



Acute Toxicity Assessment of *Centella asiatica* and *Bidens pilosa* Aqueous Crude Leaf Extracts in Pregnant Rat Model

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Abstract

There is little information on the safety of using traditional medicines during pregnancy. The current study assessed the safety of aqueous crude leaf extracts from two medicinal plants; *Centella asiatica* L. and *Bidens pilosa* L. in pregnant rats. Acute toxicity was evaluated by administering a single dose of plant extracts to pregnant rats at three standard doses of 1000, 2000 and 5000 mg/kg for 14 days. Distilled water was used as a negative control. Physiological and behavioural responses were assessed at 30 minutes, 1 hour, 4 hours, 12 hours and every other day until gestation day 22. The pregnant rats were humanely sacrificed at term after anaesthesia using CO₂. Blood was collected for haematological analysis and tissues (liver, kidney and gravid uterus) were for histopathological examination. The findings showed that the plants extracts are non-toxic at low studied dose of 1000 mg/kg when taken only once. However, *C. asiatica* and *B. pilosa* aqueous crude leaf extracts at higher doses (5000 mg/kg) caused a significant increase ($p < 0.05$) of platelets, and biochemical parameters, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) resulting in severe histopathological effects to the liver, kidney and gravid uterus. These findings provide baseline data on the safety of studied plant crude leaf extracts and exposure time to pregnant rats, as well as indications for future preclinical and clinical studies on the formulation of the plant extracts. Furthermore, the results give insights to the current users of the plant extracts to avoid their maximum consumption during pregnancy.

Keywords: Acute toxicity; Traditional medicine; Pregnant rat; *Bidens pilosa*; *Centella asiatica*

Introduction

More than 80% of the current world population in developing countries depends on traditional medicine to manage a wide range of human illnesses (WHO 2019). Despite being widely used and generally

considered harmless, medicinal plants can potentially be harmful, especially during pregnancy (Nasri and Shirzad 2013). Due to the high metabolic needs of pregnancy, various physiological and anatomical changes are required, and these changes have an effect

on several of organ systems, including the cardiovascular, pulmonary, renal, gastrointestinal and haematological (Kazma et al. 2020). The physiological and anatomical changes that occur during pregnancy can also have an impact on the process of drug absorption, distribution, metabolism and excretion and may induce birth defects. Owing to physiological changes that occur during pregnancy, most of medications are contraindicated during pregnancy (Pariente et al. 2016). Nonetheless, there is a growing trend of pregnant women using medicinal plants, such as *Vernonia amygdalina*, *Ruta chalepensis*, *Aloe vera*, *Allium sativum* (Ahmed et al. 2018), *Daucus carota* (Mills et al. 2006) despite the paucity of safety information and proof of the efficacy of their use. In Africa, the average use of pregnancy-related medicinal plants ranged from 32% in Central Africa to 45% in East Africa (Hajj and Holst 2020). Previous studies show that the use of traditional medicines during pregnancy is fairly prevalent throughout sub-Saharan Africa (Ahmed et al. 2018). In Tanzania, the use of traditional medicines ranged between 60 and 80% of the population to control a range of health conditions, including those that may emerge during pregnancy (Fukunaga et al. 2020). Although the majority of pregnant women especially in rural areas believe that traditional medicines are safer to use during pregnancy than modern ones, there are some cases reported on the toxicity of traditional medicines during pregnancy (Mawoza et al. 2019). For instance, Fukunaga et al. (2020) found a link between the use of traditional medicines and maternal complications during pregnancy in Kigoma, Tanzania. In the current study, two traditional medicines, *Centella asiatica* and *Bidens pilosa* were selected based on their availability and their traditional use in Tanzania. *B. pilosa*, is used to manage anaemia among pregnant women in Eastern Uganda (Nalumansi et al. 2017), indicating the plant extract may promote erythropoietin production, and also reported to have anti-inflammatory properties. *C. asiatica*, is also a common plant used during pregnancy and is

used for wound healing and other skin problems such as striae gravidarum (Oktavia et al. 2023), anti-inflammatory and anti-diabetic agent (Sari et al. 2014). Despite their widespread use, the safety of *Centella asiatica* and *Bidens pilosa* during pregnancy has not yet been studied. Therefore, the objective of the current study was to determine whether crude leaf extracts from *Centella asiatica* and *Bidens pilosa* are safe to consume during pregnancy.

Materials and Methods

Collection and Authentication of Plant

Materials

Two medicinal plants species *C. asiatica* and *B. pilosa* were collected in June 2021. *C. asiatica* was specifically collected from Kidia, Moshi District close to Kilimanjaro Mountain at an elevation of 1550 m above sea level, and *B. pilosa* was collected in the West Usambara Mountains in Lushoto district, Tanga region at an elevation of 500 m above the sea level. The mountains are located in north-eastern Tanzania (4° 24'–5° 00' S and 38° 10'–38° 36' E) and cover an area of 4,500 km². The annual rainfall ranges between 600 mm to 1200 mm, and the temperature ranges between 13°C and 27°C. The identification of the plants used were confirmed by the botanist, and the voucher specimen with the numbers JLP 01 for *C. asiatica* and MEJ 01 for *B. pilosa* were deposited in the herbarium of the Department of Botany at the University of Dar es Salaam.

Preparation and Extraction of Plant

Materials

Centella asiatica and *B. pilosa* leaves were washed with tap water and dried under shade at room temperature for three weeks at Zoology and Wildlife Conservation laboratory. Preparation of the aqueous crude extracts of *C. asiatica* and *B. pilosa* was done using the method of Li et al. (2000) with minor modifications. The dried leaves were ground into a fine powder using an electric blender (WARING MX-1100XT). Five hundred grams (500 g) of both powdered plant leaves were soaked in 1500 ml of distilled water for 48 hours with intermittent

shaking after every 12 hours. Using cloth gauze and Whatman filter paper 1, the mixture was decanted and filtered. In order to obtain the crude extract powder, the filtrates were dried by freeze-drying at a low temperature of -80°C and pressure (37 kPa) using the freeze-dryer (Edwards High Vacuum International Crawley, Sussex, England) at Muhimbili University of Health and Allied Sciences in Dar es Salaam. The filtrates were initially stored at -80°C at the Department of Molecular Biology and Biotechnology research laboratory. Finally, the obtained crude leaf extract was kept in airtight containers and stored in the refrigerator at 4°C in the Department of Zoology and Wildlife Conservation UDSM until the time of use.

Collection of Study Animals, Care and Ethical Consideration

A total of 21 fertile healthy male rats (14-17 weeks; 200-250 g) and 42 healthy virgin female rats (10-12 weeks; 150-200 g) of Wistar strain (*Rattus norvegicus*) were purchased from Sokoine University of Agriculture (SUA) in Morogoro, Tanzania. The animals were raised in the animal house at the Department of Zoology and Wildlife Conservation, UDSM. Animals were kept in wooden cages (100 cm long x 80 cm width) made for rats at a temperature of 22 to 30°C , a relative humidity of 40 to 60%, and a 12:12 hours light/dark cycle. Each cage contained 10-15 rats. The male and female Wistar rats were placed in separate cages of the same dimension. The bedding materials were wood shavings and were changed regularly after 2 to 3 days. Commercial rat food (broiler grower pellets) with protein 150 g/kg, moisture 120 g/kg, crude fat 25.80 g/kg, crude fibre 70.90 g/kg, calcium 8.16 g/kg and phosphorus 6 g/kg was provided daily in clean plates and clean drinking water was always available and its bottles were washed at 2 days' interval. The cages were disinfected once per week using 70% ethanol. The techniques used in this study were in accordance to the revised international standard for handling animals (OECD, 2022) guideline number 425 after obtaining an

approval of the project proposal by the Departmental Higher Degrees, Research and Publications Committee, University of Dar es Salaam with registration number 2019-06-08130. Before the start of the experiment, all rats were acclimatized for a one-week period. To reduce or eliminate pain, worry and distress before the loss of consciousness, pregnant animals were subjected to carbon dioxide in their living cages in a separate room during the experiment. This was done according to the American Veterinary Medical Association (AVMA) guidelines for euthanasia of animals; 2013 edition. Immediately after the experiment, the animals were buried deeply in the ground at a depth of approximately 1 m.

Induction of Pregnancy

After 7 days of acclimatization, the healthy virgin female rats aged 13 weeks and 160-180 g body weight were paired with healthy fertile male rats 15-18 weeks; 200-250 g in order to obtain the pregnant Wistar rats. The criteria for the female rat to be included in the pairing process were the health of the animal and the stage of oestrous cycle. Vaginal smear cytology was used to predict the oestrous cycle phase in virgin female rats. Ovulation usually occurs at proestrous and estrous stages and the female animals at these stages were placed in male cages at a 2:1 (female-male) ratio at 19:00 hours in the evening, mated during the night, and then removed at 6:00 am the next morning. Gestation day 0 was the day that overnight mating was established by the discovery of a vaginal plug and sperm in the vaginal smear of the female rat. All rats tested positive for pregnancy were given numbers and placed in separate cages. Unsuccessfully impregnated rats were returned to the pairing process for the following round until the third round before excluding them from the experiment. Pregnancy induction went on until 21 pregnant rats were obtained.

Experimental Design

Following pregnancy induction, the pregnant rats were randomly separated into

seven groups; six treatments groups and one control group. All pregnant rats were given numbers. A shuffled deck of cards was used for randomization, and animals with even numbers were put into the control group and those with odd numbers into the treatment groups. After the number of animals in the control group was completed, animals with even numbers were equally put in each of the treatment groups. Each group had three healthy pregnant Wistar rats as recommended in OECD guideline number 425 on the number of animals to be used in toxicity studies.

Acute Toxicity of *C. asiatica* and *B. pilosa* Aqueous Crude Leaf Extracts in Pregnant Wistar Rats

Acute oral toxicity test on pregnant Wistar rats was performed based on OECD guideline 425 (OECD 2022). Pregnant rats were separated into seven groups (three for each extract and one for control), fasted for 3-4 hours, and weighed before the dose was administered. Six groups of pregnant rats (three for each extract) were each given a single dose of *C. asiatica* and *B. pilosa* via oral gavage at three different standard doses (1000, 2000 and 5000 mg/kg) while the negative control group received 20 ml/kg distilled water. Dose administration took place at gestation day 7 and animals were observed for behavioural changes and general toxicity signs after dosing from 30 minutes, 1 hour, 4 hours, 12 hours, 24 hours and observation continued every other day for a total of 14 days. Behavioural changes that were monitored included tremors, salivation, diarrhoea, vomiting, touch reaction, breathing, vaginal discharge, alertness, grooming, pinna reflex, lacrimation and sleeping. Other parameters monitored were rectal temperature measured using a digital thermometer, maternal body and foetal birth weights measured by digital weighing scale (Ming Heng Digital scale).

On gestation day 22 the pregnant Wistar rats were allowed to deliver. For each rat, three to four pups were delivered. After delivery, rats were put under general anaesthesia for sample collection including

the gravid uterus and placenta of unborn pups, ending with euthanasia using carbon dioxide. The pregnant animals were housed in cages in the induction chamber, which was filled with a compressed carbon dioxide gas. This was carried out in a room apart from the animal house. Euthanasia took place after 2 to 3 minutes of carbon dioxide being filled at a rate of 30–70% of the chamber's volume per minute. This was done to induce sleeping in animals and avoid them from pain during surgical procedures. Then the rats were put on a horizontal position, abdominal and thoracic cavities were opened, 0.5 and 1 mls blood samples were collected by cardiac puncture into a vacutainer tube with anticoagulant ethylene diamine tetra acetic acid (EDTA) and plain tubes for haematological and biochemical analysis, respectively. The blood samples were put in a cool box and immediately sent to Lantent laboratory in Kinondoni, Dar es Salaam, for analysis using automated haematology analyzer (SYSMEX XN550) and biochemistry analyzer (Abbott architect ci4100 plus). Haematological parameters determined include white blood cell count (WBC), differential counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), red blood cell (RBC) count, haemoglobin concentration (HB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets count. Two liver enzymes, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), were biochemical markers assessed. Immediately following the humane sacrifice of the pregnant rats, gross pathological examination of the liver, kidney, placenta and gravid uterus was performed to check for any indication of damage to these organs as a result of administration of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts to pregnant Wistar rats. Subsequently, the tissues were processed for histopathological examination.

Data Analysis

All numerical data were expressed as Mean \pm SEM. Data analysis was performed using Paleontological Statistics (PAST) version 3.22. The Shapiro-Wilk test was used to test the normality of data on body weight, rectal temperature, litter size, haematological and biochemical markers. All parameters in the acute toxicity study were analysed using a One-Way Analysis of Variance (ANOVA), and significant comparisons were then confirmed using Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

Results

The results showed that administration of different doses of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts during 14 days of acute toxicity study, did not cause mortality

or changes in behavioural responses of the tested pregnant Wistar rats (even up to 5000 mg/kg) with respect to the negative control that received distilled water. The findings revealed the LD₅₀ for the aqueous crude leaf extracts of these plants to be above 5000 mg/kg. The parameters that were observed following the administration of the test substances are shown in Table 1. From the control group to the group that received the maximum dose, every parameter that was observed after the test materials were administered was normal. These findings confirm that the lethal dose (LD₅₀) of the aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* is estimated to be greater than 5000 mg/kg body weight when administered as a single oral dose to pregnant Wistar rats.

Table 1: Effect of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts administered orally on the overall behaviour of the tested pregnant rats

		RESPONSES													
EXTRACTS	Dose (mg/kg)	Alertness	Grooming	Convulsion	Urination	Salivation	Touch response	Diarrhea	Vomiting	Breathing	Sleeping	Pinna reflex	Vaginal discharge	Tremors	Death
<i>C. asiatica</i>	1000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
<i>C. asiatica</i>	2000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
<i>C. asiatica</i>	5000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
<i>B. pilosa</i>	1000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
<i>B. pilosa</i>	2000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
<i>B. pilosa</i>	5000	A	A	A	N	N	N	A	A	N	N	N	A	A	A
CONTROL		A	A	A	N	N	N	A	A	N	N	N	A	A	A

Control group received 20 ml/kg of distilled water at the same time during dosing of the treatment groups. A = Absent N = Normal

Assessment of Physiological and Anatomical Changes of Pregnant Wistar Rats administered with *C. asiatica* and *B. pilosa* Aqueous Crude Leaf Extracts during Acute Toxicity Study

The aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* did not affect the core body temperature of the treated pregnant rats in a 14-day of acute toxicity study when compared to the negative control group (Table 2).

Table 2: Rectal temperature of pregnant rats administered *C. asiatica* and *B. pilosa* aqueous leaf crude extracts for 14-day observation of an acute toxicity.

	Average rectal temperature (°C)						
	CONTROL Water (20 ml/kg)	<i>C. asiatica</i> 1000 mg/kg	<i>C. asiatica</i> 2000 mg/kg	<i>C. asiatica</i> 5000 mg/kg	<i>B. pilosa</i> 1000 mg/kg	<i>B. pilosa</i> 2000 mg/kg	<i>B. pilosa</i> 5000 mg/kg
GD 0	37.03 ± 0.3	37.27 ± 0.3	37.30 ± 0.3	37.57 ± 0.3	37.30 ± 0.2	37.10 ± 0.2	37.23 ± 0.2
GD 07	37.20 ± 0.3	37.40 ± 0.2	37.40 ± 0.2	37.20 ± 0.2	37.67 ± 0.2	37.17 ± 0.2	37.30 ± 0.2
GD 14	37.40 ± 0.1	37.20 ± 0.2	37.60 ± 0.2	37.27 ± 0.3	37.47 ± 0.2	37.20 ± 0.1	37.40 ± 0.2
GD 20	37.33 ± 0.1	37.60 ± 0.3	37.50 ± 0.3	37.27 ± 0.2	37.30 ± 0.2	37.63 ± 0.2	37.13 ± 0.1

Values are presented as mean ± SEM (n = 3 for each group).

There was no significant difference between treated groups of *C. asiatica* and *B. pilosa* compared to the negative control group (p = 0.35 and 0.59 respectively) which was determined by one-way ANOVA.

In addition to temperature, the mother's body weight from gestation day 0 to 20 was evaluated during the acute toxicity study. *C. asiatica* and *B. pilosa* aqueous crude leaf extracts did not change maternal body weight throughout the experiment. In all treatment

and the control groups, there was a minimal rise in body weight from gestation day 0 to 7 but a large increase was recorded in the later gestation days due to pregnancy, which means that the extracts did not affect maternal growth. Maternal body weights increase due to pregnancy for *C. asiatica* and *B. pilosa* aqueous extracts, respectively (Figures 1 and 2).

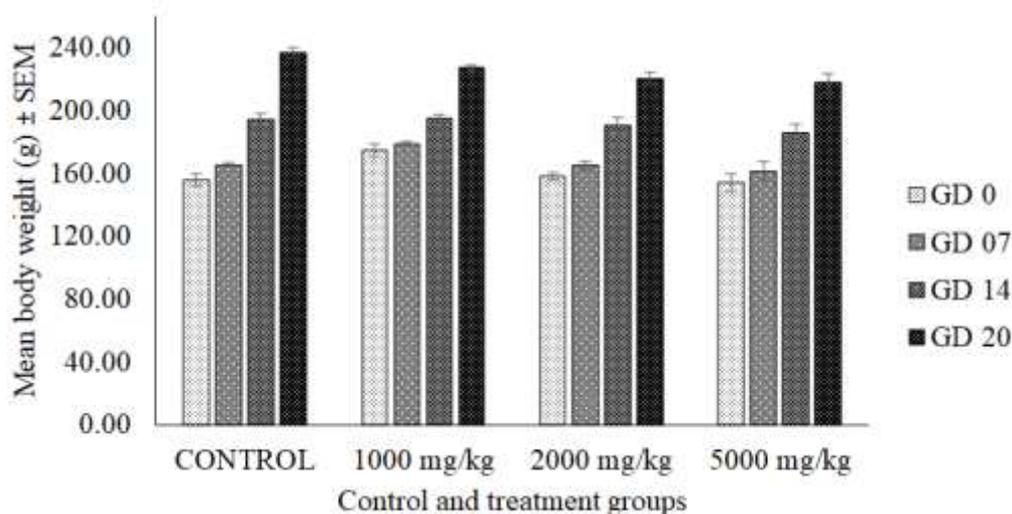


Figure 1: Mean maternal body weight of pregnant rats administered with aqueous crude leaf extracts of *C. asiatica* as a single dose in a 14-day acute toxicity study. Control group received 20 ml/kg distilled water during the dosing of treatment groups. Values are presented as mean ± SEM (n = 3 for each group). There was no significant difference in maternal body weight between the treatment groups and negative control (p = 0.94, one-way ANOVA). GD = Gestation day (s).

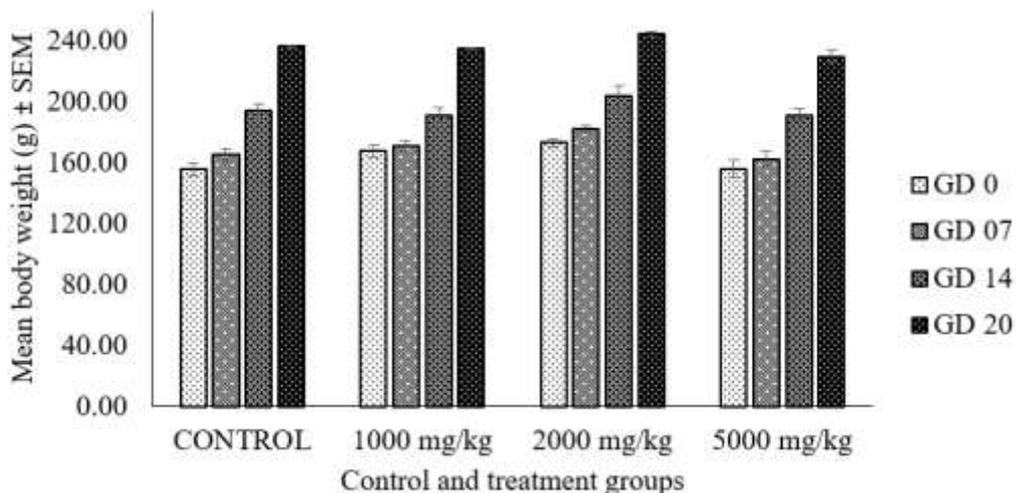


Figure 2: Mean maternal body weight of pregnant rats administered with aqueous crude leaf extracts of *B. pilosa* as a single dose in a 14-day acute toxicity study. Control group received 20 ml/kg distilled water during the dosing of treatment groups. Values are presented as mean \pm SEM ($n = 3$ for each group). There was no significant difference in maternal body weight between the treatment groups and negative control ($p = 0.86$, one-way ANOVA). GD = Gestation day (s).

As shown in Figure 3, pregnant rats administered with varying dosages of aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* during acute toxicity delivered pups with comparable birth weights to the control

group. Additionally, the number of newborn per single mother did not change significantly in the treated groups compared to the negative control ($p < 0.05$, Figure 4).

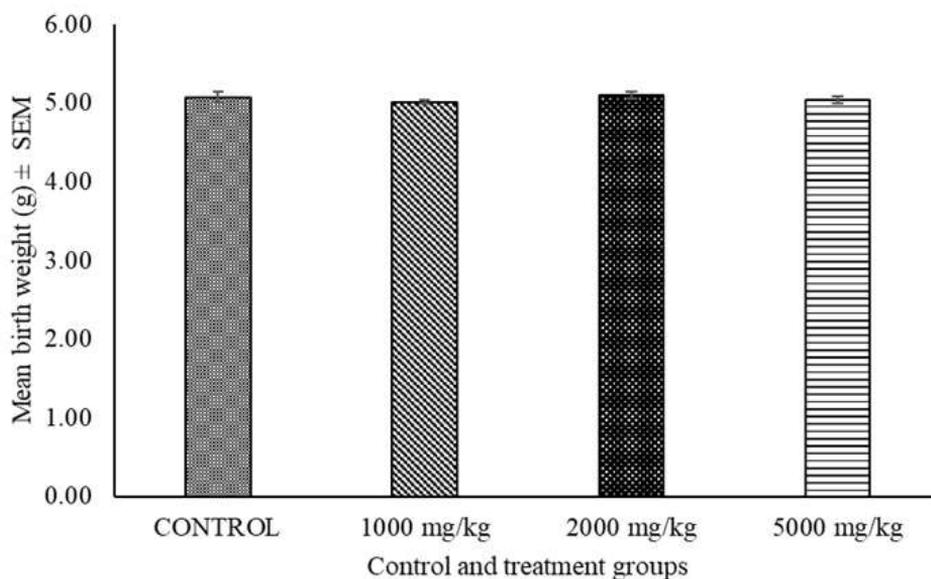


Figure 3: Birth weight of pups delivered by pregnant rats administered with aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* in a 14-day acute toxicity study. Control group received 20 ml/kg distilled water during the dosing of the treatment groups. Values are presented as mean \pm SEM (n = 3). There was no significant difference between the treated groups and negative control (p = 0.68, One-way ANOVA).

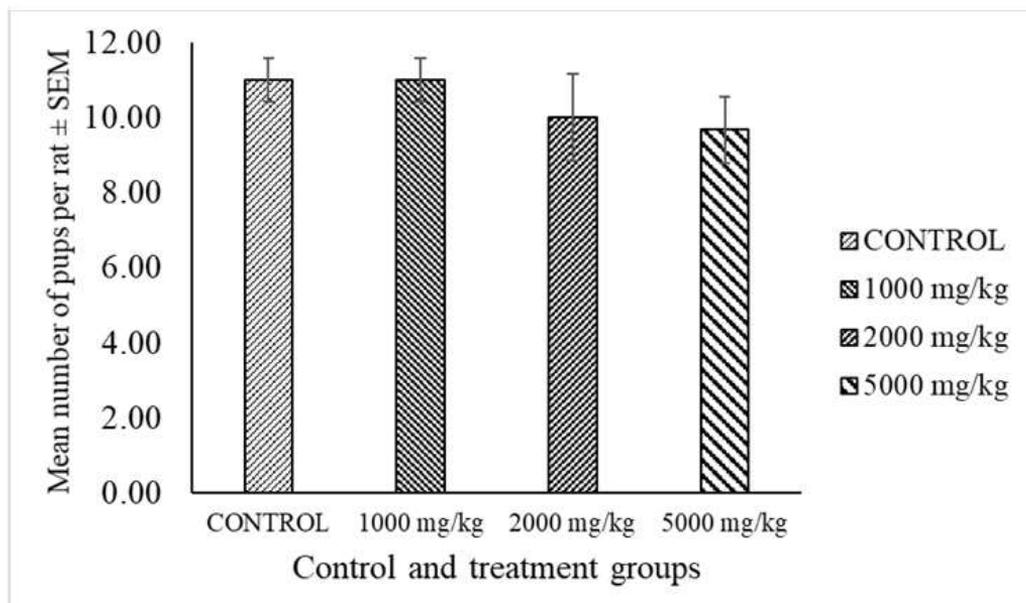


Figure 4: Average number of pups produced by pregnant rats administered with aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* as single dose in a 14-day acute toxicity study. Control group received 20 ml/kg distilled water during the dosing of treatment groups. Values are presented as mean \pm SEM (n = 3). There was no significant difference between the treated groups and negative control (p = 0.70 determined by one-way ANOVA).

The Effect of *C. asiatica* and *B. pilosa* Aqueous Crude Leaf Extracts on Haematological and Biochemical Parameters in Acute Toxicity Study

The quantity and quality of red blood cells were assessed following the administration of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts as a single dose in a 14-day acute toxicity study. The results show no significant variation in the mean values of red blood cell quantity (RBC count, HB, and HCT) and red blood cell quality (MCV, MCH and MCHC) in the treatment groups compared to the negative control (p > 0.05). Likewise, the effect of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts on white blood cells and their differentials was assessed in this study. These parameters are

the first line of cellular defence and usually increase in response to pathogenic infections, tissue damage and inflammatory processes. The findings showed that both extracts did not significantly affect the mean values of leukocytes and their differentials in all treatment groups in comparison to the negative control group after 14 days of acute toxicity study (Tables 3 and 4).

Furthermore, the effect of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts on the platelets count of the tested pregnant rats was determined. The results showed that mean values of platelets count were dose dependent and Tukey's multiple comparison analysis among the treated groups indicated no significant difference between the pregnant rats administered the doses (1000 mg/kg and

2000 mg/kg) in comparison with the negative control. However, there was a significant elevation of mean platelets count between the group that received the highest dose of the extracts (5000 mg/kg) with respect to negative control (* p < 0.05, Tables 3 and 4). In this study, two markers of liver damage: aspartate aminotransferase and alanine aminotransferase were examined. The

findings showed that the mean values of AST and ALT were dose dependent. Tukey's analysis revealed a significant elevation of these parameters in the groups that received the higher doses (2000 and 5000 mg/kg) in comparison to the negative control (* p < 0.05, Tables 3 and 4).

Table 3: Mean values of haematological and biochemical parameters of pregnant Wistar rats administered with various doses of aqueous leaf crude extracts of *C. asiatica* during acute toxicity study.

	CONTROL	1000 mg/kg	2000 mg/kg	5000 mg/kg
RBC (X 10¹²/L)	6.46 ± 0.24	6.34 ± 0.06	6.37 ± 0.07	6.30 ± 0.08
HB (g/dL)	12.10 ± 0.21	12.07 ± 0.12	12.41 ± 0.01	12.15 ± 0.15
HCT (L/L)	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
MCV (fL)	53.53 ± 0.55	53.87 ± 0.15	54.17 ± 0.72	54.03 ± 0.39
MCH (pg)	18.00 ± 0.12	17.83 ± 0.15	17.80 ± 0.12	17.93 ± 0.15
MCHC (g/dL)	33.77 ± 0.55	33.60 ± 0.38	33.33 ± 0.35	33.80 ± 0.17
PLTS (X 10⁹/L)	636.67 ± 8.09	638.00 ± 10.21	670.67 ± 10.59	848.67 ± 22.56 *
WBC (X 10⁹/L)	6.05 ± 0.12	6.07 ± 0.05	6.22 ± 0.08	6.30 ± 0.10
NEU (X 10⁹/L)	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
BASO (X 10⁹/L)	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
EOSI (X 10⁹/L)	0.02 ± 0.006	0.02 ± 0.006	0.02 ± 0.06	0.02 ± 0.06
LYMPH (X 10⁹/L)	5.68 ± 0.15	5.73 ± 0.08	5.76 ± 0.09	5.70 ± 0.11
MONO (X 10⁹/L)	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
ALT (IU/L)	49.53 ± 0.96	52.67 ± 1.68	56.90 ± 0.67 *	61.13 ± 1.04 *
AST (IU/L)	99.10 ± 0.61	100.47 ± 1.52	125.10 ± 1.24 *	128.97 ± 1.49 *

Significant different from control, * P < 0.05 determined by one-way ANOVA followed by Tukey's multiple comparison test. Results are expressed as mean ± SEM, n = 3.

Table 4: Mean values of haematological and biochemical parameters of pregnant Wistar rats administered with various doses of aqueous leaf crude extracts of *B. pilosa* during acute toxicity study.

	CONTROL	1000 mg/kg	2000 mg/kg	5000 mg/kg
RBC (X 10¹²/L)	6.46 ± 0.24	6.37 ± 0.17	6.34 ± 0.12	6.54 ± 0.20
HB (g/dL)	12.10 ± 0.21	12.08 ± 0.12	12.57 ± 0.15	12.70 ± 0.21

HCT (L/L)	0.34 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.01
MCV (fL)	53.53 ± 0.55	53.57 ± 0.35	52.10 ± 0.46	52.37 ± 0.66
MCH (pg)	18.00 ± 0.12	17.97 ± 0.18	17.70 ± 0.21	17.77 ± 0.22
MCHC (g/dL)	33.77 ± 0.55	33.60 ± 0.21	34.13 ± 0.15	33.33 ± 0.59
PLTS (X 10⁹/L)	636.67 ± 8.09	650.00 ± 16.65	673.00 ± 19.60	969.67 ± 8.09 *
WBC (X 10⁹/L)	6.05 ± 0.12	6.07 ± 0.05	6.22 ± 0.08	6.30 ± 0.10
NEU (X 10⁹/L)	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
BASO (X 10⁹/L)	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
EOSI (X 10⁹/L)	0.02 ± 0.006	0.02 ± 0.006	0.02 ± 0.06	0.02 ± 0.06
LYMPH (X 10⁹/L)	5.68 ± 0.15	5.73 ± 0.08	5.76 ± 0.09	5.70 ± 0.11
MONO (X 10⁹/L)	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
ALT (IU/L)	49.53 ± 0.96	50.63 ± 0.90	55.18 ± 0.67 *	75.97 ± 0.64 *
AST (IU/L)	99.00 ± 0.60	99.00 ± 1.00	110.00 ± 1.70 *	115.00 ± 2.00 *

Significant difference from control, * P < 0.05 determined by one-way ANOVA followed by Tukey's multiple comparison test. Results are expressed as mean ± SEM, n = 3.

Gross Morphological Examination of Visceral Organs in Pregnant Rats Administered with Aqueous Crude Leaf Extract of *C. asiatica* and *B. pilosa* during Acute Toxicity Study

Gross pathology of the liver, kidney and gravid uterus was assessed in the current study and the findings showed that administration of aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* at 1000, 2000 and 5000 mg/kg b.w for 14 days of acute toxicity induced no obvious pathological changes in terms of colour, texture and injury in organs of pregnant rats as shown in Figures 5 and 6 for liver, 7 and 8 for kidney and 9 and 10 for gravid uterus, respectively.

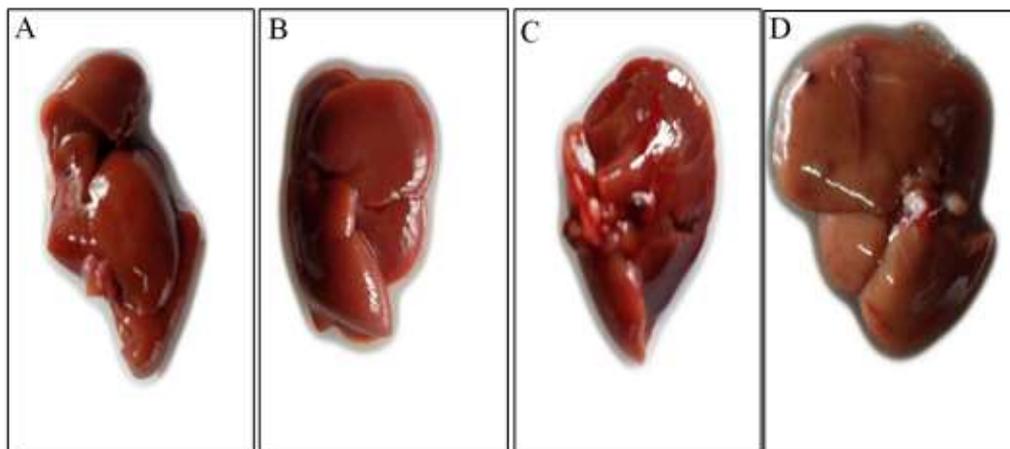


Figure 5: Gross appearance of the liver of pregnant rats administered with a single dose of *C. asiatica* aqueous crude leaf extract during acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D).

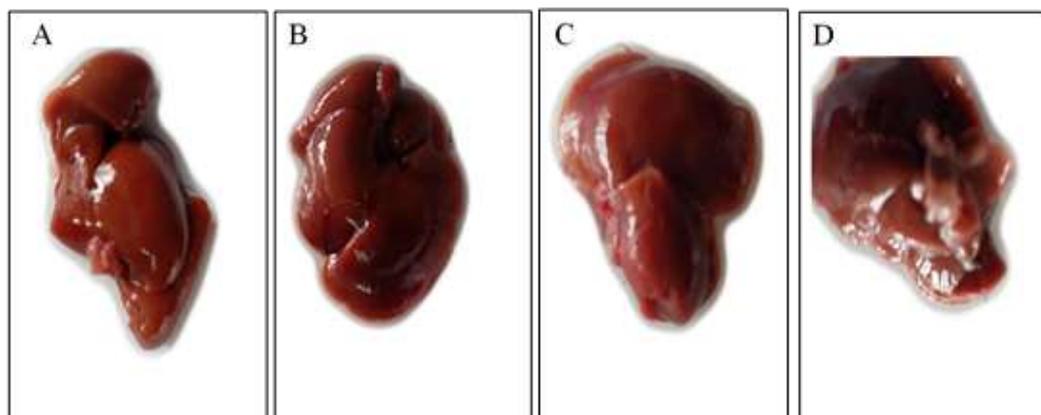


Figure 6: Gross appearance of the liver of pregnant rats treated with a single dose of *B. pilosa* aqueous crude leaf extract during acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D).

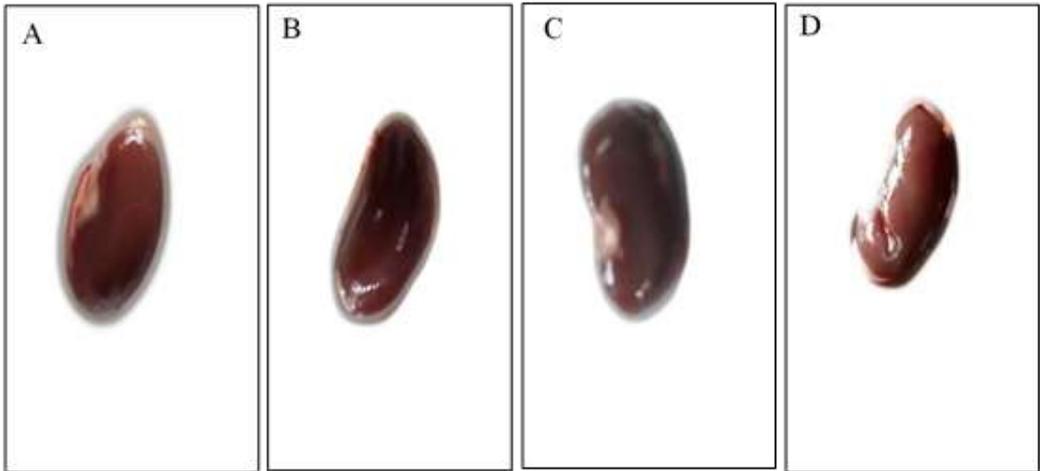


Figure 7: Gross appearance of the kidney of pregnant rats administered with a single dose of aqueous crude leaf extract of *C. asiatica* in a 14-day acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D).

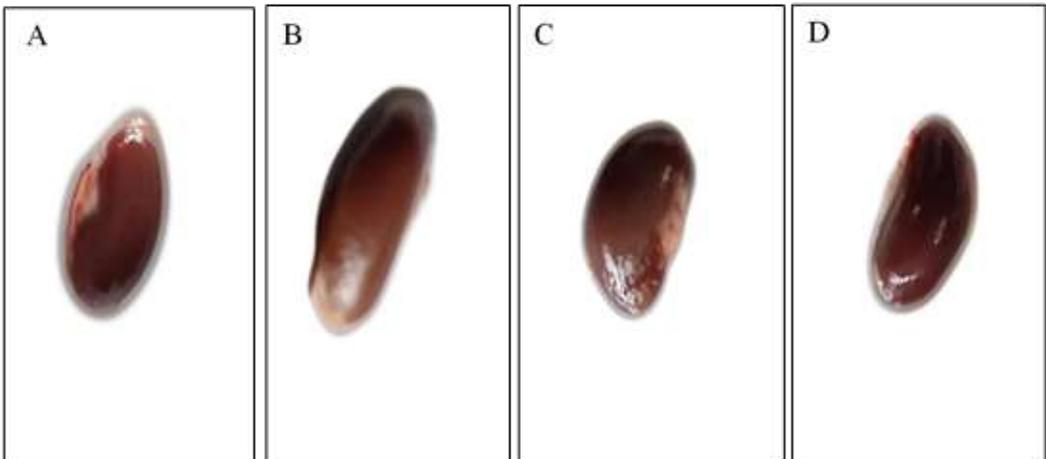


Figure 1: Gross appearance of the kidney of pregnant rats administered with a single dose of *B. pilosa* aqueous crude leaf extract in a 14-day acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D).

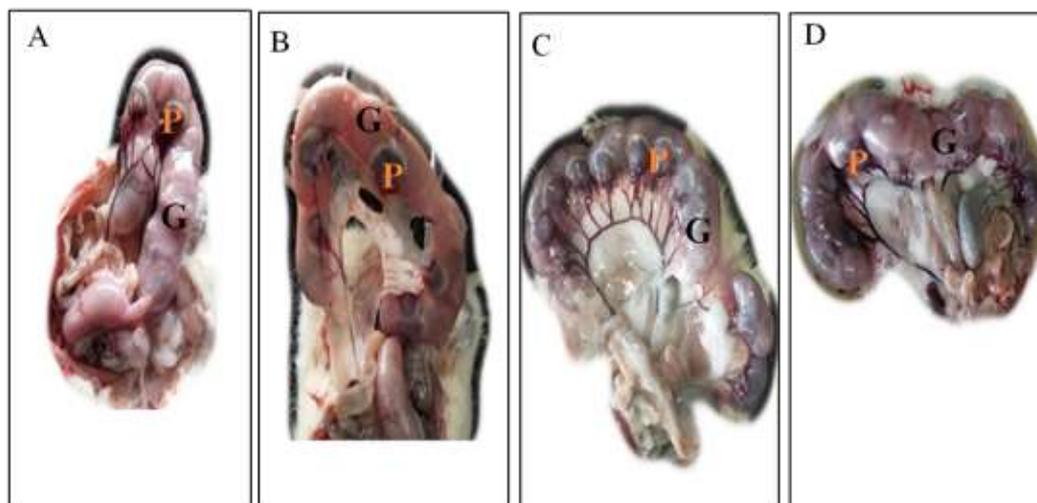


Figure 9: Gross appearance of the gravid uterus and placenta of pregnant rats administered with a single dose of *C. asiatica* aqueous crude leaf extract in a 14-day acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D)). P = Placenta and G = Gravid uterus.

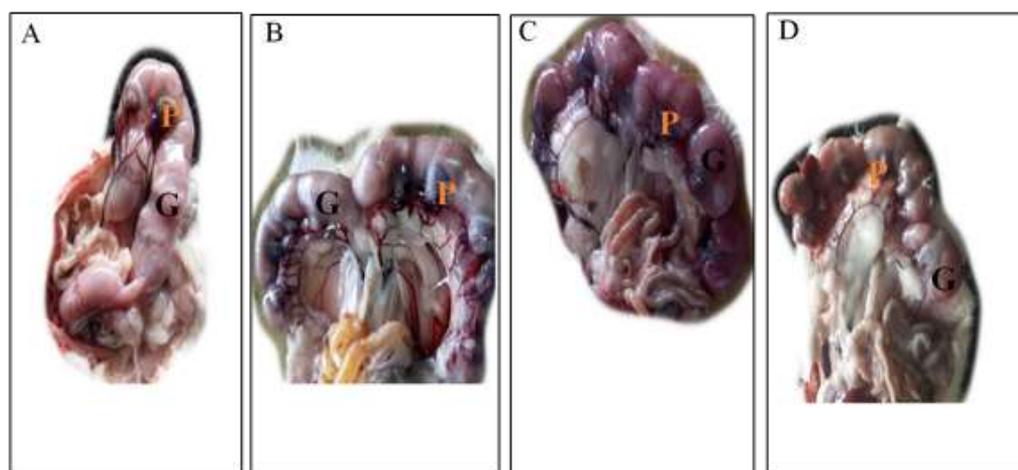


Figure 10: Gross appearance of the gravid uterus and placenta of pregnant rats administered with a single dose of *B. pilosa* aqueous crude leaf extract in a 14-day acute toxicity study. Control (A), rat treated by 1000 mg/kg (B), rat treated by 2000 mg/kg (C) and rat treated by 5000 mg/kg (D)). P = Placenta and G = Gravid uterus.

Histopathological Assessment of Visceral Organs in Pregnant Rats Administered with Aqueous Crude Leaf Extract of *C. asiatica* and *B. pilosa* during Acute Toxicity Study

To determine whether organs or tissues had been harmed by the aqueous leaf crude extracts of *C. asiatica* and *B. pilosa*

administered orally to pregnant Wistar rats, the liver, kidney and gravid uterine tissues underwent histopathological analysis. For *C. asiatica* there were no changes in the liver microscopic structures in the group that administered a lower dose of 1000 mg/kg compared to the negative control that received distilled water (Figures 11A and B)

while in *B. pilosa* at this dose level the minor damage was empty spaces in the cytoplasm of hepatocytes (CS), blue arrow in Figure 11E. All other treated groups displayed severe microscopic changes in the liver structure, including the central vein (CV) filled with blood (congestion, green arrows in

Figures 11D and G), hepatocytes show empty spaces in the cytoplasm of hepatocytes (CS) (vacuolation, blue arrows in C-G) and haemorrhage (He), yellow arrows in C. Some showed enlargement of hepatocytes (H) and nuclei of hepatocytes pushed to the periphery due to necrosis (Figure 11 D and G).

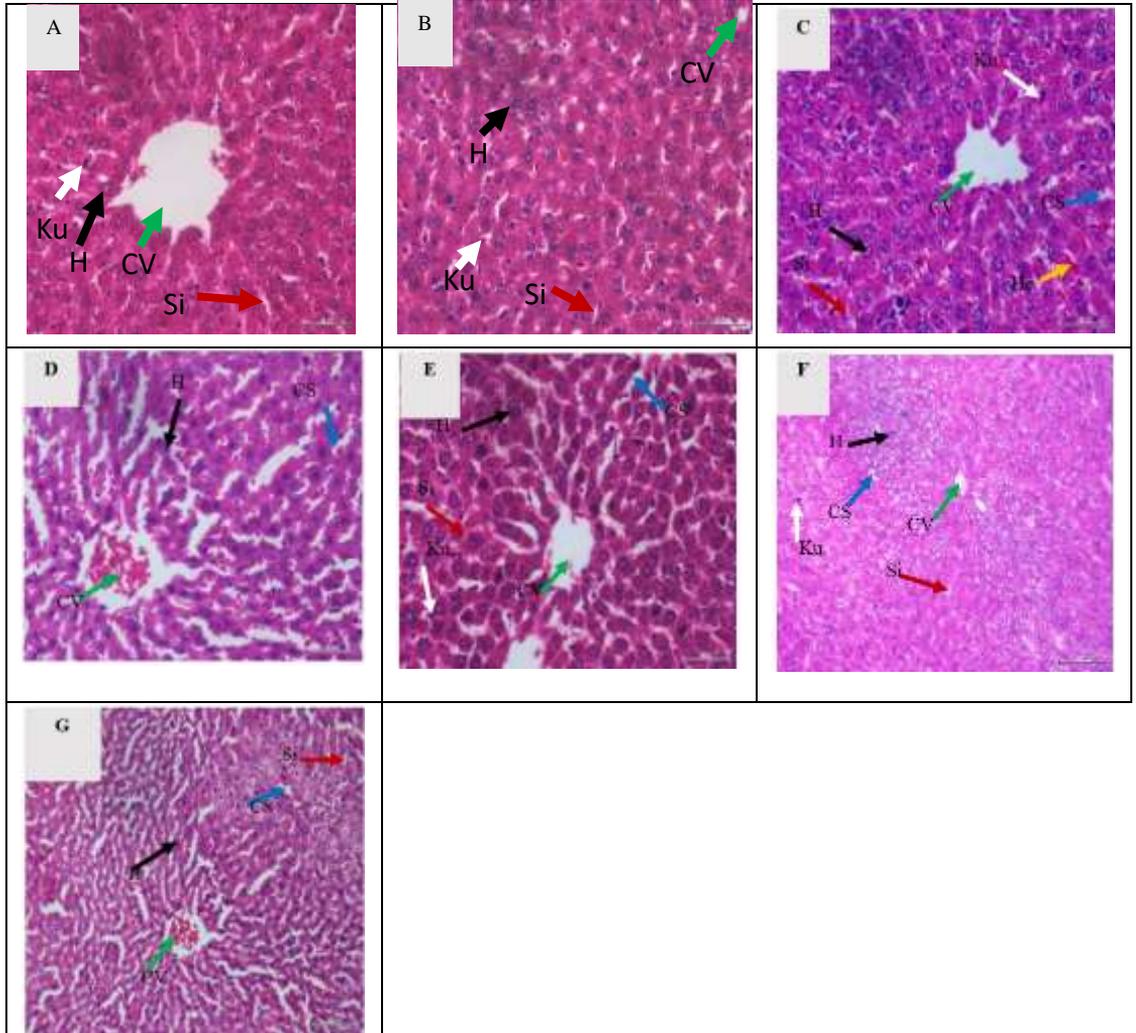


Figure 11: Liver sections stained with haematoxylin and eosin showing the effect of *C. asiatica* and *B. pilosa* aqueous leaf crude extracts administered to pregnant Wistar rats during acute toxicity study. (A) Control group; (B) 1000 mg/kg *C. asiatica*; (C) 2000 mg/kg *C. asiatica*; (D) 5000 mg/kg *C. asiatica*; (E) 1000 mg/kg *B. pilosa*; (F) 2000 mg/kg *B. pilosa*; (G) 5000 mg/kg *B. pilosa*. Figures A and B show normal kupffer (Ku, white arrows), hepatocytes (H), central vein (CV) and sinusoidal cells (Si). There is an enlargement of hepatocytes (H, black arrows) in C while in D and E hepatocytes are shrunken causing cytoplasmic spaces or vacuolation (CS, blue arrows). However, in figures F and G there are dark stained

nuclei of hepatocytes to indicate condensation of the nucleus [pyknosis] (H), blood appeared in the central vein (CV, green arrows) in G and sinusoids (Si, red arrows) to indicate congestion. kupffer cell and hepatocyte nuclei appear pushed to the periphery. Magnification Scale bar: A-D 50 μ m or Magnification: A-D: 200X; F: 100X.

In the case of the kidney, there were no changes in the microscopic structures of the pregnant rats administered the low dose (1000 mg/kg) compared to the negative control for both plant extracts (Figure 12A, B and E). However other treated groups showed severe microscopic changes in the kidney structure in comparison to the negative control. Some of the signs of the damage were haemorrhage (He, green arrow in Figure

12C), cytoplasmic spaces (CS, red arrows in Figure 12F and G), shrunken of the glomerulus (GI) yellow arrows in C, D, F and G and widen of the Bowman's capsule (BC) white arrows in C, D, F and G. Laminal area of distal convoluted tubules (Dt, black arrow) and proximal convoluted tubules (Pt, blue arrow) decreased due to the thickening of epithelium (necrosis) [Figure 12C, D, F and G].

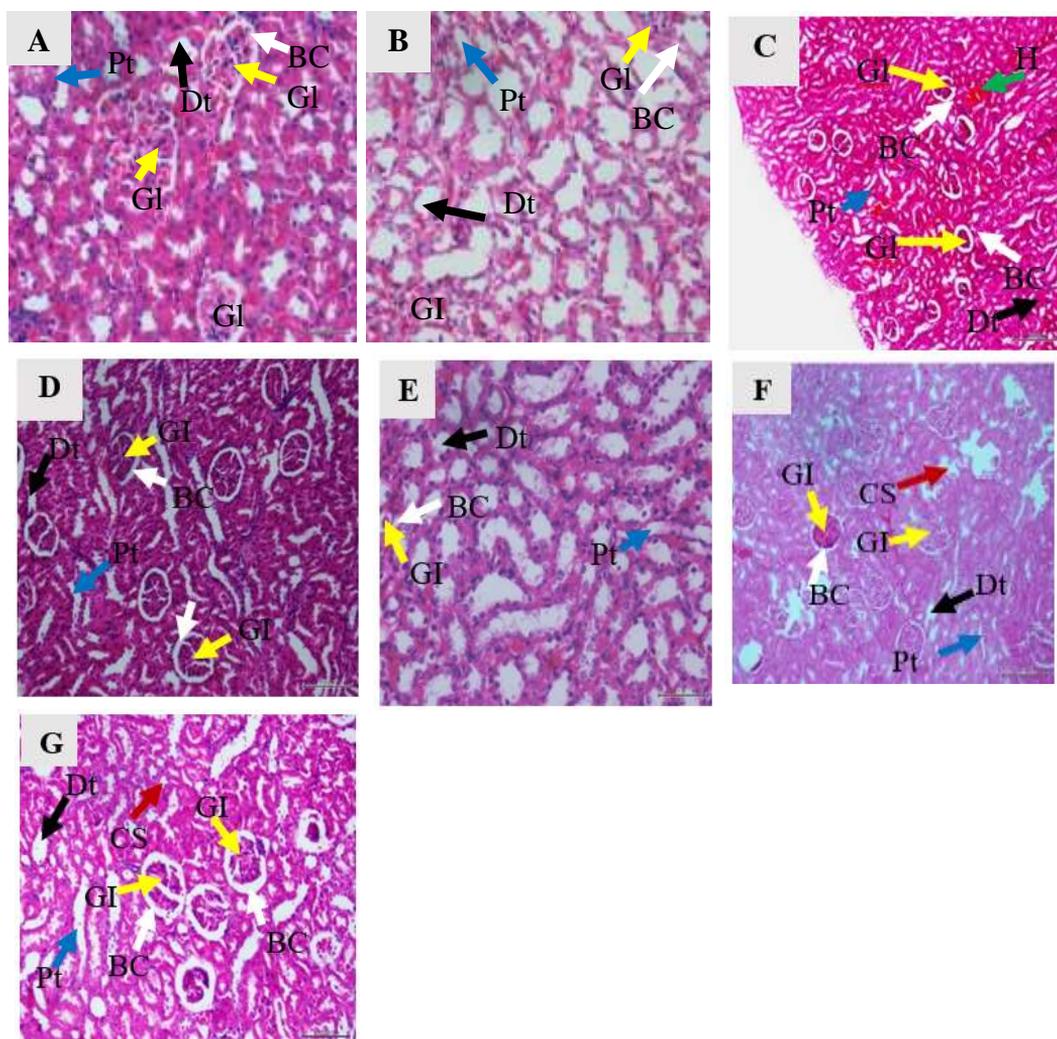


Figure 12: Kidney sections stained with haematoxylin and eosin showing the effect of *C. asiatica* and *B. pilosa* aqueous leaf crude extracts administered to pregnant Wistar rats during acute toxicity study. (A) Control group; (B) 1000 mg/kg *C. asiatica*; (C) 2000 mg/kg *C. asiatica*; (D) 5000 mg/kg *C. asiatica*; (E) 1000 mg/kg *B. pilosa*; (F) 2000 mg/kg *B. pilosa*; (G) 5000 mg/kg *B. pilosa*. Figures A, B and E show normal glomeruli (GI, yellow arrows), Bowman's capsule (BC, white arrows), proximal convoluted tubule (Pt, blue arrows) and distal convoluted tubule (Dt, black arrows). In Figures C, D, F and G the Bowman's capsule is widened and the glomerulus was shrunken. Also, the luminal area of the distal and proximal convoluted tubule decreased due to necrosis and haemorrhage (H, green arrow) appeared in Figure C. Magnification Scale bar: A-D 50 μ m or Magnification: A-D: 200X; F: 100X.

Regarding to the gravid uterus, the results demonstrated that the gap between the mother's placental labyrinth and the foetus's yolk sac increased with increasing doses (** in Figures 13). The negative control and the low dose groups (1000 mg/kg) for both

extracts did not show great separation between the maternal placental labyrinth and foetal yolk sac. However, there was an enlargement in the gap between these tissues at higher doses of 2000 and 5000 mg/kg. (Figures 13C and 13D).

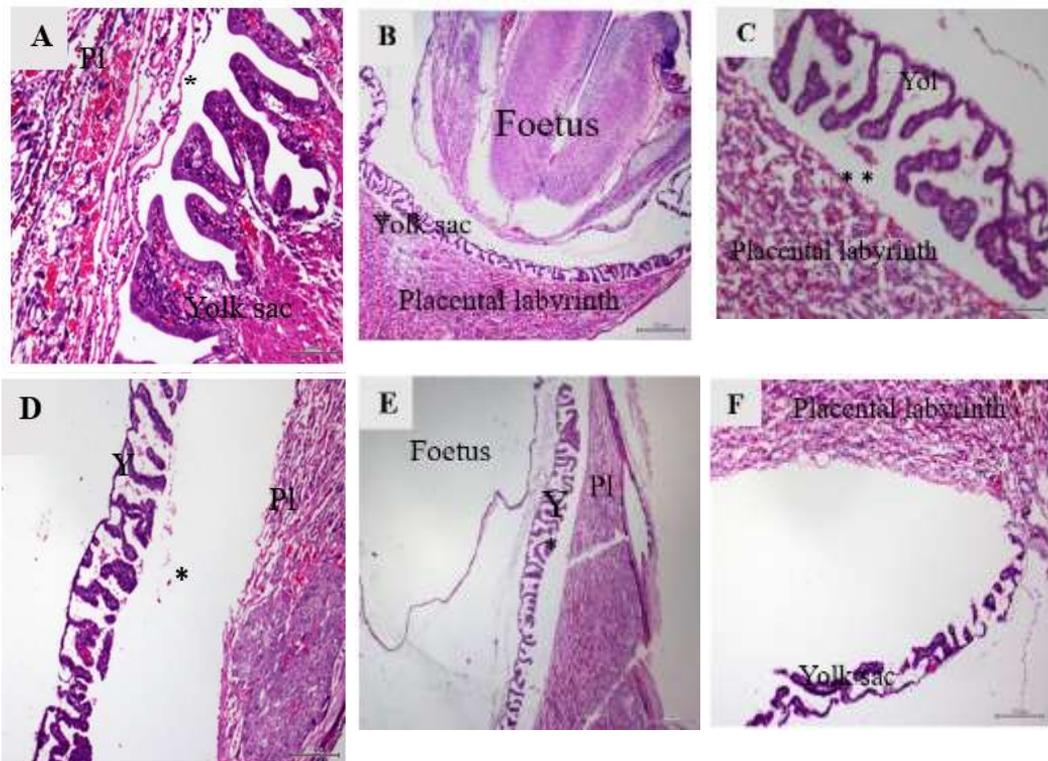




Figure 13: Gravid uterine sections stained with haematoxylin and eosin showing the effect of *C. asiatica* and *B. pilosa* aqueous leaf crude extracts administered to pregnant Wistar rats during acute toxicity study. (A) Control group; (B) 1000 mg/kg *C. asiatica*; (C) 2000 mg/kg *C. asiatica*; (D) 5000 mg/kg *C. asiatica*; (E) 1000 mg/kg *B. pilosa*; (F) 2000 mg/kg *B. pilosa*; (G) 5000 mg/kg *B. pilosa*. * * = Gap between mother's placental labyrinth and foetal yolk sac. Figures A, B and E show the standard gap between maternal placental labyrinth and foetal yolk sac (**) while figures C, D, F and G show an increase in these gaps. Scale bar: A-D 100 μ m or Magnification: A-D: 200X; F: 100X.

Discussion

This study aimed to assess the acute toxicity of *C. asiatica* and *B. pilosa* aqueous crude leaf extracts during pregnancy using a rat model. Oral administration of aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* of up to 5,000 mg/kg did not cause mortality in the pregnant Wistar rats. These findings imply that the oral LD₅₀ of these extracts is more than 5,000 mg/kg. According to two previous studies, the LD₅₀ for the aqueous and ethanolic extract of *C. asiatica* and *B. pilosa* in non-pregnant animals was reported to be greater than 2000 mg/kg (Deshpande et al. 2015, Subhuti 2000). Therefore, it can be inferred that *C. asiatica* and *B. pilosa* aqueous crude leaf extracts are safe when taken orally by pregnant animals according to OECD guideline number 425 (OECD 2022).

One of the sensitive indicators in determining toxicity following exposure to harmful chemicals is body weight loss (Vahalia et al. 2011). This study showed no statistically significant difference in body weight gain between the treatment groups and the negative control. Body weight gain is indicative of animals' healthy status (Tousson

et al. 2011). Additionally, there were no significant changes in the birth weights of newborns delivered between the treatment and the negative control groups. These findings indicate that both *C. asiatica* and *B. pilosa* aqueous crude leaf extracts are non-toxic to the foetus at the tested dose levels.

Blood profile provides critical information on how the body reacts to stress, injury and other variables (Tan et al. 2008). Blood parameters observed in this study (RBC count, HB, HCT, MCV, MCH and MCHC) were not significantly affected in the treatment groups as compared to the control group in the acute toxicity study. This is an indication that both aqueous crude extracts of *C. asiatica* and *B. pilosa* did not induce both microcytic and macrocytic anaemia in pregnant Wistar rats. Likewise, a study by Deshpande et al. (2015) demonstrated that administration of *C. asiatica* standardized leaf extract at 1000 and 2000 mg/kg to non-pregnant rats during acute toxicity did not significantly affect haematological parameters. Also Liang et al. (2020) reported that *B. pilosa* aqueous leaf extract administered to non pregnant mice up to 1g/kg did not change the haematological

values of treatment in comparison to the control groups during acute toxicity study.

The study found that while *C. asiatica* and *B. pilosa* aqueous crude leaf extracts did not cause tissue damage or inflammatory responses in pregnant Wistar rats at low doses, the highest acute dose resulted in tissue damage and a significant elevation of platelets count, possibly indicative of secondary thrombocytosis due to inflammatory responses (Koupenova et al. 2018).

Medical professionals are concerned that traditional remedies may affect important organs like the liver and kidneys (Ogbe et al. 2012). Biochemical markers assessed in the current study were AST and ALT. Interestingly, the mean values of AST and ALT at 1000 mg/kg were not altered. These findings are consistent with that of Deshpande et al. (2015) and Liang et al. (2020) where administration of *C. asiatica* and *B. pilosa* at 1000 mg/kg during acute toxicity did not significantly affect biochemical parameters including AST and ALT of the tested non-pregnant rats and mice, respectively. However, significant elevation of AST and ALT observed in this study, especially at dose levels of 2000 and 5000 mg/kg suggests that *C. asiatica* and *B. pilosa* aqueous crude leaf extracts are hepatotoxic when taken once at higher doses. Histopathological analyses offer data to support the conclusions of haematological and biochemical indicators. In the current study, the liver, kidney and gravid uterus sections of pregnant Wistar rats administered with the aqueous crude leaf extracts of *C. asiatica* and *B. pilosa* did not exhibit any damage upon gross pathological examination. However, microscopic examination revealed severe damage in the liver, kidney and gravid uterus during the administration of the high dose of 5000 mg/kg in an acute toxicity study. Compared to controls, some revealed enlarged hepatocytes and hepatocyte nuclei that were pushed to the periphery due to necrosis. This is consistent with the biochemical alterations of the liver damage marker particularly ALT found in this study.

The placenta is an organ in charge of shielding the developing embryo from xenobiotics throughout pregnancy. The labyrinth zone aids in exchanging materials between mother and foetus and is particularly susceptible to the target site in placental toxicity (Moreno et al. 2013). Toxic chemicals can affect any process in a way that causes placental malfunction, which can lead to miscarriage or foetal death (Furukawa et al. 2008). In the current study, the gap between the mother's placental labyrinth and foetus's yolk sac at 1000 mg/kg low dose group during acute toxicity was not affected in comparison to that of the control for both plant extracts. Studies backed the hypothesis that the labyrinth and fetal development are directly related (Perez-Garcia et al. 2018). However at acute test, higher doses of (2000 and 5000 mg/kg) there was the enlargement of the gap between the labyrinth and yolk sac, which means that some of the phytochemicals in these plants extracts were possibly toxic to the labyrinth zone, warranting further studies for their identification. The labyrinth zone is a hormone-dependent tissue, and toxins inhibitors cause the labyrinth zone to enlarge (Moreno et al. 2013).

However, the plant species assessed in this study were collected from only two regions in Tanzania, Kilimanjaro and Tanga which might affect its generalizability. In addition, the impact of seasonal variation on the use of these traditional medicines was not considered. Our research was constrained by the limited size and resources available, which impacted our ability to comprehensively identify the phytochemicals present in these plants. and evaluate the toxicity of the young pups, which have to be considered in future study.

Conclusion

The results of this study demonstrate that the administration of *C. asiatica* and *B. pilosa* in pregnant rats is safe at a low dose of 1000 mg/kg b.w when administered once but toxic at higher doses. These findings could be used to help set optimal doses of extract formulation for future preclinical and clinical

studies, and give insights to the current users. Scientific evidence on the safety of commonly used traditional medicines during pregnancy is crucial for informed traditional complementary and alternative medicine for improved maternal and neonatal outcomes.

Further studies can be conducted by assessing the safety of these plant species during pregnancy from other geographical regions in Tanzania including their phytochemical composition.

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Declaration of Interest

The authors declare that they have no competing or incompatible interests.

References

- Ahmed SM, Nordeng H, Sundby J, Aragaw YA, de Boer HJ 2018 The Use of Medicinal Plants by Pregnant Women in Africa: A systematic review. *J. Ethnopharmacol.* 224: 297-313
- AVMA 2013 Guidelines for the Euthanasia of Animals.
- Deshpande P, Mohan V, Thakurdesai T 2015 Pre-Clinical Safety Assessment of Standardized Extract of *Centella asiatica* Urban Leaves. *J. Toxicol. Int.* 22(1): 10-20.
- Fukunaga R, Morof D, Blanton C, Ruiz A, Maro G, Serbanesai S 2020 Factors Associated with Local Herb Use During Pregnancy and Labor Among Women in Kigoma Region, Tanzania, 2014-2016. *BMC Preg. Childbirth.* 20(1): 122.
- Furukawa S, Hayashi S, Usuda K, Abe M, Ogawa I 2008 Histopathological Effect of Ketoconazole on Rat Placenta. *J. Vet. Med. Sci./Jpn. Soc. Vet. Sci.* 70(11): 1179-1184.
- Hajj M and Holst L 2020 Herbal Medicine Use During Pregnancy, a review of the literature with a special focus on sub-Saharan Africa. *Front. Pharmacol.* 11: 866.
- Kazma S, Anker S, Allegaert K, Dallmann A, Ahmadzia H 2020 Anatomical and Physiological Alteration of Pregnancy. *J. Pharmacokinet. Pharmacodyn.* 47(4): 271-285.
- Koupenova M, Clancy L, Corkrey H, Freedman J 2018 Circulating Platelets as Mediators of Immunity, Inflammation and Thrombosis. *Circ Res.* 122: 337-51.
- Liang Y, Lin C, Yang C, Chen Y, Yang M, Chou F, Yang W, Chang C 2020 Toxicity Study of *Bidens pilosa* in Animals. *J. Tradit. Complement. Med.* 10: 150-157.
- Li X, Motwani M, Tong W, Bornmann W, Schwartz G 2000 Huanglian, A Chinese Herbal Extract Inhibits Cell Growth by Suppressing the Expression of Cydin B1 and Inhibiting CDC2 Kinase Activity in Human Cancer Cells. *Mol. Pharmacol.* 58: 1287-1293.
- Mawoza T, Nhachi C and Magwali T 2019 Prevalence of Traditional Medicine Use during Pregnancy, at Labour and for Postpartum Care in a Rural Area in Zimbabwe. *Clin. Mother Child Health.* 16(2): 321.
- Mills E, Dugoau J, Perri D, Koren G 2006 Herbal Medicine in Pregnancy and Lactation. An Evidence Based Approach. *CRC Press.* 1st Ed. London.
- Moreno R, Fussel K, Groters S, Schneider S, Strauss V, Stinchcombe S, Fegert I, Veras M, Van B 2013 Epoxiconazole-induced Degradation in Rat Placenta and the Effect of Estradiol Supplement. *Birth Defects Res B. Dev. Reprod. Toxicol.* 98: 208-221.
- Nalumansi P, Mugisha M, Anywar G 2017 Medicinal Plants used during Antenatal

- Care by Pregnant Women in Eastern Uganda. *Afr. J. Reprod. Heal.* 21(4): 33.
- Nasri H and Shirzad H 2013 Toxicity and safety of medicinal plants. *J. Herb Med. Pharmacol.* 2(2): 21-22.
- OECD 2022 Test Guideline Number 425: Acute Oral Toxicity: Up-and- Down Procedure for Testing of Chemicals 2022:17.
- Oktavia E, Hadisaputro S, Rahayu S 2023 The Effectiveness of Topical Gel Formulation Extract *Centella asiatica* and *Curcuma domestica* to Fading Striae Gravidarum. *Medisians.* 21(3): 64-68.
- Ogbe R, Yahaya A, Anefu E 2012 Aqueous Ethanolic Extract of *Mangifera indica* Stem Bark Effect on Biochemical and Haematological Parameters of Albino Rats. *Arch. App. Sci. Res.* 4: 1618-1622.
- Pariante G, Leibson T, Carls A, Adams-Webber T, Ito S, and Koren G 2016 Pregnancy Associated Changes in Pharmacokinetics; A Systematic Review. *PLoS Med.* 13(11): e1002160. <https://doi.org/10.1371/journal.pmed.1002160>.
- Perez-Garcia V, Fineberg E, Wilson R, Murray A, Mazzeo C, Tudor C, Sienerth A, White K, Tuck E, and Ryder J 2018 Placentation Defects Are Highly Prevalent in Embryonic Lethal Mouse Mutants. *Nature.* 555: 463–468.
- Sari D, Aswin S, Susilowati R 2014 Ethanol Extract of *Centella asiatica* Leaf Improve Memory Performance in Rats After Chronic Stress via Reducing Nitric Oxide and Increasing Brain-Derived Neurotrophic Factor (BDNF) Concentrations. *Int. J. Psychol* 1(1): 61-67.
- Subhuti D 2000 Bidens: A popular Remedy Escapes Notice of Western Practitioners. ITM, India.
- Tan P, Mezui V, Enow-Orock V, Njikam G, Dimo N, Bitolog P 2008 Teratogenic Effects, Acute and Sub-Chronic Toxicity of the Leaf Aqueous Extract of *Ocimum suave* Wild (Lamiaceae) in Rats. *J. Ethno pharmacol.* 115(2): 232–237.
- Tousson E, El-Moghazy M and El-Atrsh E 2011 The Possible Effect of Diet Containing *Nigella sativa* and *Thymus vulgaris* on Blood Parameters and Some Organs Structure in Rabbit. *Toxicol Ind Health.* 27(2): 107-116.
- Vahalia M, Thakur K, Nadkarni S and Sangle V 2011 Chronic Toxicity Study for TamraBhasma (A generic ayurvedic mineral formulation) in Laboratory Animals. *Res. Sci. Tech.* 3: 76-79.
- WHO 2019 Global Report on Traditional and Complementary Medicine. Global survey. Geneva.