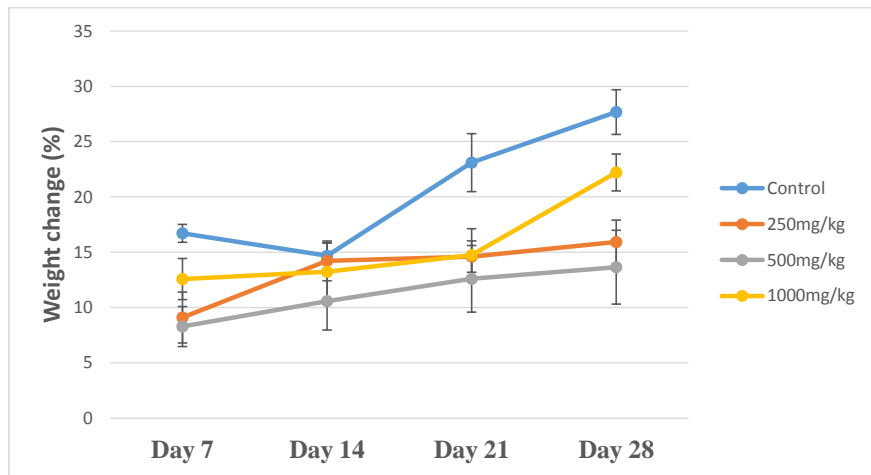


Figure 3 Weekly percentage weight changes of control and *C. edulis* treated rat

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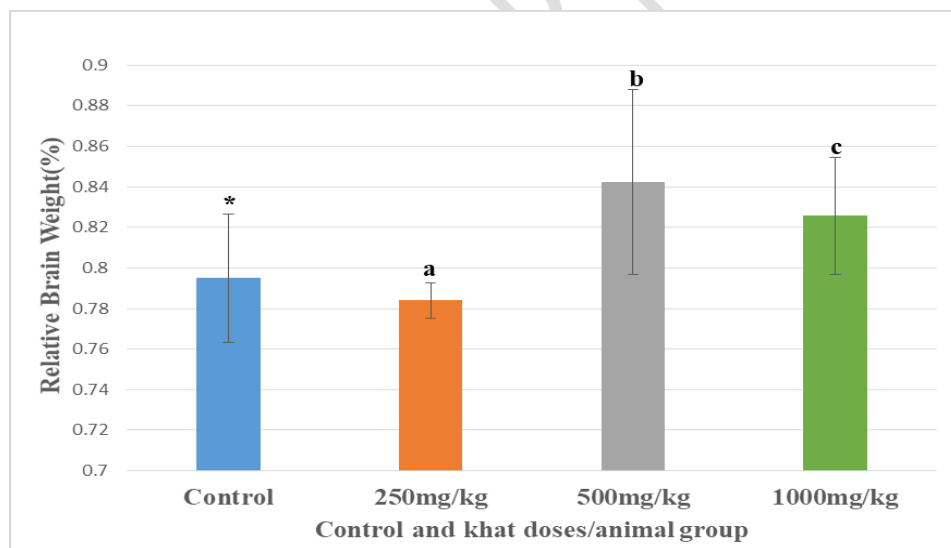


Figure 4 Relative brain weight of control and *C. edulis* treated rat

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3.2 EFFECT OF ETHANOL LEAF EXTRACT OF *C. EDULIS* ON OXIDATIVE BIOMARKERS

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3.2.1 SOD ACTIVITY

The average values of SOD activity of *C. edulis* treated group dosed 250, 500, 1000mg/kg (respectively 63.90 ± 1.19 , 63.07 ± 1.99 and 54.47 ± 1.47) were significantly ($p < 0.05$) reduced when compared to the control group (71.10 ± 2.75). There was statistical difference in SOD activity when compared among the *C. edulis* treated groups (Figure 5).

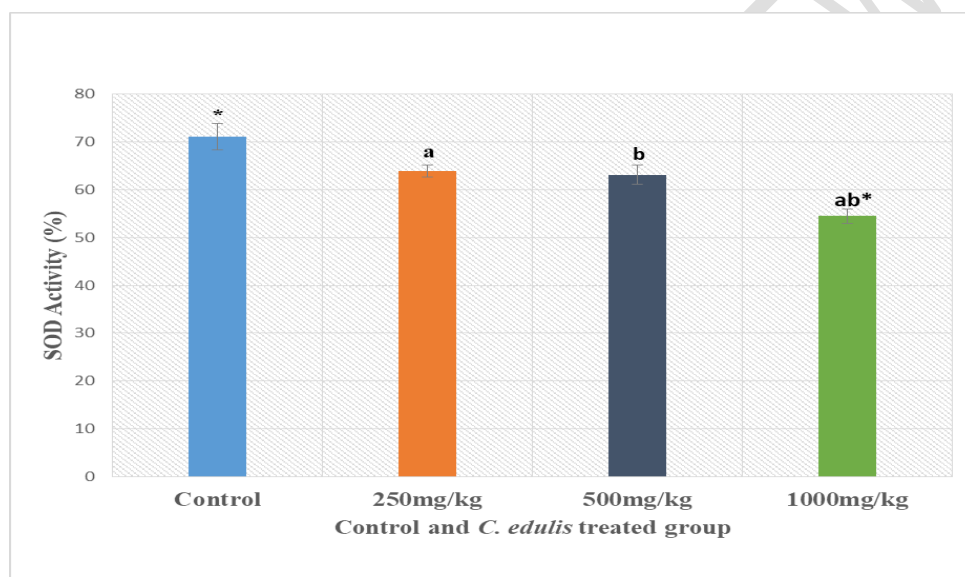


Figure 5 Effect of *C. edulis* extract on SOD activity in the prefrontal cortex. Data are presented as Mean \pm SEM, Mean values with different letters represent statistical significant differences ($p < 0.05$), * represent statistical difference compared to the control.

3.2.2 CAT ACTIVITY

The average values of CAT activity of *C. edulis* treated group dosed 250, 500, 1000mg/kg (respectively 467.1 ± 29.03 , 439.7 ± 34.99 and 421.2 ± 50.39) did not show significant difference ($p > 0.05$) when compared with the control group (489.4 ± 26.16). However, the CAT activity of *C. edulis* treated groups was significantly reduced when compared to the control group (Figure 6).

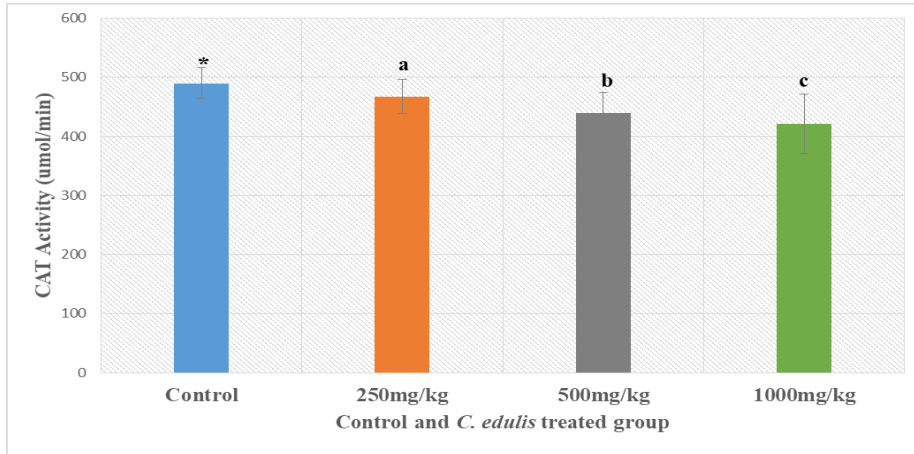


Figure 6 Effect of *C. edulis* extract on CAT activity in the prefrontal cortex. Data are presented as Mean \pm SEM.

3.2.3 MDA LEVELS

The mean values of the MDA levels of the *C. edulis* treated group dosed 250, 500, 1000mg/kg (respectively 3.19 ± 0.38 , 4.92 ± 0.42 and 5.22 ± 0.75) were significantly ($p < 0.05$) increased when compared with the control group (3.05 ± 0.24) this is shown in Figure 7.

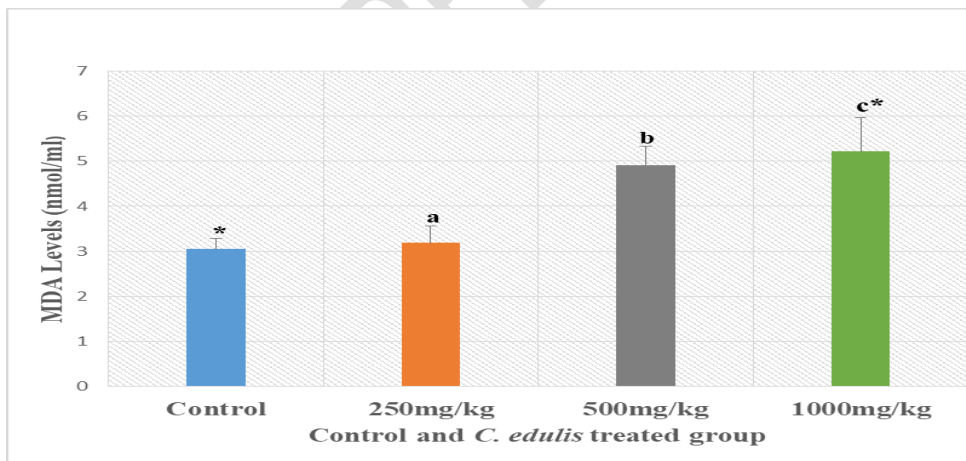


Figure 7 Effect of *C. edulis* extract on MDA levels in the prefrontal cortex. Data are presented as Mean \pm SEM, Mean values with different letters and asterisks (*) represent statistical significant differences ($p < 0.05$).

3.3 EFFECT OF ETHANOL LEAF EXTRACT OF *C. edulis* ON HEMATOLOGICAL PROFILE

The sub-acute administration of ethanol leaf extract of *C. edulis* did not cause any significant difference ($p>0.05$) in the hematological parameters of the treated groups when compared to the control group. However, slight alterations in some hematological parameters of the *C. edulis* treated groups compared to the control group were observed. The WBC of group dosed 100mg/kg of the extract was slightly increased (6.70 ± 2.35) when compared with the control group (5.35 ± 2.00), the MCV of groups dosed 500 and 1000mg/kg (51.70 ± 1.12 and 53.78 ± 1.84 respectively) were reduced when compared with the control (65.45 ± 0.87) and the MCHC of groups dosed 500 and 1000mg/kg of the extract (29.20 ± 0.34 and 29.50 ± 0.60 respectively) slightly increased when compared with the control group (28.60 ± 0.47) as shown in Table 1.

Table 1: Hematological Profile of Control and *C. edulis* treated Wistar Rats

Parameters	Control (10MI)	LD (250mg/kg)	MD (500mg/kg)	HD (1000mg/kg)
WBC ($\times 10^9/L$)	5.35 ± 2.00	5.43 ± 0.37	5.18 ± 1.75	6.70 ± 2.35
Platelet ($\times 10^9/L$)	732.30 ± 72.38	741.80 ± 20.50	771.00 ± 62.53	734.50 ± 82.48
RBC ($\times 10^{12}/L$)	8.10 ± 0.21	7.99 ± 0.19	7.93 ± 0.46	8.25 ± 0.41
Hb (g/dl)	15.15 ± 0.36	15.00 ± 0.27	15.10 ± 0.70	15.53 ± 0.53
HCT (%)	52.98 ± 1.85	52.65 ± 0.82	51.70 ± 2.34	53.78 ± 1.84
MCV (fl)	65.45 ± 0.87	65.93 ± 0.62	51.70 ± 1.12	53.78 ± 8.89
MCH (pg)	18.68 ± 0.37	18.75 ± 0.19	19.05 ± 0.33	18.88 ± 0.30
MCHC (g/dL)	28.60 ± 0.47	28.45 ± 0.19	29.20 ± 0.34	29.50 ± 0.60

The values are presented as Mean \pm SEM; n=4, WBC: White Blood Cells, RBC: Red Blood Cells, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration.

4. DISCUSSION

4.1 LETHAL DOSE DETERMINATION

Acute toxicity study is an initial procedure for screening of substances, phytochemical, synthetic chemical and pharmacological agents for toxicity [17]. Acute toxicity mainly gives an idea of the median lethal dose (LD₅₀). In this study, the median lethal dose (LD₅₀) of the *C. edulis* leaf extract was > 5000mg/kg. The only remarkable behavioral signs or pattern observed with the treatment groups was corner sitting, restlessness and diarrhea (occurring in the early days of administration). This present study agrees with the study by Ketema et al., (2015) who did not record any mortality in an acute toxicity study of *C. edulis* extract on mice [34]. The findings of this study also confirms the behavioural patterns or symptoms reported in previous studies which suggest that prolonged effects of *C. edulis* among users include insomnia, anxiety, and irritability [61, 47], increased locomotor activity [38]. Similarly, the present study aligns with previous human studies which reported no death related to *C. edulis* use [4, 46]. The findings of the present study therefore suggest that the oral consumption of *C. edulis* leaves at dose less than or equal to 5000 mg/kg b.w. may be acutely safe, however, the sub-acute (repeated administration) test of the plant has shown deleterious effects on the brain cell, neurons, hepatocytes, heart and lungs. Increase risk for psychosis [47], increase in blood pressure and heart rate [32] as such prolonged consumption of *C. edulis* could cause adverse effects.

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4.2 BODY AND RELATIVE ORGAN WEIGHTS OF STUDY RATS

In this study, the body weight of the treated rats compared to the control group increased gradually with rats in 1000mg/kg group showing a decline in weight at week 3 and a sharp increase at week 4. This study findings correlated with previous study by Echoru et al., (2018) who reported general body weight increase of khat-treated rats in the first 3 weeks followed by gradual decline in the body weight from week 4 to 6 [20]. This present study result also agrees with the study conducted by Alele and Rujumba, (2011) whose administration of khat to male rats showed no weight loss [2]. In the present study, the relative brain to body weight of the khat-treated animals compared to the control was consistent. This study suggests that oral treatment of *C. edulis* extract does not lead to observable negative effects on the body weights and brain of khat treated animals.

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4.3 EFFECT OF ETHANOL LEAF EXTRACT OF *C. edulis* ON THE PREFRONTAL CORTEX OXIDATIVE BIOMARKER ACTIVITIES

The brain is one of the most targeted organs by the constituents of stimulant drugs or herbs. Unlike other tissues, the brain has weak endogenous antioxidant defense system which makes it vulnerable to imbalance in redox homeostasis [31]. Metabolism in the brain generates high amounts of H₂O₂ hence, requiring an unimpaired antioxidant defense enzymes (importantly SOD, CAT and GPx) to keep these ROS within the required physiological levels. SOD is a very powerful endogenous first line

detoxification antioxidant found in cells. SOD initiates the detoxification process in the cells through dismutation of superoxide radicals (O_2^-) or singlet oxygen $^1\text{O}_2$ generated in tissues via cellular metabolism to hydrogen peroxide (H_2O_2). CAT completes the detoxification process initiated by the SOD by catalyzing the breakdown of hydrogen peroxide (H_2O_2) to molecular oxygen and water [29]. In the current study, activities of SOD and CAT of *C. edulis* treated groups compared with the control group were significantly ($p < 0.05$) reduced. This study findings agreed with previous study by Fahaid et al., (2011) which reported reduced SOD and CAT activities in khat treated rats [22]. The present findings further correlated with previous reports that stimulants and most used herbs like *C. edulis* have amphetamine-like properties which induces oxidative instability [35, 25, 24]. The study therefore suggests that with reported evidence of weak endogenous antioxidant system in the brain, with particular evidences of low catalase levels in most regions of the brain [27, 31], the PFC of the brain is therefore prone to accumulation of H_2O_2 given the reduction in CAT activities induced by the *C. edulis* extract. Lipid peroxidation on the other hand indicates cellular membrane damage due to oxidative stress. MDA which constitute one of the byproducts of lipid peroxidation is mostly used to estimate oxidative stress conditions [26]. In this study, the MDA levels increased among the 500-1000mg/kg b w *C. edulis* treated groups and the rat group dosed 1000mg/kg b w was significantly ($p < 0.05$) increased when compared to the control group. The current study agreed with previous studies which reported increased levels of MDA caused by Khat use [8, 10]. The findings of this present study showed that the elevated MDA levels due to lipid peroxidation in the PFC can be attributed to the high concentration of polyunsaturated fatty acids present in membrane lipids of the brain [58].

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4.4 EFFECT OF ETHANOL LEAF EXTRACT OF *C. edulis* ON HEMATOLOGICAL PARAMETERS

Evaluation of hematological parameters helps to reveal the abnormalities in the body physiologic or metabolic processes and further gives an insight into toxicity related information which may have slipped from detection during physical assessment of tissues and body weight. The response of the body to stress, puncture, injury and imbalance is therefore usually obtain through the blood profile [14]. Hence, to ascertain the effect of substances and plant extract like *C. edulis*, the evaluation of hematological profile is important [53]. In this study, the complete blood counts (CBC) in *C. edulis* treated groups showed no significant differences ($p > 0.05$) compared with the control. However, the red blood indices mainly, the mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) which constitute the vital indicators for diagnosing anemia [59] showed slight difference when compared to the control group (Table 1). This study findings agreed with previous study which reported no significant differences in the blood counts among khat treated male rats [1]. This study findings indicates that despite the statistically insignificant differences, the slight variations in the MCH, MCV and MCHC of khat treated groups compared to control group showed that the ethanol *C. edulis* extract has capacity to induce toxicity on the RBC counts when administered orally for a long

time. Therefore the ethanol *C. edulis* leaf may cause anemia [54]. This agrees with previous study by Ketema et al., (2015) which reported that khat use was strongly linked to severe anemia [34].

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The white blood cell (WBC) count is another important hematological parameter whose increase may indicate the effect of a given substance or plant extracts in triggering response from the immune system of the administered animals [55]. Gross reduction of the WBC on the other hand signals a decrease in leukocytes (mainly leukopenia) production which will suggest inability of the body to fight invading pathogens or infections. In this study, the total WBC count after 28 day sub-acute administration of *C. edulis* extract indicated that there was no significant difference ($p>0.05$) in the treated groups compared to the control group. However, the WBC counts slightly increased in 1000mg/kg khat treated group when compared with the control group. This present study agrees with the findings of Alele et al., (2013) who reported that there was no statistical difference in WBC counts between the khat treated groups [1]. The current study result therefore suggest that the ethanol leaf extract of *C. edulis* does not contain phytochemicals or if present were not sufficient to induce leukocytosis which is characterized by an abnormally increased WBC circulating in the blood or in suppression of normal production of WBC [59]. Also the duration of administration (28 days) in this study may not be too sufficient to see the chronic effects of the *C. edulis* extract on the WBC counts of the administered rats.

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CONCLUSION

C. edulis extract contains phytochemical constituents which are responsible for its pharmacological properties. The median lethal dose (LD_{50}) of the *C. edulis* leaf extract is greater than 5000mg/kg, hence, it may be considered acutely safe. However, the adverse health effects, behavioral signs, effects on oxidative biomarkers and alterations in some hematological indices indicates the potential toxic effect of khat extract. Long term chewing therefore can lead to oxidative stress and hematological conditions like anemia. Chronic *C. edulis* chewers are advice to take caution.

ETHICAL APPROVAL

Ethical approval was sought and obtained from the Kampala International University Research Ethics Committee. Approval to conduct the study was given with the Approval no: Nr. KIU-2021-8. The animals were handled according to the guidelines of the Institute for Laboratory Animal Research (2011). Guide for the care and use of laboratory Animals (8thed.).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use

these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCE

- [1] Alele P. E., Abayomi M. A., Lawrence I. Chronic khat (*Catha edulis*) and alcohol marginally alter complete blood counts, clinical chemistry, and testosterone in male rats. *Journal of Experimental Pharmacology*. 2013: 5: 33–44.
- [2] Alele PE, Rujumba JB. Khat (*Catha edulis*) and ethanol co-dependence modulate seizure expression in a pentylenetetrazol seizure model. *Journal of Ethnopharmacology*. 2011: 137 (3): 1431–1436
- [3] Al-Habori, M. The potential adverse effects of habitual use of *Catha edulis* (khat). *Expert Opinion on Drug Safety*. 2005: 4(6), 1145–1154.
- [4] Al-Habori M, Al-Aghbari AM, Al-Mamary, et al. Toxicological Evaluation of *Catha Edulis* Leaves. A Long Term Feeding Experiment in Animals. *Journal of Ethnopharmacology* 2002: 83: 209-17.
- [5] Al-Motarreb, A., Baker, K., & Broadley, K. J. Khat: Pharmacological and medical aspects and its social use in Yemen. *Phototherapy Research*. 2002: 16, 403-413.
- [6] Al-Motarreb, A., Al-Habori, M. & Broadley, K. J. Khat chewing, cardiovascular diseases and other internal medical problems: The current situation and directions for future research. *Journal of Ethnopharmacology*. 2010:132 (3), 540-548.
- [7] Al-Qirim, T. M, Shahwan, M, Zaidi, K. R, Uddin, Q. & Banu, N. Effect of Khat. Its Constituents and Restraint Stress on Free Radical Metabolism of Rats. *Journal of Ethnopharmacology*. 2003: 83, 245-50.
- [8] Al-Qirim TM, Shahwan M, Zaidi KR, Uddin Q, Banu N. Effect of khat, its constituents and restraint stress on free radical metabolism of rats. *Journal of Ethnopharmacology*. 2002:83: 245-250.
- [9] Alsalahi A., Abdulla, M. A. Al-Mamary M. et al., Toxicological features of *Catha edulis* (Khat) on livers and kidneys of male and female Sprague-Dawley rats: A subchronic study. *Evidence-Based Complementary and Alternative Medicine*. 2012: 829-401.
- [10] Al-Zubairi, A., Al-Habori, M. & Al-Geiry, A. Effect of *Catha Edulis* (khat) Chewing on Plasma Lipid Peroxidation. *Journal of Ethnopharmacology*. 2003:87, 3-9.
- [11] Angrist B. and Sudilovsky, A. Central nervous system stimulants: historical aspects and clinical effects in Stimulants. Springer. 1978: 99–165, US, Boston, MA, USA.
- [12] Arbos KA, Claro LM, Borges L, Santos CA, Weffort-Santos AM. Human erythrocytes as a system for

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Comment [SP29]:

Comment [SP30]:

evaluating the antioxidant capacity of vegetable extracts. *Nutrition Research*. 2008: 28, 457-63.

- [13] Bellomo, G. Cell damage by oxygen free radicals. *Cytotechnology*. 1991: 5, 71–73.
- [14] Bosco AD, Gerencser Z, Szendro Z, Ugnai C, Cullere M, et al. Dietary supplementation of spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on rabbit meat appearance, oxidative stability and fatty acid profile during retail display. *Meat Science*. 2014: 96, 114-119.
- [15] Brenneisen, R., Geissshusler, S. & Schorno, X. Metabolism of cathinone to (-)- norephedrine and (-)- norpseudoephedrine. *Journal of Pharmacology*. 1986: 38, 298–300.
- [16] Cheung CC, Zheng GJ, Li AM, et al. Relationship between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis* *Aquat Toxicology*. 2001:52:189– 203.
- [17] Chiendu, E.; Arome, D.; and Ameh, F.S. A New method for Determining Acute Toxicity in Animal Models. *Toxicology International Journal*. 2013: 20 (3): 224-226.
- [18] Cohen G., Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*. 1970:34, 30-8
- [19] Dimba, E., Gjertsen, B. T., Francis, G. W. *Catha Edulis* (Khat) Induces Cell Death by apoptosis in Leukemia Cell Lines. *Annals of New York Academic of Science*. 2003:101, 384-8.
- [20] Echoru, I., Bukenya, E. M., Edmund, M. G., Owembabazi, E., Lemuel, A. M. & Ahimbisibwe, J. Khat distorts the prefrontal cortex histology and function of adult Wistar rats. *Anatomy Journal of Africa*. 2018:7 (1), 1121 – 1131.
- [21] Ersche K. D, Clark L, London M, Robbins T. W, Sahakian B. J. Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology*. 2006:31 (5): 1036– 47.
- [22] Fahaid, H. A., Ismaeel, B., Mohammad, A. D., Luke, O. N., Mahmoud, A., Hussein, F. S., et al., Khat (*Catha edulis*) Extract Increases Oxidative Stress Parameters and Impairs Renal and Hepatic Functions in Rats. *Bahrain Medical Bulletin* 2011:33(1), 1–9.
- [23] Ferrari CKB, França EL, Honorio-França AC. Nitric Oxide, Health and Disease. *Journal Applied Biomedical*. 2009:7, 163–173.
- [24] Geissshüsler, S. Brenneisen, R. The content of psychoactive phenylpropyl andphenylpentenyl khatamines in *Catha edulis* Forsk of different origin. *Journal of Ethnopharmacology*. 1987: 19, 269-277.

Comment [SP31]:

- [25] Graziani, M., Michele, S. and Paolo, N. Khat chewing from the pharmacological point of view: an update. *Substance Use and Misuse*. 2008: 43: 762 – 783.
- [26] Halliwell B. and Gutteridge J. M. C. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Archives of Biochemistry and Biophysics*. 1986: 246 (2) 501–514.
- [27] Halliwell B Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*. 2006:141, 312–322.
- [28] Hodge, A. and Sterner, B. Toxicity Classes. In: Canadian Center for Occupational Health and Safety. 2005. <http://www.ccohs.ca/oshanswers/chemicals/id50.htm>
- [29] Ighodaro, O. M. & Akinloye, O. A. First line defense antioxidants-superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPX): Their fundamental Role in the entire Antioxidant Defense Grid. *Alexandria Journal of Medicine*. 2018:54, 287–293.
- [30] Institute for Laboratory Animal Research (2011). Guide for the care and use of laboratory Animals (8thed.) Washington, D. C. The national academies press.
- [31] James Nathan Cogley, Maria Luisa Fiorello, Damian Miles Bailey. 13 Reasons why the brain is susceptible to oxidative stress Redox Biology. 2018:15 (2018) 490–503
<https://doi.org/10.1016/j.redox.2018.01.008>
- [32] Kalix, P. Pharmacological properties of the stimulant khat. *Pharmacology Therapy*. 1990: 48, 397-416.
- [33] Glenice Cox & Hagen Rampes. Adverse effects of khat: a review. *Advances in Psychiatric Treatment*. 2003:9, 456-463
- [34] Ketema Tsige, Moti Yohannes, Esayas Alemayehu and Argaw Ambelu. Effect of chronic khat (*Catha edulis*, Forsk) use on outcome of Plasmodium berghei ANKA infection in Swiss albino mice *BMC Infectious Diseases*. 2015:15, 170. DOI 10.1186/s12879-015-0911-2
- [35] Khalid, M. N., Maher, A. A., Adnan, A. A., Qais, Y. M. Abdullah, R. Nagesh, B. et al., Effect of Daily Chewing Soft Buds and Leaves of *Catha edulis* (Khat) on the Antioxidant Defense System and Oxidative Stress Markers in Blood. *Arabian Journal of Science and Engineering*. 2015: 40, 1–6.
- [36] Kimani S, Nyongesa A. Effects of single daily khat (*Catha edulis*) extract on spatial learning and memory in CBA mice. *Behavioural Brain Research*. 2008:195 (1) 192–197.
- [37] Lorke, D. A New Approach to Practical Acute-Toxicity Testing. *Achieves of Toxicology*. 1983: 53, 275-289.

Comment [SP32]:

- [38] Maitai CK. The toxicity of the plant *Catha edulis* in rats. *Toxicon*. 1977;15, 363-366.
- [39] Mates, J.M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*. 2000;153, 83-104.
- [40] Mehmet S. and Marc M. Cholinergic Functioning in Stimulant Addiction: Implications for Medications Development CNS Drugs. 2009;23(11): 939–952.
- [41] Miller E.K, Cohen J.D. An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience*. 2001;24:167–202.
- [42] Misra H. P. and I. Fridovich. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The *Journal of Biological Chemistry*. 1972: 247, (10) 3170–3175.
- [43] Mohammed Sadeg Abdullah Al-Awar, Mohammed Abdullah Yahya Al-Eryan. Effect of the Ethanolic Extract of *Catha edulis* Leaves on the Electrical Activity of Some Brain Centers of Male Rabbit. *Int. Biol. Biomed. Journal. Summer*. 2017: 3(3).
- [44] Mohan, S. Abdelwahab, S. Hobani Y et al., *Catha edulis* extract induces H9c2 cell apoptosis by increasing reactive oxygen species generation and activation of mitochondrial proteins. *Pharmacognosy Magazine*. 2016: 12(46) 321.
- [45] National Institute on Drug Abuse (NIDA). The science of drug abuse and addiction, NIDA Info facts: Khat. 2010. www.nida.nih.gov/infofacts/khat.html.
- [46] Nencini, P. & Ahmed, A. M. Khat consumption: a pharmacological review. *Drug Alcohol Depend*. 1989;23 (1), 19–29.
- [47] Odenwald, M. Chronic khat use and psychotic disorders: A review of literature and future prospects. *SUCHT*. 2007;53, 9-22.
- [48] Odenwald M., F. Neuner, M. Schauer et al., Khat use as risk factor for psychotic disorders: A cross-sectional and case-control study in Somalia. *BMC Medicine*. 2005: 3(5).
- [49] OECD 407. Guidelines for the testing of chemicals. Acute oral toxicity. Fixed dose procedure. 2001:OECD/OCDE 407. Paris: OECD Publishing. [https:// doi.org/10.1787/9789264071001-en](https://doi.org/10.1787/9789264071001-en)
- [50] OECD 407. Guidelines for the testing of chemicals. Repeated dose 28-day oral toxicity study in rodents. 2008: OECD/OCDE 407. OECD Publishing.
- [51] Patel, N. B. Natural Amphetamine Khat: A Cultural Tradition or a Drug of Abuse? *International Review of Neurobiology*. 2015: 120, 235–255.

- [52] Pisoschi, A. M., Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. *European. Journal of Medical Chemistry*. 2015: 97, 55–74.
- [53] Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA. Effect of prolonged Vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. *PharmaceScience*. 2002:70, 135-145.
- [54] Rogers K. Blood: Physiology and Circulation (The Human Body), 1st edition. *Encyclopedia Britannica*. 2011:42, 121-225.
- [55] Tousson E, El-Moghazy M, El-Atrsh E. The possible effect of diets containing *Nigella sativa* and *Thymus vulgaris* on blood parameters and some organs structure in rabbit. *Toxicology and Industrial Health*. 2011:27,107-116.
- [56] UNODC, World Drug Report 2010 (United Nations Publication, Sales No. E.10.XI.13).
- [57] Vladimirov YA, Proskurnina EV. Free radicals and cell chemiluminescence. *Biochemistry* 2010:74, 1545–1566.
- [58] Watson, B. D. Evaluation of the concomitance of lipid peroxidation in experimental models of cerebral ischemia and stroke. In *Progress in Brain Research*; Elsevier: Amsterdam. The Netherlands. 1993:96,69–95.
- [59] Weingand K, Brown G, Hall R, Davies D, Gossett K, et al. Harmonization of animal clinical pathology testing in toxicity and safety studies. The Joint Scientific Committee for International Harmonization of Clinical Pathology Testing. *Fundam Applied Toxicology* 1996:29: 198-201.
- [60] Yagi S. K. Lipid peroxides and human diseases. *Chemistry and Physics of Lipids*. 1987: 45 (2-4),337–351.
- [61] Yousef, G., Huq, Z., & Lambert T. Khat chewing as a cause of psychosis. *British Journal of Hospital Medicine*. 1995:54 (7), 322–6.