

Full Length Research Paper

Derangement of hemopoiesis and hematological indices in Khat (*Catha edulis*) - treated rats

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The purpose of this study was to identify the sub-acute toxic effects of Khat (*Catha edulis*) on hemopoiesis and hematological indices of white albino rats. Two groups, each of 10 rats, were used. In the experimental group, a hydro-ethanol extract of *C. edulis* was administered orally to rats, daily, in single doses of 500 mg/kg body weight, for for weeks. The control group received equivalent amounts of normal saline. Our results show, for the first time, that oral administration of *C. edulis* hydro-ethanol extract caused significant derangement in hemopoiesis and in gross hematological indices in rats, characterized by macrocytic anemia and leukopenia. Our data show statistically significant decreases in total leukocytes count (TLC) in which, hemoglobin concentration (Hb. conc.), packed cell volume (PCV), and red cell count (RCC), accompanied by significant increases in mean cell volume (MCV), red blood cell distribution width (RDW) and platelets count with no change in mean hemoglobin concentration (MHC). In peripheral blood smears (PBS) of treated rats, there were evidences of dyserythropoiesis- impaired hemoglobinization, macrocytosis, poikilocytosis and anisocytosis, and dysgranulopoiesis- giant forms, hypersegmented neutrophils and bizarre nuclear shapes. In conclusion, our results indicate that oral administration of a hydro-ethanol extract of *C. edulis* adversely affected blood cell formation and induced macrocytic anemia and leukopenia in rats. However, the exact mechanisms of these hematological changes produced by Khat are still in need for further studies.

Key words: *Catha edulis*, hemopoiesis, anemia, leukopenia, rats.

INTRODUCTION

Khat refers to the young leaves and shoots of the plant *Catha edulis* (a species of the Celastraceae family), a flowering evergreen tree or shrub native to East Africa and the Arabian Peninsula. Its local names include qat (Yemen), eschat (Ethiopia) and miraa (Kenya). The habit of Khat chewing has prevailed for centuries among populations in the horn of Africa and the Arabian Peninsula. The leaves and shoots are chewed and the

juice swallowed to induce a stimulant and euphorogenic effect in the user (Kalix, 1984). In many societies, Khat chewing plays a significant role in traditional culture. It is also used to stay alert and overcome fatigue, hunger and thirst, similar to the use of Coca leaves by inhabitants of Central and parts of South America. The most widely reported adverse side effects of Khat chewing include anorexia, insomnia, mydriasis, hyperthermia and endo-

crinological disturbances (Brenneisen et al., 1990; Nencini et al., 1983; Nencini and Ahmed, 1989). Various detrimental effects of the active principle of Khat in men and animals have been described (Kalix, 1990; Kalix and Khan, 1984; Al-Habori et al., 2002). Khat has been shown to exert a mutagenic effect on bacteria and rodent cells (Hannan et al., 1985; Qureshi et al., 1988), and more recently on the exfoliated cells of volunteers who chewed Khat on a regular basis (Kassie et al., 2001). Khat-induced analgesia has also been reported (Connor et al., 2000), but its mechanism of action, central or peripheral, is not known. Acute autonomic responses, such as elevated blood pressure and tachycardia, have also been reported (Wilder et al., 1994).

The determination of levels of constituents of blood and plasma in mammals and humans has continued to play a valuable role in the assessment of normal function of living organisms as deviations in their normal levels have been associated with disease states (Cheesborough, 1991). Assessment of hematological parameters may be used to determine the presence and extent of deleterious effects of foreign compounds including plant extracts on humans and animals. It has also been used to unravel blood-related functions of chemical compounds including plant extracts (Yakubu et al., 2007). The effects of medicinal plant products on hematological parameters of experimental animals have been performed by several workers (Akpanabiatu et al., 2005; Aboderin and Oyetayo, 2006). These studies have been particularly helpful in assessing the safe use or otherwise of such compounds and plant extracts. Despite the wide use of Khat, very little work has been done to study its toxicological effects on blood and related systems. There is a dearth of scientific literature on the effects of Khat on hematological parameters. Motivated by this, the current study was designed to assess the sub-acute effect(s) of oral administration of a hydro-ethanol Khat extract (70/30%, V/V) on some hematological parameters in white albino rats.

MATERIALS AND METHODS

Preparations of *Catha edulis* shrub extract and dose selection

This study was carried out at the laboratories of the Department of Physiology, College of Medicine, King Khalid University during the spring of 2010. Fresh shrubs (stem tips and leaves) of *C. edulis* were obtained through the Local Office of the General Directorate of Narcotics Control in Aseer region, southwestern of Saudi Arabia. The General Directorate of Narcotics Control is the only legitimate source of Khat in Saudi Arabia, as both the peddling and consumption of Khat are prohibited in the country. The plant material was washed, dried and extracted with 500 ml of water-ethanol mixture (70/30%, V/V) at room temperature overnight, and then filtered. The hydro-ethanol extraction procedure selected here is according to previous extraction procedures for extraction of phenolic active compound in plants as reported and followed in previous studies (Dallak et al., 2010; Al-Hashem et al., 2011). The filtrate was evaporated in a vacuum at 40°C. The hydro-ethanol

extract (20 g) obtained, constituted 10.7% of the original dry material. It was dissolved in freshly prepared normal saline to a final concentration of 200 mg/ml.

About 0.5 ml of the solution was administered orally to the animal. The dose of the extract used was 100 mg/rat (equivalent to 500 mg/kg as dry plant). Dose selection was based on the average amount of Khat leaves chewed daily by Khat chewers (Al-Zubairi et al., 2003; Nencini and Ahmed, 1989).

Experimental animals

Male rats weighing between 180 and 200 g were obtained from the animal house at the College of Medicine of King Khalid University. The rats were housed in cages (10 rats per cage) in a climatically controlled room - temperature, 22°C and humidity, 55% with 12 h light/12 h dark cycles. Water and standard pellet diet were available *ad libitum* throughout the experimental period. The rats were acclimatized for 10 days prior to the experiment. The rats were given single daily doses of extract or normal saline orally using special cavage needles, as follows: Group 1: Control rats received normal saline. Group 2: Treated rats received about 0.5 ml *C. edulis* hydro-ethanol extract at a final concentration of 0.5 mg/kg. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (1996) and were approved by the Physiology Department Research Committee of King Khalid University (approval number: 2010-0313).

Hematological analysis

After treatment (24 h after the last administration), the animals were sacrificed by decapitation between 8:00 and 10:00 am and fresh blood was immediately collected into EDTA test tubes for routine hematological analysis. Packed cell volume (PCV), hemoglobin concentration (Hb. conc.), red cell count (RCC), mean cell volume (MCV), mean cell hemoglobin (MCH), red blood cells distribution width (RDW) and total white blood cell (WBC), leukocytes count (TLC) were determined using the Automated Hematological Analyzer (Sysmex, SE 9500, Kobe, Japan).

Preparation of peripheral blood smears (PBS)

Peripheral blood smears slides were made using the wedge technique. They were air-dried, fixed in alcohol and stained with Wright's stain. The slides were mounted in a Nikon microscope (Nikon Eclipse 50i, Tokyo, Japan) and viewed at 60x magnification. Parts of the slide were then digitally micro-photographed using a connected Nikon digital camera (Nikon DS-Fi1, Tokyo, Japan).

Statistical analysis

All data obtained were statistically analyzed using students' t-test. The data were expressed as mean \pm standard deviation and values of $P < 0.05$ were considered statistically significant.

RESULTS

Experimental data of *C. edulis* treated rats as compared to the control rats received normal saline depicted in Tables 1, 2 and Figure 1. Oral administration of a hydro-ethanol extract of *C. edulis* to rats induced a statistically significant decrease in red blood cell count (RBC's). The

Table 1. Results of hematological screening of normal and *Catha edulis* treated rats.

Hematological parameter	Control	<i>Catha edulis</i> treated rats
RBC count ($\times 10^6 / \mu\text{l}$)	9.21 \pm 0.54	6.35 \pm 1.84*
Hb.conc. (g/dl)	16.74 \pm 0.96	10.74 \pm 2.16*
HCT (PCV) (%)	46.10 \pm 3.2	37.40 \pm 5.4*
MCV (fl)	50.06 \pm 2.21	73.08 \pm 2.47*
MCH (pg)	18.36 \pm 0.51	18.52 \pm 0.72
RDW (fl)	23.82 \pm 1.23	45.9 \pm 2.09*
TLC ($\times 10^3 / \mu\text{l}$)	16.84 \pm 2.46	10.83 \pm 3.38*
Plt.count ($\times 10^3 / \mu\text{l}$)	309.8 \pm 43.10	707.5 \pm 127.03*

Results are mean \pm SD., $n = 10$; *Significantly different from control: $P < 0.05$; RBC, Red Blood Cell; Hb. conc., hemoglobin concentration; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; RDW, red cell distribution width; TLC, total leucocytes count; plt. Count, platelets count.

Table 2. Results of total leukocytes count (TLC) and differential count in the blood of normal and *Catha edulis* treated rats.

Hematological parameter	Control	<i>Catha edulis</i> treated rats
TLC ($\times 10^3 / \mu\text{l}$)	16.84 \pm 2.46	10.83 \pm 3.38*
Lymphocytes (%)	52.63 \pm 3.11	32.81 \pm 2.34*
Neutrophils (%)	29.87 \pm 2.69	55.11 \pm 3.02*
Monocytes (%)	9.11 \pm 1.34	7.32 \pm 1.21*
Basophils (%)	7.65 \pm 0.93	5.98 \pm 1.76*
Eosinophils (%)	2.23 \pm 0.12	1.14 \pm 0.87*

Results are mean \pm SD., $n = 10$; *Significantly different from control: $P < 0.05$.

percentages of decrease in RBC in *C. edulis* treated rats were 31.1%. Also, this decrease in RBC's in those rats treated with *C. edulis* was accompanied by a significant decrease in the packed cell volume (PCV) and hemoglobin concentrations (Hb). The decreases in these two hematological parameters were 35.82 and 18.9%, respectively. Moreover, when compared to the control rats, *C. edulis* treated rats showed a marked increase in the value of mean cell volume (MCV) (46.04%). Mean cell hemoglobin levels (MCH) were unaffected. Associated with these data, Table 1 also shows about 35.6% significant increase in the value of red blood cell distribution width (RDW) in the blood of rats treated with *C. edulis* hydro-ethanol extract. Results of platelets count and total leukocytes count (TLC) showed a significant thrombocytosis and leukopenia *C. edulis*-treated rats in comparison to normal control rats (Tables 1 and 2). The major increase in TLC was monitored in the neutrophils (84.5%) while the other leukocytes including lymphocytes, monocytes, basophils and eosinophils showed significant decreases in *C. edulis*-treated rat. The percentages of decreases in these cells after *C. edulis* administration were 38.82, 19.6, 21.83 and 48.81%, respectively.

Peripheral blood smears (PBS) from the blood of *C. edulis*-treated rats showed different abnormalities. Those abnormalities were characterized by the presence of round-oval macrocytes, anisopoikilocytosis, increased numbers of polychromatophilic RBCs and bizarre segmentation of neutrophils (Figure 1C to I).

DISCUSSION

Blood is an important index of the physiological and pathological status of the living body. Certain medicinal herbal preparations and even some conventional drugs and chemicals have been shown to adversely affect blood components (Bowman and Rand, 1980; Synder et al., 1977; Ajagbonn et al., 1999; Dioka et al., 2002). Deviations in blood cell counts and depletion/elevation of plasma constituents outside reference ranges often indicate hematoxicity (Watt and Breyer-Brandwijk, 1962; Cheeke, 1998). Ingestion of some plant materials (either in the raw form or as extracts) has been reported to cause anemia, which may result from splenic sequestration of red bloods, impaired red blood cell production or primary bone marrow dysfunction (Straus,

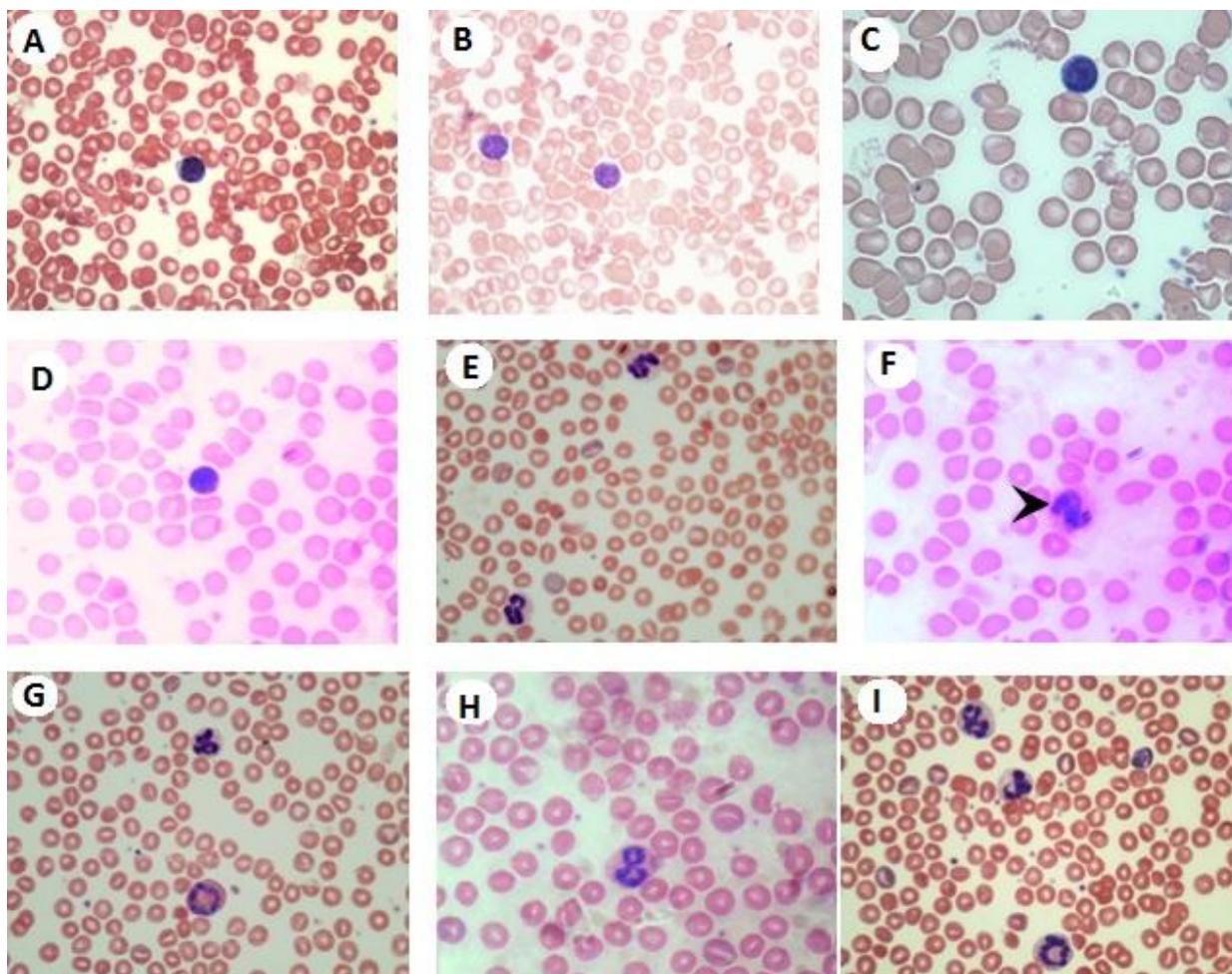


Figure 1. Microphotographs of blood smears of the rats. A and B are blood smears of control rats showing normocytic, normochromic RBC's and a normal lymphocyte. C to I are blood smears of *Catha edulis*-treated rats in which (C and D) show macrocytic RBCs; (E and F) show anisopoikilocytosis, round-oval RBCs, bizarre segmentation (arrow). (G) shows anisopoikilocytosis, round-oval RBCs, hypersegmented and ring nucleus bizarre neutrophils; (H) shows Cabot-ring like nucleus, bizarre segmentation; (I) shows hypersegmentation, bizarre segmentation of neutrophils in the shape of ring nucleus with polychromatophilic RBCs.

1998; Muller and Tobin, 1980). The current study shows that rats administered *C. edulis* hydro-ethanol extract, orally for 28 days, developed anemia, usually, a manifestation of an underlying disease process (Shakoori et al., 1990). There were significant decreases in PCV and Hb concentration in the blood of *C. edulis* treated rats. These findings could result from increased hemolysis and/or reduced erythropoiesis. Decreased hemoglobin concentration could also result from extract-induced impairment of biosynthesis of heme in the bone marrow (Olson et al., 1984). The anemia seen in this study is macrocytic and normochromic with significantly increased RDW but no change in MCH, suggesting a degree of anisocytosis.

Macrocytic anemia may result from a variety of disorders and demands further clinical and laboratory investigation to more appropriately determine its cause.

Macrocytic anemia is broadly divided into two categories, megaloblastic and non-megaloblastic, based on further investigations including bone marrow examination. This categorization is helpful in determining the etiology of the anemia. The spectrum of etiologies associated with macrocytic anemia with high RDW in humans includes vitamin B12 or folate deficiency, liver disease, myelodysplasia, thyroid disease, auto-immune hemolytic anemia (AIHA), alcoholism, cytotoxic drugs and cold agglutinin disease. In this study, samples of aspirated bone marrow were not examined to establish the nature of the observed macrocytic anemia. However, examination of stained smears of peripheral blood revealed features of non-megaloblastic macrocytic anemia which appear to absolve vitamin B12 or folate deficiency as the cause of the macrocytic anemia. The megaloblastic response to vitamin B12 deficiency is be-

lieved to be unique to man. Vitamin B12-deficient rats (Toyoshima et al., 1996), monkeys (Kark et al., 1974) and fruit bats *Rousettus aegyptiacus* (Green et al., 1975) developed neuropathy, but have unimpaired hematopoiesis and do not show megaloblastosis. Ebara et al. (2003) have now shown that when vitamin B12-deficient rats are subjected to the additional stress of hypoxia to induce hematopoiesis, they do indeed develop megaloblastosis not explained entirely by vitamin B12 deficiency. *C. edulis* extract caused a statistically significant decrease in total leukocytes count (TLC). This could be ascribed to a suppression of leucocytosis in the bone marrow. Ingestion of a wide variety of plants has been reported to cause bone marrow infiltration and suppression of hematopoiesis (Lund, 2000). Such an effect by khat would be expected to compromise cellular inflammatory processes, with resultant immunosuppression (Hogg, 1987; Young, 1989). Dimba et al. (2004) concluded that Khat induces rather swift and sensitive cell death by apoptosis through mechanisms involving activation of caspase-1 and -8. They observed that cell death from Khat was more sensitively induced in leukemia cell lines than in human peripheral blood leukocytes (Dimba et al., 2004).

The significant increase in the neutrophils, and the associated concomitant significant decreases in the other types of leukocytes seen in the current study could be explained by the fact that *C. edulis* have stimulating effect on adrenocortical function in animals (Ahmed and El-Qirbi, 1993). It is well reported that glucocorticoids increase the concentration of neutrophils and decrease the concentration of lymphocytes, monocytes, eosinophils and basophils (Al-Hegami, 2001). The decrease in the number of lymphocytes in the peripheral blood of the *C. edulis* treated rats is possibly due to the fact that these cells migrate to the affected damaged tissue after *C. edulis* intoxication as reported in our previous studies (Dallak et al., 2010; Al-Hashem et al., 2011). Katzung (2009) reported that the reduction in circulating lymphocytes is the result of their movements from the vascular beds to lymphoid tissues. Beside the effect of glucocorticoids, the increase in the neutrophils could be possibly due to the increase in flow into blood from bone marrow and decrease migration from blood vessels. Our data are in the same line with the study of Al-Hegami (2001) who reported that administration of *C. edulis* to rabbits resulted in a significant decrease in RBC's and significant decreases in TLC with a significant increase in granulocytes with a significant decrease in lymphocytes. However, our study are not in agreement with the study carried by Alsalahi et al. (2012) who reported no significant changes in the RBC's, Hb and PCV values where they reported significant increases in TLC. This variation with our results could be due to the type of extract used where they used water extract in their study, and possibly due to the dose of *C. edulis* selected as they have used doses higher than 500 mg/g.

In the current study, we found that *C. edulis* extract caused bicytopenia, dyserythropoiesis, and dysgranulopoiesis combined with other altered blood parameters suggesting acquired myelodysplastic syndrome. The peripheral blood smear showed evidence of dyserythropoiesis including the presence of impaired hemoglobinization, round-oval macrocytes, poikilocytosis and anisocytosis. Also, there was evidence of dysgranulopoiesis, characterized by the presence of giant forms, hypersegmented neutrophils, and bizarre nuclear shapes in the form of ring- and Cabot-ring like nuclei. The combination of dyserythropoiesis and dysgranulopoiesis, with altered blood indices seen in this study point to acquired myelodysplastic syndrome (Bennett et al., 2004). Myelodysplastic syndrome (MDS) is categorized into two major subtypes - primary and drug-induced subtypes. MDS develops from a number of heterogeneous pathways. DNA damage is a common factor, with evolution of clonal cytogenetic abnormalities and frequent abnormalities in DNA methylation and epigenetics. Certain genetic abnormalities, such as inactivation of the p53 tumor suppressor gene, are associated with MDS (Greenberg et al., 2006; Aul et al., 2002). The term myelodysplastic syndrome (MDS) is used, indeed, to describe a heterogeneous group of disorders that are characterized by clonal and ineffective hematopoiesis, morphological dysplasia, peripheral blood cytopenia and progressive bone marrow failure (Greenberg et al., 2006; Benetatos and Bourantas, 2005). MDS transforms to acute myeloid leukemia (AML) in approximately 30% of cases (Aul et al., 2002).

The observed thrombocytosis could be reactive to haemorrhagic complications of Khat chewing for example, haemorrhagic gastric or duodenal ulcer, cerebral hemorrhages and/or chronic hemorrhoids. This suggestion could also explain the observed polychromasia. Hannan et al. (1985) showed that extracts of *C. edulis* leaves exert a mutagenic effect on bacteria while De Hondt et al. (1984) observed chromosomal aberrations after subcutaneous treatment of rats with methanolic Khat extract. Furthermore, Khat extract was reported to increase the frequency of micronucleated polychromatic erythrocytes, induce bone marrow depression and reduce the mitotic index of somatic cells (Qureshi et al., 1988). Other studies have shown a correlation between Khat chewing, DNA damage and cancer. In a survey that reviewed cancer cases over two years in the Aseer region of Saudi Arabia (Soufi et al., 1991), 28 head and neck cancer patients were found and 10 of them showed a history of Khat chewing. All were non-smoking chewers and all of them had used Khat over a period of 25 years or longer. Eight of these ten presented with oral cancers. In some cases, the malignant lesion occurred at exactly the same site where the Khat bolus was held. The authors concluded that a strong correlation between Khat chewing and oral cancer existed. The authors considered Khat as an important contributing factor. It was also re-

ported that 50% of Khat chewers develop oral mucosal keratosis (Hill and Gibson, 1987). Keratosis of the oral buccal mucosa is considered as a pre-cancerous lesion that may develop into oral cancer (Goldenberg et al., 2004).

Recently, Ali et al. (2004) reported that 22.4% of Khat chewers had oral keratotic white lesions at the site of Khat chewing, while only 0.6% of non-chewers had white lesions in the oral cavity. The prevalence of these lesions and their severity increased with frequency and duration of Khat use. In another study performed in Yemen, 30 of 36 patients suffering from squamous cell carcinoma were habitual Khat chewers from childhood (Nasr and Khatri, 2000). In human leukaemia cell lines and in human peripheral blood leukocytes, Khat extracts- cathinone and cathine produced a rapid and synchronized cell death with all the morphological and biochemical features of apoptotic cell death (Dimba et al., 2004). Based on these studies, a possible defect in DNA synthesis caused by *C. edulis* could be responsible for the blood picture obtained in the present study suggesting a possible sub acute drug-induced MDS. Clearly, further studies are required to elucidate this hypothesis.

Conclusion

Our results suggest that oral administration of a hydro-ethanol extract of Khat induced moderate macrocytic anemia and moderate leucopenia in rats. However, the precise mechanism of the type of bicytopenia produced by this plant is still ill-understood. It would most probably be multi-factorial; hence, future prospective studies on Khat chewers with deranged hematological parameters should include complete blood count (CBC), microscopic examination of routinely-stained blood smear, reticulocyte count, Coomb's test, microscopic examination of routinely stained bone marrow aspiration and biopsy smears, complete liver functions tests, kidney functions tests and if indicated thyroid function tests.

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