

# Toxic Effects of Aqueous Leaf Extract of *Vernonia bipontini* Vatke on Blood, Liver and Kidney Tissues of Mice

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## ABSTRACT

The present paper evaluates the acute and chronic toxicity of aqueous crude leaf extract of *Vernonia bipontini* Vatke (*V. bipontini* V) in mice model. Leaves of the plant were collected from Bale, Ethiopia, dried under shade, crushed into powder and soaked in water to yield the extract. Lethal dose of aqueous leaf extract of the plant was determined using nine groups of mice to which the aqueous leaf extracts of *V. bipontini* V was administered at doses ranging from 1250 to 3250mg/kg. All animals were closely observed for any physical and behavioral alterations for acute toxicity evaluation. For long-term toxicity evaluation, animals were subjected to oral administration of the extract at 400 and 800mg/kg, at 24 hours intervals for 45 days. The treated animals survived for 45 days. Body weights of the mice were recorded. Blood sample was collected from experimental and control groups for hematological studies and biochemical analysis on the 46<sup>th</sup> day after anesthesia. The liver and kidney of each animal were taken and examined by light microscope for any anatomical abnormalities. The LD<sub>50</sub> was found to be 2500.62±5.24 mg/kg. The extract had no significant effect on liver and kidney weights, hematological (RBC, WBC, platelet, Hgb, Hct, Mcv, Mch and L) and biochemical parameters such as liver AST, ALT and ALP; and kidney urea at all doses (P>0.05). Light microscope examination of liver and kidney tissue of mice treated with 400 and 800mg/kg of the extract did not show structural abnormalities. The results suggest that the extract of this plant may be safe, even when administered at a dose of 800mg/kg for 45 days. This is in agreement with the traditional claim of the water preparation of *V. bipontini* Vatke leaves.

**Key words:** *V. bipontini* Vatke, Swiss Albino mice, Hematological and Biochemical parameters.

## 1. INTRODUCTION

Medicinal plants contain potentially useful chemicals that serve as basis for the manufacturing of modern medicines (Okigbo et al., 2009). In recent years, clinical importance of herbal medicines

has received considerable attention. However, poisoning of animals with plants is a common clinical phenomenon (Abebe et al., 2003; Nwafor, 2004); and some medicinal plants may produce adverse long-term effects such as hepatotoxicity (Steenkamp et al., 2006). Ethiopian medicinal plants are shown to be very effective against some ailments of human and domestic animals (Endashaw, 2007). Furthermore, over 95% of traditional medicinal preparations are of plant origin in Ethiopia (Kassaye et al., 2006). Thus, medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs in the country (Endashaw, 2007).

*V. bipontini* V is a herb claimed to be useful for the treatment of malaria and malaria related symptoms, and it was found to be effective at 400mg/kg/day against *Plasmodium berghei* in mice model (Ashenafi et al., 2007). Its aqueous and methanol leaf extracts showed 52.7% and 40% inhibition, respectively against *Plasmodium berghei* in mice (Ashenafi et al., 2007). It is for this reason that this study is primarily designed to determine LD<sub>50</sub> and long-term effects of aqueous leaf extracts of *V. bipontini* V at doses of 400mg/kg and 800mg/kg that might probably have effects on hematological and biochemical parameters (liver AST, ALT and ALP; and kidney urea) and on liver and kidney tissues. In Ethiopia, people use *V. bipontini* V against malaria without the knowledge of its side effects especially on liver, kidney and blood tissues which are commonly affected by toxic ingredients in medicinal plants (Obboh, 2006; WHO, 2000). Many developing countries still rely on traditional medicinal plants for primary health care (WHO, 2011; Cunningham, 1993; Kassaye et al., 2006; Endashaw, 2007). This is also true in Ethiopia; where majority of the total population depends on traditional medicine (WHO, 2011; Fullas, 2001; Kassaye et al., 2006; Tilahun and Mirutse, 2007); however, currently, the use of traditional medicine seems to decline because of the recent advent in health extension program which has enabled Ethiopia to increase primary health care coverage from 76.9% to 90% (Banteyerga, 2011).

Although *V. bipontini* V is used traditionally as antimalaria, antispasmodic, antisnake bite, antivenereal diseases, purgative and vermifuge (Ashenafi et al., 2007), only a preliminary antimalaria investigation of *V. bipontini* V has been carried out to evaluate its effectiveness at the dose of 400mg/kg (Ashenafi et al., 2007). People living in areas, where *V. bipontini* V grows use water preparation of the plant leaves for treating malaria and malaria related symptoms.

*Vernonia*, *V. amygdalina* is one of the pharmacologically useful plants (Ojiako & Nwanjo, 2006). Both aqueous and alcoholic extracts of stem-bark, root and leaves of *V. amygdalina* are reported to be extensively used as anti-malaria, purgative, and in the treatment of eczema or inflammatory conditions of the skin (Ojiako and Nwanjo, 2006). Microscopic examination of liver and kidney tissue sections has showed no morphological abnormalities the same as controls after 42 days of oral administration of aqueous leaf extract of *V. amygdalina* (Amole et al., 2006).

Antimicrobial activity of the leaf extract of *V. amygdalina* has been reported (Abosi and Raseroka, 2003). Aqueous leaf extract has been shown to reduce blood sugar levels in rabbits (Abosi and Raseroka, 2003). It is also reported that the leaf and root-bark extracts of this species showed decreases in trichomonas vaginalis parasitaemia (Yeap et al., 2010) resulting in parasite inhibition ranging from 41.5% to 67.0% for the leaf extract and 38.5% to 53.5% for the root-bark extract (Abosi and Raseroka, 2003). Dichloromethane extract of *V. amygdalina* recorded the greatest antiplasmodial activity *in vitro* (Melariri et al., 2011). The effects of various concentrations of aqueous extract of *V. amygdalina* leaves on some biochemical indices of liver function in albino Wistar rats showed that *V. amygdalina* has nutritional, clinical and veterinary relevance considering the diverse applications of the plant in almost all African countries (Ojiako and Nwanjo, 2006). The crude extracts of *V. amygdalina* produce significant changes in packed cell volume (Pcv), red blood cell and hemoglobin concentration. This extract also significantly reduces the fecal egg count of helminths in the dung of chimpanzee (Yeap et al., 2010). It could be a potential source of a new lead on anthelmintic agent (Adedapo et al., 2007; Yeap et al., 2010). On the other hand, it is also indicated that the aqueous leaf extract of *V. amygdalina* produced no significant changes in Pcv, white blood cell and platelet counts (Amole et al., 2006).

## **2. MATERIALS AND METHODS**

### **2.1. Collection of Plant Materials**

Leaves of *Vernonia bipontini* Vatke were collected from Bale, Delomenna Awraja 524 kms southeast of Addis Ababa in December 2007. Species identification of the collected plant specimen was done both at Herbarium of Department of Drug Research (DDR), Ethiopian Health and Nutrition Research Institute (EHNRI) and at the National Herbarium of the Department of Biology, Addis Ababa University with a Voucher number of (05/MEB).

## 2.2. Processing of the Plant Materials

Fresh leaves of *Vernonia bipontini* Vatke were cleaned with tap water and dried under shade and ground using a manual tick grinder. The powder leaf material (2.2kg) was macerated with distilled water for 24 hours with intermittent agitation by Orbital shaker (DS-500 model 0.75” 1.9cm Cat. No. 444.7018). Supernatant of agitated material was separated from the un-dissolved portion of the plant. The supernatant was then filtered with filter paper. The filtrate was deep frozen and lyophilized to obtain the crude extract.

## 2.3. Experimental Animals

The animals used for this study were adult male and female Swiss Albino mice. The mice were obtained from Research Animal Breeding Laboratory of DDR, EHNRI, Ethiopia. The mice were acclimatized to laboratory condition for a week before subjected to experiment. Mice of the same sex were grouped into 9 experimental and 1 control groups for LD<sub>50</sub> determination, and 20 experimental and 10 control groups for long term administration of aqueous leaf extracts. Finally, all mice were housed in common metallic cage under 23±2<sup>0</sup>C. They had unrestricted access to a standard pellet diet and tap water. The animals were maintained under 12 hours light-dark cycle throughout the study period.

Each group of mice was given 1250mg/kg, 1500mg/kg, 1750mg/kg, 2000mg/kg, 2250mg/kg, 2500mg/kg, 2750mg/kg, 3000mg/kg, and 3250mg/kg doses of aqueous leaf extracts orally using intragastric catheter. These extracts were given once after the animals had fasted for 18 hours for LD<sub>50</sub> determination. However, in long-term toxicity study, mice were administered with 400mg/kg and 800mg/kg doses of aqueous leaf extracts for 45 days (WHO, 2000) after 7 days acclimatization.

Lethal doses for fifty percent of the mice (LD<sub>50</sub>) for aqueous leaf extract were determined using a total number of 100 Swiss albino mice that were divided into 10 groups of 10 mice. Two months old, mice ranging from 25 to 35g in weight were selected. Nine groups of mice were formed to administer the aqueous leaf extracts of *V. bipontini* V at doses from 1250mg/kg to 3250mg/kg interval of 250mg/kg. The number of death in each group within 24 hours was recorded. Besides, delayed mortality up to 3 days was considered as lethal dose. This was done by observing the mice for toxicity signs (Nwinyi et al., 2004). The dose increment was limited to 3250mg/kg because all mice died at this dose.

The long-term toxicity study was carried out using 30 female and male Swiss Albino mice. Animals were kept in environment of  $23\pm 2^{\circ}\text{C}$  with 12 hours light-dark cycle. Food and water were freely available for a week before the beginning of administration of aqueous leaf extract and throughout the duration of the study. Out of 30 mice, 20 were randomly assigned to 2 experimental groups of 10 mice each and the male and female mice were placed in separate cages. The remaining 10 mice were also randomly assigned to 1 control group. Then, the animals were randomly assigned into one (1<sup>st</sup>) control group and two (2<sup>nd</sup> and 3<sup>rd</sup>) experimental groups for aqueous leaf extract administration.

The 1<sup>st</sup> control group for aqueous leaf extracts received 0.5ml of distilled water. The two experimental two (2<sup>nd</sup> and 3<sup>rd</sup>) groups were administered with aqueous leaf extract at doses of 400mg/kg and 800mg/kg, respectively. Aqueous leaf extract was administered in 24 hours intervals for 45 days (Amole et al., 2006; WHO, 2000). Standard pellet (132g) was fed within 24 hours intervals to a cage of 5 mice. All groups were closely observed for acute symptoms such as food intake, behavioral changes and signs of abnormalities throughout the study. Signs of abnormalities were measured as compare to the control group. Finally, tissues were taken from all groups of mice after blood samples were collected for histopathological evaluation based on the method Histological Techniques for Marine Bivalve Molluscs (Kim et al., 2006).

Initial body weights of all groups of mice were taken before the commencement of the first oral administration using SCIENTECH Mode No SL 3100D Rev-c accuracy class (II) measuring balance. Body weights of all groups were also taken on the last day of oral administration and these were considered to be the final body weight.

Blood collection was performed by placing each animal in airtight dissector jar with cotton soaked in diethyl ether anesthesia; experimental animals should be least stressful because stress will affect the outcome of the study (Parasuraman et al., 2010). Blood was collected from each animal by cardiac puncture using sterile needle and 5ml syringe. The sample was put in an ethylene-diamine-tetra-acetic acid (EDTA) bottles to prevent adhesion proteins (coagulation factors) in cell-cell and cell-matrix interactions for hematological determinations (Gabriel et al., 2008) using automated hematological analyzer, SYSMEX KX-ZIN (Adebayo et al., 2005) at EHNRI, Addis Ababa, Ethiopia. Hematological parameters including total counts of RBC and WBC, hemoglobin (Hgb), hematocrit (Hct), mean cell volume (Mcv), mean corpuscular

hemoglobin (Mch), mean corpuscular hemoglobin concentration (Mchc), lymphocytes (L), and platelet were measured (Selmanoglu et al., 2001; Tuffery, 1987).

Biochemical investigation was performed after blood sample was collected without using EDTA. The sample was kept at 4<sup>0</sup>C for 4 hours to let it clot. The clotted blood was centrifuged (using Humax 4k bench top Centrifuge with a capacity of 12x15ml; Germany, Max-Planck-ring 21D-65205 Wiesbaden) at 5000 RPM maximum speed for 10 minutes to obtain the serum. The serum samples were kept in -22<sup>0</sup>C refrigerator until used for biochemical analysis (Mohan, 2007). Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and urea were measured (Ege et al., 2008; Selmanoglu et al., 2001).

#### **2.4. Animal Dissection and Organs Weight Measurement**

Animals of each group were sacrificed at the end of 45 treatment days after body weight of mice were taken one by one on a digital electronic balance. Animals lay up on a dissecting board after blood sample was collected. A vertical midline incision with scissors cut from the neck to pubis and opens the peritoneum. Then, 3-4mm wide strips of tissue samples were randomly taken from right lobe of liver and coronal section of right kidneys were cut lengthwise with a scalpel through the renal pelvis after each of these organs was weighed with 0.001 precision automatic internal calibration CX series balance.

#### **2.5. Collection of Tissue Sample**

Tissue samples were taken immediately after sacrifice from the right lobe of liver and coronal section of right kidney and transferred by a blunt forceps to a test tube containing 10% buffered neutral formalin that completely immerses the tissues for the purpose of fixation after blood collection (Lamberg and Rothstein, 1978).

#### **2.6. Tissue Preparation and Staining**

Tissue processing starts with fixation to inhibit the decay and autolysis of tissues. This process prevents degeneration of tissue. The formalin fixed tissues were washed in running tap water for 8 hours to allow paraffin wax to infiltrate into the tissue easily (Singh, 2006).

Following washing, tissues were dehydrated in a series of an increasing graded ethanol i.e. in 70%, 80%, 95%, 100% I and 100% II for 1 hour each based on the guideline of histological technique because as ethanol concentration increases, dehydration of the tissues increases (Mohan, 2007).

In clearing step, immersing of tissues with xylene two times was used for one hour each to remove ethanol from the tissue and replace it with fluid that is miscible with paraffin (Singh, 2006). Tissues were infiltrated by two changes of paraffin wax which had a melting point of 56°C (52-64°C) for 1½ hours in each change (Mohan, 2007). The tissues were embedded in paraffin wax with the help of Electro-thermal Wax Dispenser to form tissue blocks in squared metallic plates block moulds. The blocks were then labeled, sealed in plastic bags with examining surface downward prior to sectioning, and placed in refrigerator until sectioned (Mohan, 2007). This process enables the specimens too small and/or delicate to be surrounded with wax that impart firmness without producing any injuries on the tissue (Singh, 2006).

Rotary microtome was used for sectioning of tissue blocks manually at a thickness of 5 µm. The paraffin block having tissue was put in the rotary microtome. The ribbon of sections was carefully picked from the knife by a blunt forceps to float in a water-bath of 40°C (slightly below the melting point of wax) to remove folds in the sections. Unfolded sections were picked by clean microscopic glass slides and were placed in an oven maintained at a temperature of 56°C for 20-30 minutes for proper drying and better adhesion. At this stage, the sections are ready for staining (Mohan, 2007).

Staining solutions were prepared using the formula given by Clopton (2006). The paraffin wax was removed from the tissue sections. The sections were then immersed in a series of descending alcohol concentration to remove xylene after which distilled water was used to hydrate the tissue. The hydrated sections were immersed in hematoxylin for 3-5 minutes with an eosin counterstained and agitated with acid alcohol to prevent over staining. Sections were immersed in a mixture of sodium bicarbonate, ethanol, and distilled water and tap water to give blue color to the nucleus. Finally, it was immersed in 95% alcohol and eosin to give pink color to the cytoplasm (Clopton, 2006; Mohan, 2007).

Finally, tissue sections were dehydrated in 95% alcohol, cleared in xylene, and mounted by adding a drop of DPX (Dibutyl phthalate in xylene) mounting medium on the section to cover the microscopic glass with cover glass and to increase the refractive index of the tissue under light microscope. This was done with care to prevent bubble formation between the tissue and the glass cover (Singh, 2006).

## **2.7. Tissue Examination for Histopathology**

The researchers examined the histological slides of the tissues which have been administered the aqueous leaf extract of *V. bipontini V* for 45 days and found no pathological changes at the doses of 400mg/kg and 800mg/kg as compare to control group.

## **2.8. Statistical Method**

Data were digitally analyzed using the statistical software package SPSS version 14. All values were expressed in mean  $\pm$  SEM. Treatment effects over time were compared between control and treated groups by analysis of covariance. The results were analyzed statistically using probit analysis of regression to determine LD<sub>50</sub> and analysis of variance one-way ANOVA to identify possible difference of body, liver, and kidney weights, and hematological and biochemical values. P values less than 0.05 were considered statistically significant.

## **3. RESULTS**

### **3.1. Physical Signs of Toxicity**

Mice were observed for signs of abnormalities before and after sacrificed. Treated mice showed low locomotion, weakness, erection of hairs, and white color of the eyes in the course of acute study to determine the dose at which 50% of the test population is died (LD<sub>50</sub>). During long-term administration of the extract both treated and untreated groups showed no physical changes in their appearances and signs of toxicity at 400 and 800mg/kg body weight of aqueous leaf extract of *V. bipontini V*.

### **3.2. Determination of LD<sub>50</sub>**

The acute toxicity study in mice showed LD<sub>50</sub> value of 2500.62 $\pm$ 5.24 mg/kg per body weight of mice for aqueous leaf extracts (Fig 1). The probit responses are indicated in vertical line marked by horizontal arrow and doses are indicated in horizontal line marked by vertical arrow below.

### **3.3. Effects of Aqueous Leaf Extract of *V. bipontini V* on the Body Weight of Mice**

The chronic effect of aqueous leaf extract of *V. bipontini V* on the general body weight of the mice is illustrated in table 1. The aqueous leaf extract of the plant has no significant effect on the mean values of the body weights of the mice at all doses (P=0.069 for 400mg/kg and P=0.062 for 800mg/kg). Even though it was not statistically significant as compared to the control, increased body weights in the mean values of the mice treated with 400 and 800mg/kg body weight of the extract were observed (Table 1).

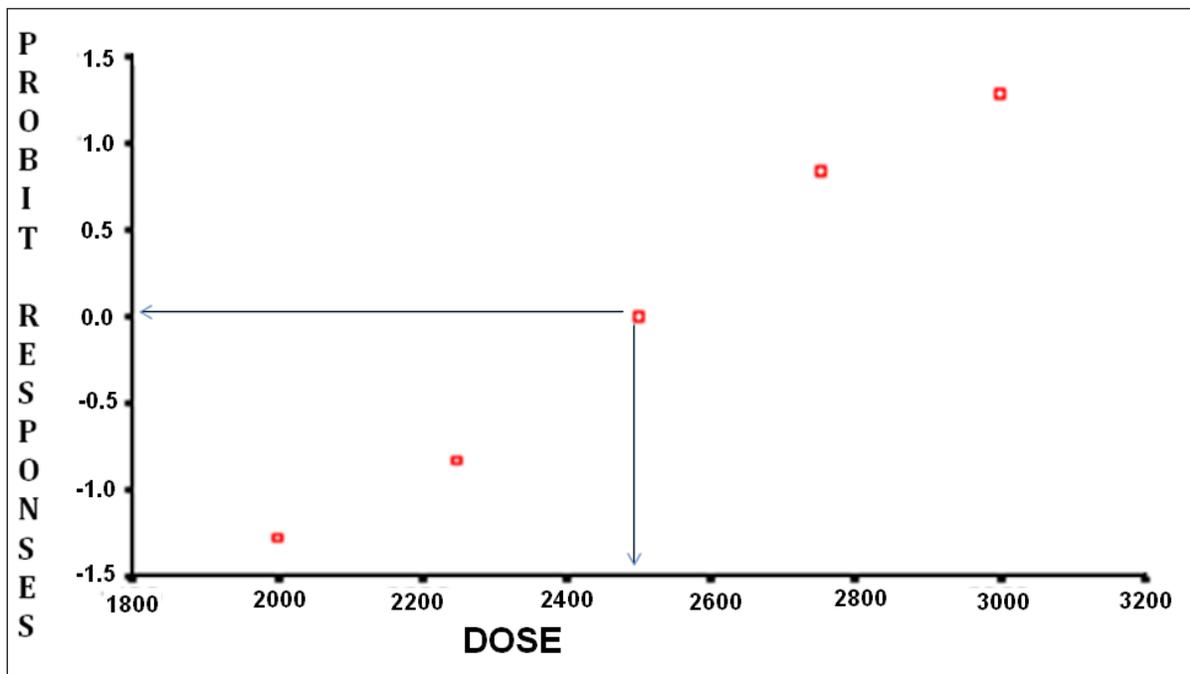


Figure 1. LD<sub>50</sub> curve for aqueous leaf extract of *V. bipontini* V results which is marked by vertical arrow (2500.62±5.24 mg/kg)

Table 1. Effect of aqueous leaf extract of *V. bipontini* V on the body weight of mice treated at doses of 400 and 800mg/kg.

| Group   | Treatment (mg/kg) | Mean Initial weight ± SE | Mean Final weight ± SE | Mean Weight change ± SE |
|---------|-------------------|--------------------------|------------------------|-------------------------|
| Control | -                 | 27.49±2.01               | 32.29±2.09             | 4.71±3.54               |
| 1       | 400               | 30.71±3.48               | 32.7±4.12              | 1.99±3.47 (0.069)       |
| 2       | 800               | 31.5±3.05                | 33.6±2.46              | 1.91±2.49 (0.062)       |

### 3.4. Effects of Aqueous Leaf Extract of *V. bipontini* V on Hematological and Biochemical Parameters

The chronic effect of the aqueous leaf extract of *V. bipontini* V on hematological and biochemical parameters of blood is illustrated in table 2. There was no significant difference in hematological composition of blood parameters between control and mice treated at doses of 400 and 800mg/kg of the plant extract. However, a decrease in RBC (M/UL) for mice treated at doses of 400 and 800mg/kg was observed. Total WBC (K/UL) also decreased insignificantly at doses of 400 (P=0.13) and 800mg/kg body weight (P=0.03) of the extract. Moreover, the platelet count (K/UL) has also shown insignificant decrease at doses of 400 (P=0.41) and 800mg/kg (P=0.07) of the extract. Similarly, Hgb concentration insignificantly decreased at doses of 400

and 800mg/kg body weight of the extract. Hematocrit percentage also showed non-significant decrease at 400 and 800mg/kg of the extract. Slight non-significant decrease in MCV was also observed for mice treated at a dose of 400 and 800mg/kg of the extract. Lymphocyte count has shown insignificant decrease in mice treated at a dose of 400mg/kg of the extract. But lymphocyte percentage of mice treated at 800mg/kg dose increased (P=0.13). However, the mean values of MCH and MCHC remained relatively the same as compared to the control group (Table 2).

Similarly, there was no significant difference between the various biochemical parameters of blood in the three groups of mice at all doses (Table 2). As shown in the same Table, serum AST level of mice treated at a dose of 400mg/kg of the extract increased insignificantly but AST level of mice treated at a dose of 800mg/kg of the extract showed non-significant decrease. In the same way, serum ALT level decreased in a dose dependent manner when treated with 400 and 800mg/kg of the extract (Table 2). Even though it was not statistically significant, an increase in serum ALP level was observed at doses of 400 (P=0.71) and 800mg/kg (P=0.71) of the extract.

Table 2. Hematological and biochemical parameters between aqueous leaf extract of *V. bipontini* V treated groups at doses of 400mg/kg, 800mg/kg, and control group.

| <b>Hematological &amp; Biochemical Parameters</b> | <b>Control with distilled water</b> | <b>Aqueous extract treated groups</b> |                     |
|---|-------------------------------------|---------------------------------------|---------------------|
|   |                                     | <b>400mg/kg</b>                       | <b>800mg/kg</b>     |
| RBC (M/UL)  | 8.03±0.62                           | 7.66±0.49 (0.13)                      | 7.63±0.43 (0.09)    |
| WBC (K/UL)  | 5.3±0.8                             | 4.89±0.59 (0.13)                      | 4.8±0.55 (0.06)     |
| Platelet (K/UL)                                   | 1030.6±23.83                        | 987.95±160 (0.41)                     | 935.9±112.64 (0.07) |
| Hgb (g/dl)  | 12.24±0.79                          | 11.52±0.51 (0.06)                     | 11.57±1.08 (0.08)   |
| Hct (%)   | 40.25±1.78                          | 38.42±1.5 (0.21)                      | 37.73±5.05 (0.09)   |
| Mcv (fl)  | 51.99±1.87                          | 50.3±1.82 (0.08)                      | 50.59±2.46 (0.14)   |
| Mch (pg)  | 12.48±0.47                          | 12.05±0.54 (0.01)                     | 12.05±0.69 (0.1)    |
| Mchc (g/dl)                                       | 25.35±1.03                          | 24.7±0.54 (0.09)                      | 24.85±0.85 (0.19)   |
| L (%)   | 83.7±3.27                           | 81.84±8.86 (0.51)                     | 88.1±5.37 (0.13)    |
| AST (IU/L)  | 132.6±26.54                         | 139±41.65 (0.8)                       | 127±38.3 (0.7)      |
| ALT (IU/L)  | 49.6±5.62                           | 48.9±6.48 (0.7)                       | 45.8±4.54 (0.1)     |
| ALP (IU/L)  | 46.7±47.67                          | 63.0±85.99 (0.71)                     | 72.2±138 (0.56)     |
| Urea (mg/dl)                                      | 55.9±7.01                           | 54.9±9.89 (0.8)                       | 60±6.6 (0.13)       |

The increase in ALP level was relatively high for those treated at a dose of 800mg/kg of the extract. However, ALT serum level insignificantly decreased at 400 and 800mg/kg of the plant extract as compared to the control. Similarly, at 800mg/kg AST serum level insignificantly

declined (P=0.7). Blood urea also insignificantly decreased in mice treated with 400 mg/kg and increased in mice treated with 800mg/kg of the extract.

**3.5. Effects of Aqueous Leaf Extract of *V. bipontini* V on the Weights of Liver and Kidney of Mice**

The chronic effect of aqueous leaf extract of *V. bipontini* V on the weights of liver and kidney of mice is shown in table 3. There were no significant changes in the liver and kidney weights of the mice treated with 400mg/kg and 800mg/kg doses of the extract as compared to the control group (Table 3).

Table 3. Effects of aqueous leaf extract of *V. bipontini* V on the weights of liver and kidney of mice.

| Group   | Treatment (mg/kg) | Mean Liver weight ± SE | Mean Kidney weight ± SE |
|---------|-------------------|------------------------|-------------------------|
| Control | -                 | 1.66±0.07              | 0.25±0.13               |
| 1       | 400               | 1.62±0.07 (0.172)      | 0.24±0.08 (0.23)        |
| 2       | 800               | 1.59±0.07 (0.054)      | 0.24±0.01 (0.08)        |

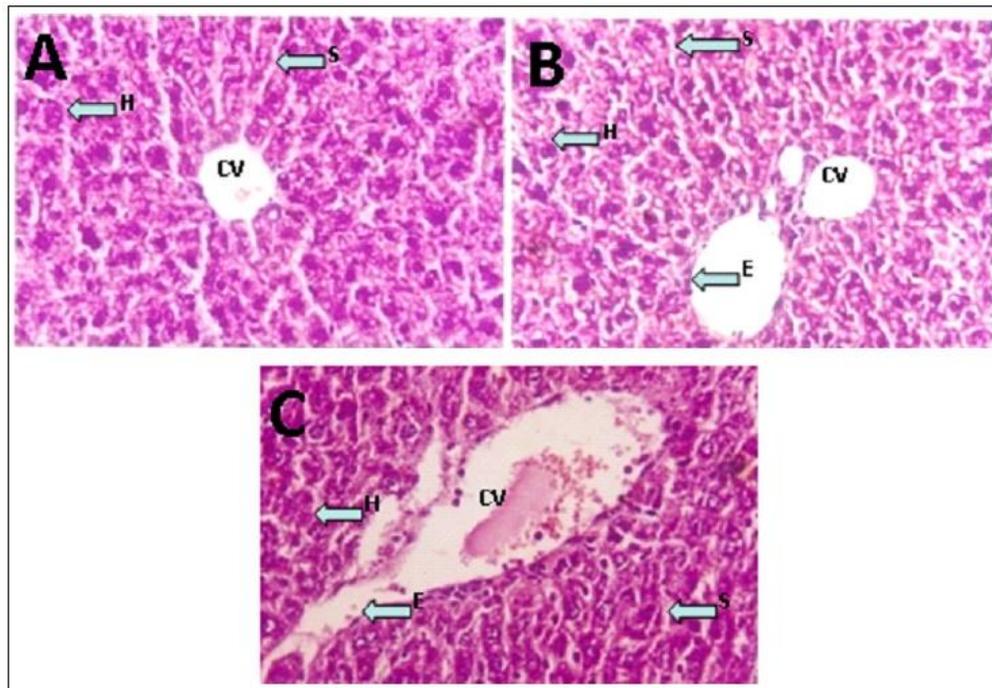


Figure 2. Photomicrographs of liver sections of the mice treated with 400mg/kg (B) and 800mg/kg (C) aqueous leaf extract of the plant showing no histopathological changes as compared to the control groups (A) (H&E, x 4216).

**Note:** H = Hepatocyte; S = Sinusoid; CV = Central vein; E = Endothelial cell.

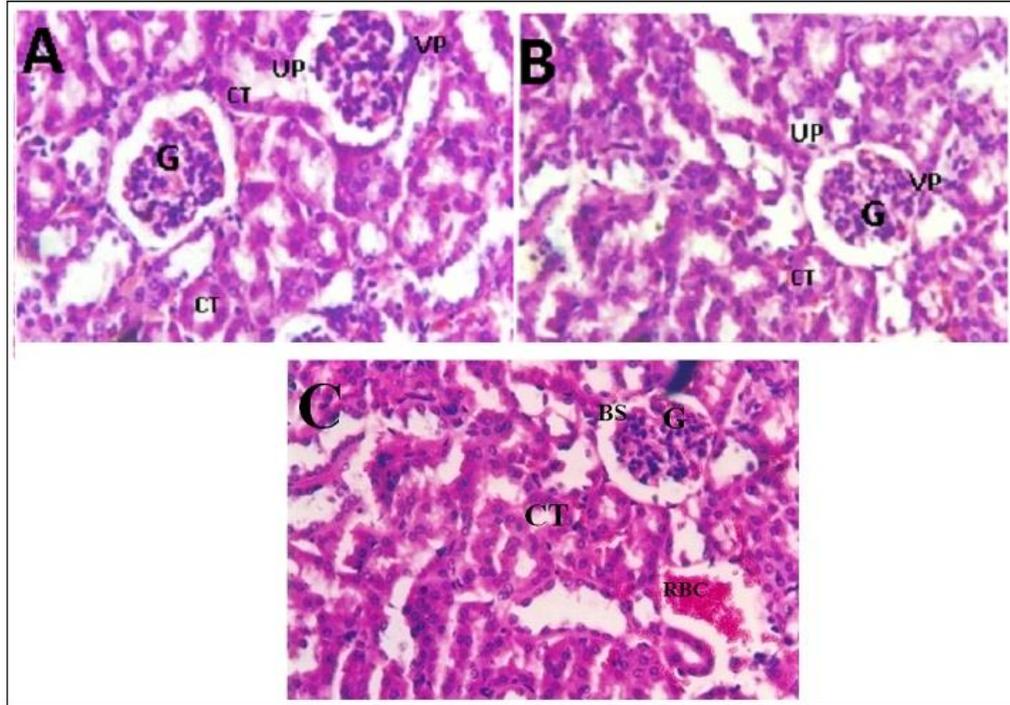


Figure 3. Photomicrographs of kidney sections of control mouse (Fig.3. A) showing no histopathological change as compared to the mice treated with 400 (B) and 800mg/kg (C) aqueous leaf extract of the plant (H&E, x4216).

**Note:** RBC = Red blood cells; G = Glomerulus; UP = Urinary pole; CT = Convoluted tubule; VP = Vascular pole; BS = Bowman's space.

### 3.6. Microscopic Observation

#### 3.6.1. Effects of Aqueous Leaf Extract of *V. bipontini* V on Histopathology of Liver

Microscopic examination of the liver sections of different groups of mice showed a normal architecture. Mice treated orally with the aqueous leaf extract of *V. bipontini* V for 45 days (Fig 2 B & C) showed no significant changes in their liver architecture at all doses as compared with the control (Fig 2A). The liver histology of both the control and mice treated with 400 and 800mg/kg of aqueous leaf extract showed normal features: there is no significant difference in the shape of the central vein, size of hepatic sinusoids and hepatocytes (Fig 2A, B& C) (H and E, x 4216).

#### 3.6.2. Effects of Aqueous Leaf Extract of *V. bipontini*V on Histopathology of Kidney

The histopathological effect of the aqueous leaf extract of *V. bipontini*V was done on the kidney section that was stained with hematoxylin and eosin. Microscopic observation indicated that there was no difference observed between the kidney sections of the control (Fig 3A) and mice treated with doses of 400mg/kg and 800mg/kg aqueous leaf extract of the plant (Fig 3B &C).

Renal histology of both treated and untreated groups exhibited normal features: no tubular necrosis was seen, size of Bowman's space and convoluted tubules and glomeruli were normal and clearly visible as compared to the control (Fig 3A, x4216).

#### 4. DISCUSSION

The widely used medicinal plants have formed the basis of health care throughout the world since the earlier days of humanity and have considerable importance (Ebong et al., 2008). Traditional medicine still remains the main resource for a large majority of the people in Ethiopia for treating health problems (WHO, 2011; Tilahun and Mirutse, 2007; Endashaw, 2007; Kassaye et al., 2006); however, currently, the use of traditional medicine seems to decline in Ethiopia because of the recent advent in health extension program (Banteyerga, 2011). Thus, knowledge of uses and side effects of medicinal plants provide a vital contribution to human health care. *Vernonia* species are the sources of many local medicines (Amole et al., 2006). People living in areas, where *V. bipontini* *V* grows use the plant for treating malaria and malaria related symptoms.

Mice treated with 400mg/kg and 800mg/kg of aqueous leaf extract did not show the signs of toxicity during long-term experiment. This is in agreement with traditional claim of the water preparation of *V. bipontini* *Vatke* leaves for treatment of malaria and malaria related symptoms (Ashenafi et al., 2007).

In the present study, aqueous leaf extract of *V. bipontini* *V* did not produce any significant increase in the mean values of body weights of mice treated at all doses. Similar results were previously reported in a study of a related plant extracts (*V. amygdalina*) by Amole et al. (2006). In this study, treatment of mice at 400 and 800mg/kg of aqueous leaf extract of *V. bipontini* *V* did not alter hematological and some biochemical composition of blood. Our findings revealed that hematological and biochemical parameters remained within normal limits after chronic treatment with all doses of aqueous leaf extract of *V. bipontini* *V*. These findings are supported by previous reports that described the absence of any significant effect on the hematological (Amole et al., 2006, Eleyinmi et al., 2006) and biochemical (Ojiako and Nwanjo, 2006) parameters of blood after chronic administration of related species (*V. amygdalina*) in mice.

There was no change in the liver and kidney weights of mice treated with aqueous leaf extract of *V. bipontini* V at all doses. These results are in line with the findings of Amole et al. (2006); Igile et al. (1995); and Eleyinmi et al. (2006), who reported no significant differences in weights of the organs.

In the present study, treatment with aqueous leaf extract of the plant did not cause any pathologic lesions in the liver and kidneys even at 800mg/kg. It might be due to the absence of cyanogenic glycoside in the plant extract that is responsible for histopathological changes (Nwanjo, 2005). This observation is in agreement with those of Amole et al. (2006) who described that microscopic observation of the tissue sections of liver and kidney showed no morphological abnormalities as compared to the controls after chronic oral administration of aqueous leaf extract of the related species (*V. amygdalina*).

## 5. CONCLUSION

The results suggest that the extract of this plant may be safe, even when taken for 45 days at dose of 800mg/kg. This is in agreement with traditional claim of the water preparation of *V. bipontini* *Vatke* leaves.

On the basis of the results, we would like to recommend investigation on the histopathology of other organs and further study is also recommended to examine effects of the plant extract on blood parameters and histopathology of different organs in prenatal mice and other animals' model.

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