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Articles

Toxicological Effects of Aqueous Leaf Extract of Bitter (*Vernonia Amygdalina*) on Liver Enzymes of Albino Rats

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Abstract

The research was conducted to ascertain the hepatotoxic potentials of the leaves extract of Vernonia amygdalina on liver enzymes of apparently healthy albino rats. A total of sixteen (16) albino rats were clustered into four (4) groups of four (4) rats each designated as group A-D, Group A served as control while groups B, C and D were treated with 200mg/kg, 300 mg/kg, and 400 mg/kg aqueous leaves of extract of *Vernonia amygdalina* respectively for a period of two weeks. The liver enzymes were determined using spectrophotometric method. The results revealed a significant ($P < 0.05$) decrease in the activity of serum ALP of the rats treated with 400 mg/kg body weight of *Vernonia amygdalina* extract when compared with control rats. In conclusion, acute oral administration of aqeous extract of *Vernonia amygdalina* was found to be relatively safe at a low dosage. However, the extract at the dose of 400 mg/kg brought about alterations in the serum ALP activity.

Keywords: Vernonia amygdalina, Hepatic, ALP, AST, ALT, Liver and Enzymes.

1. Introduction

Nigeria is blessed with an abundance of rich genomic resources of cultivated, semi-wild and wild species of crops being used as traditional vegetables and different types are consumed by various ethnic groups for different reasons (Billah, Kabir, 2015). Edible leaves from vegetable plants are eaten as supporting food or main dishes. They may be aromatic, bitter or tasteless (Jaca, Kambizi, 2011), but they are the cheapest and most accessible source of proteins, vitamins, minerals, essential amino acids (Billah, Kabir, 2015; Alara et al., 2018). Leaf vegetables are highly beneficial for maintenance of health and prevention of diseases. They contain valuable source of food ingredients that can be utilized to build up and improve the body successfully (Alara et al., 2018). They contain high carbohydrate vitamin and mineral contents (Ülger et al., 2018).

Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in tropical Africa. *Vernonia amygdalina* is commonly called bitter leaf in English because of its bitter taste (Oyeyemi et al., 2018). The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa. Africa common names includes Grawa (Amharic), Ewuro

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(Yoruba), Etidot (Ibibio), Onugbu (Igbo), Nyuna (Tiv), Oriwo (Edo), Labwori (Acholi), Olusia (Luo) and ndoleh (Cameroon) (Michael, Stanley, 2018).

Vernonia amygdalina is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDs, infusion of the plant is taken as needed (Michael, Stanley, 2018). The leaves have a sweet and bitter taste, they are sold fresh or dried, and it is a typical ingredient in egusi soup (Oyeyemi et al., 2018).

These leaves are exported from several Africa countries and can be purchased in grocery stores aiming to serve African clients. The roots *V. amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity (Ülger et al., 2018).

Medicinal plants used to treat illness are of considerable interest for ethnobotanical community (Chekole, 2017; Jima, Megersa, 2018). They are recognized to contain valuable medicinal properties in different parts of the plant. Various plants have shown varying degree of ethnobotanical medicines. Most of these plants have been claimed to possess medicinal properties but most claims are hearsay and few have received adequate medical or scientific evaluation (Olufunmilayo, 2017). Little toxicological (adverse effect) information exists concerning traditional medicinal plants (Ibisi et al., 2017). The study was designed to investigate the toxicological effects of Vernonia amygdalina with a view to providing valuable information which may lead to the development of alternative drugs and therapeutic strategies with little or no side effects.

2. Materials and methods

Plant Materials

The fresh leaf of *Vernonia amygdalina* was purchased from Muda Lawal market in Bauchi State, Nigeria and was taken to the Biological Science Department, Abubakar Tafawa Balewa University Bauchi.

Preparation of the Extract

The leaves were sorted out separately to obtain only fresh leaves and washed with distilled water without squeezing to remove debris and dust particle. They were air-dried and ground into coarse powder using pestle and mortar and sieved to fine powder. 150 g of the fine powder was extracted or cold macerated into 900 ml of distilled water for 24 hours and the macerated mixture was then filtered through muslin cloth. It was then filtered to obtain the *Vernonia amygdalina* and mixture aqueous extract through filter paper. The filtrated was concentrated in an electric oven at 50^{o}C until a semisolid residue dark solid extract was obtained.

Experimental Animals

Sixteen (16) white albino rats with weighed between 80-100 g were purchased from National Veterinary Research Institute (NVRI) Vom, Plateau state. The animals were placed in cages and fed appropriately at biological science department, Abubakar Tafawa Balewa University Bauchi.

Experimental Design

At the end of the seven days' acclimatization period, the animals were randomly assigned into four different groups of four rats each, designated as groups of A – D. Group A received water and feed only and serves as control, group B were administered orally with 200 mg/kg, group C were administered orally with 300 mg/kg and group D were administered orally with 400 mg/kg doses of the extract for the period of fourteen days. On the 15th day all the rats were sacrificed and blood sample collected.

Administration of the Extract

Administration of the extract was done via oral route with the aid of oral cannula and syringe. Animals received their doses once per day for the period of two weeks. They were observed daily for clinical signs of toxicity or pharmacological signs, throughout the period of study.

Collection of Blood

At the end of the two weeks of extract administration, the albino rats were slaughtered to obtain blood from jugular vein. The collected blood sample from each rat were allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Serum was obtained used for the assay of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP).

Blood Analysis

Hepatic analysis of the serum enzymes for ALT and AST was done by the method of Reitman and Frankel (1957), ALP was assayed according to the method of Rec (1972).

Estimation of Parameters

Aspartate Aminotransferase (AST) assayed using Colorimetric method of Reitman and Frankel, 1957.

Alanine Aminotransferase (ALT) assayed by Colorimetric method of Reitman and Frankel, 1957.

ALKALINE PHOSPHATASE (ALP) assayed by method of Rec, 1972.

3. Results and discussion

From the Table 1 below, the results revealed a significant ($P < 0.05$) decrease of serum ALP activity (97.06±0.65) was observed in the rats treated with 400 mg/kg body weight of the *Vernonia amygdalina* but no significant changes were observed at lower doses. The extract appears to have no significant effect ($P < 0.05$) on serum AST, ALT activity and the Albumin concentration when compared with control rats.

Table 1. Effect of aqueous leaf extract of *Vernonia amygdalina* on liver enzymes in normal albino rats

Grouping	Parameters Assayed		
	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Group A (Control)	5.6±0.15	2.8±0.31	106.26±8.51
Group B (200 mg/kg)	6.4±0.50	2.64±0.19	108.56±1.30
Group C (300 mg/kg)	6.0±0.47	2.7±0.07	104.88±2.25
Group D (400 mg/kg)	6.0±0.52	2.6±0.04	97.06±0.65*

Table 1 showed the effect of aqueous leaf extract of *Vernonia amygdalina* on liver enzymes in normal albino rats. The activity of AST was slightly increased to 6.4±0.50 in the rats treated with 200 mg/kg body weight of the extract but slightly decreased to 6.0±0.47 and 6.0±0.52 in the rats treated with 300 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (5.6±0.15) with no significant ($P > 0.05$) differences. However, the activity of ALT was slightly increased to 2.7±0.07 in the rats treated with 300 mg/kg body weight of the extracts but slightly decreased to 2.64±0.19 and 2.6±0.04 in the rats treated with 200 and 400 mg/kg body weight of the extracts respectively when compared with untreated group (2.8±0.31) with no significant ($P > 0.05$) difference. The result of ALP showed a significant ($P < 0.05$) decrease of serum ALP activity (97.06±0.65) was observed in the rats treated with 400 mg/kg body weight of the extracts but no significant ($P > 0.05$) increase 108.56±1.30 and 104.88±2.25 was observed in the rats treated with 200 and 300 mg/kg body weight of the extracts respectively when compared with untreated group (106.26±8.51).

4. Conclusion

Acute oral administration of the extracts was found to be relatively safe at a low dosage. However, at higher dose of 400 mg/kg the extract brought alteration in the serum ALP activity.

5. Recommendation

Further studies should be carried out by increasing the number of experimental animals, so that larger data could be obtained so as to reach a better conclusion. Biochemical parameters associated with liver function test such as bilirubin, albumin and total protein should also be analyzed so as to find out the detail hepatotoxic effect of *Vernonia amygdalina*.

Histological analysis of the liver of albino rat should also be conducted.

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Anti-Malarial Activity of the Ethanolic Extract of *Vernonia Amygdalina* (Bitter Leave) on PCV and Parasiteamia of Experimental Mice

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Abstract

Malaria is one of the most important infectious diseases in the tropics and sub-tropics. The search for antimalarial compounds has been necessitated by *P. falciparum* resistance to almost all antimalarial drugs. In this study, the in vivo antimalarial activities of ethanolic extracts of *Vernonia amygdalina,* a plant used by traditional healers to treat malaria and other diseases was carried out. Twenty-one (21) Albino mice were randomly divided into seven groups of three mice each (n = 3). Group 1, 2, and 3 were the experimental group, while group 4, 5, 6, and 7 were treated/untreated, normal, uninfected/treated and standard respectively. Groups 1, 2, and 3 were treated with dose of 200 mg/kg. bwt, 300 mg/kg. bwt and 400 mg/kg. bwt respectively. The average daily parasitaemia level of the *Plasmodium berghei* infected mice treated with 300 mg/kg. bwt of the ethanolic leaf extract of *Vernonia amgdalina*extract and 25 mg/kg. bwt of chloroquine significantly ($P < 0.05$) reduce when compared with negative control group. However, there is no significant ($P > 0.05$) different in the level of parasitaemia in 200 mg/kg. bwt and 400 mg/kg. bwt at the varying concentractions of the ethanolic extract's dosage, Therefore, this shows that *Vernonia amygdalina*has both curative and suppressive activities since at lower concentrations, the parasite was seen to reduce from the initial load before administration and at higher concentrations, the parasite was seen to reduce to the lowest level indicating that if treatment period is extended concentration is administered, the parasite will be cleared completely, thus, the curative activities of the *Vernonia amygdalina*leaf extract.

Keywords: Ethanolic, Parasitaemia, *Vernonia amygdalina*, PCV, *Plasmodium berghei,* extract, leaf, treatment, concentrations.

1. Introduction

Malaria is one of the most important tropical diseases and the greatest cause of hospitalization and death among children age 6 months to 5 years (Molta et al., 2006). The World Health Organization reported that there were an estimated 246 million malaria cases distributed among 3.3 billion people at risk in 2006, causing at least a million deaths. These were mostly children under five years. One hundred and nine countries were endemic in 2008 and 45 within the WHO African region (WHO, 2008). Approximately 80 % of malaria cases in the world are estimated to be in Africa where the disease is endemic (WHO, 2008). The disease is a major cause of the continent high infant mortality, killing 1 in every 20 children below 5 years of age. In Nigeria,

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malaria transmission occurs all-year round in the South, and is more seasonal in the North. The country accounts for a quarter of all malaria cases in the WHO African region (WHO, 2008). The problem is further compounded by the upsurge in the resistance strain of the parasite. Thus, the continuous search for novel and more effective anti-malarial compounds especially from medicinal plants extracts has been of utmost importance in view of the success of artemisinin, the active principle of an ancient Chinese herbal remedy for fevers (Osamor, Owumi, 2010).

In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria. *Vernonia amygdalina* Delile (VA), family Asteraceae or compositae are plants that are consumed locally as food and serve important ethno medicinal uses. Many parts of the plants are useful, they are used locally for the treatment of fever, stomach disorder, jaundice, worm infestation, constipation, malaria, hiccups, kidney problems, amoebic dysentery, schistosomiasis, cough, wounds, diabetes, laxative, veneral diseases and other bacterial and protozoa infection. *Vernonia amygdalina*, popularly known as bitter leaf, is an under shrub of variable height with petiolate green leaves of about 6 mm diameter. The leaves are usually bitter and are very popular soup vegetable in Nigeria (Ojiako, Nwanjo, 2006).

Malaria remains a major public health burden and resistance has emerged to every antimalarial on the market, including the frontline drug, artemisinin. Our limited understanding of Plasmodium biology hinders the elucidation of resistance mechanisms. In this regard, traditional method using plants like *Vernonia amygdalina* can facilitate the integration of existing experimental knowledge and further understanding of these mechanisms which will lead to finding cure to this parasitic disease which over the year as continue to develop resistant to existing drugs.

2. Materials and methods

Plant Identification and Authentication:

Fresh leave of *Vernonia amygdalina* were collected from Bukuru express in Gyel in 2019. It was then taken to University of Jos, Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria for identification and it was subsequently identified and given a Voucher number: JUHN20000304, it was subsequently air dried at room temperature for 20 days and pounded into its powdered form using mortar and pestle.

Ethical Clearance:

Ethical clearance was obtained with approval number UJ/FPS/F17- and white albino mice species which has susceptibility to *Plasmodium berghei* mosquito parasite were obtained from the University of Jos animal house.

Chemicals and Reagents Used:

Dragendorf's reagent, Ammonia solution, Acteone, lead acetate, Sodium Hydroxide, Sulphuric acid, Glacia Acetic acid, 1 % Hydrochloric acid, Distilled water, Ferric chloride, chloroform, normal saline, Giemsa stain, methanol.

The following equipment and reagents were used: Olympus microscope, hemocytometer with aspirating pipette, capillary tube, microhematocrit centrifuge, PCV reader, cover slip and slides, weighing balance, measuring cylinder, sample container, and magnetic stirrer, Centrifuge, hawksely micro-haematocrit reader, vacuum pump, porter and pestle, standard laboratory glass wares, heparinized tubes, Giemsa stain were purchased from Sigma-Andrich Chemical Company (St. Louis, USA). All other chemicals and reagents used for this study were of analytical grade.

Materials for Inoculation of Experimental Animals (White Albino Mice):

Razor blade, Butterfly needle, 2 ml syringe, Beaker, Cotton wool, Slides, Giemsa stain, and microscope.

Experimental design:

GROUP 1: 3 parasitized mice administered 200 mg/kg of extract per body weight per day.
GROUP 2: 3 parasitized mice administered 300 mg/kg of extract per body weight per day.
GROUP 3: 3 parasitized mice administered 400 mg/kg of extract per body weight per day.
GROUP 4: Infected/untreated.
GROUP 5: Neural.
GROUP 6: uninfected/ treated
GROUP 7: 3 parasitized mice administered 25 mg/kg of chloroquine per body weight per day
GROUP F: 3 uninfected and untreated mice.
Mode of administration; oral administration.

Experimental animal:

Male and female albino mice (weighing about 15.2- 30.2) were used for this experiment. They were obtained from animal farm, University of Jos, and were fed with standard commercial feed (vital feed, top feed).

Phytochemical analysis of the plant extract:

The ethanolic extract of *Vernonia amygdalina*was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as: Saponins, tannins, alkaloids, flavonoids, steroids and terpenes, cardiac glycosides, balsam, carbohydrates, phenols and resins according to the method of Harborne (1984) with slight modification.

Test for alkaloids: To 2 mls of extract, few drops of dragendorf's reagent was added to give an orange colouration which indicated the presence of alkaloids.

Test for flavonoids: To 2 mls of the extract, few drops of 5 % lead acetate was added to give a cream light colour which indicated the presence of flavonoids.

Test for tannins: To 2 mls of the extract, few drops of 10 % ferric chloride were added to give a deep bluish or greenish colour which indicated the presence of tannins.

Test for Saponins: To 1 ml of the extract, 4 mls of distilled water was added and shaken vigorously. Formation of froth indicated the presence of Saponins.

Test for terpens and steroids: To 1 ml of the extract, 2 mls of concentrated Sulphuric acid was added along-side of the test tube. Formation of reddish brown ring at the interphase indicated the presence of terpens and steroids.

Test for cardiac glycosides (Salkowski's test): 2 mls of the extract was dissolved in 2 mls of chloroform and Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase indicated the presence of cardiac glycosides.

Test carbohydrates: 5 drops of the extract were added to 2.0 mls of Benedict's reagent, placed on a hot plate for 5 minutes and was observed for the formation of brick red precipitation which indicated the presence of carbohydrates.

Test for phenol: To 2 mls of the extract, 2 mls of ferric chloride was added and observed for the formation of a deep bluish-green colouration which indicated the presence of phenol.

Test for resins: To 2 mls of the extract, 2 mls of acetic anhydride was added and drops of concentrated Sulphuric acid were added to observe for a violet colour which indicated the presence of resins.

Preparation of ethanolic extract of *vernoniaamygdalina:*

Fresh leaves of *V. amygdalina* were cut into pieces and air dried inthe laboratory. The dried pieces were pulverized using a laboratory grinder. 80 g of the dried powderedform of the plant materials were extracted with ethanol using soxlet apparatus for 72 hours. All theextracts were concentrated to dryness on a water bath and weighed. The extracts were then stored in well-closed containersand kept at room temperature to protect from light and moisture till used (Sutharson et al., 2007).

Acute Oral Toxicity Test:

Acute oral toxicity test of the solvent fractions was performed on randomly selected 3 non-infected female mice following the Organization for Economic Corporation and Development (OECD) guideline. The mice were fasted overnight and weighed before the test. A loading dose of 2000 mg/kg b. wt and 5000 mg/kg b. wt of the extract was administered to single mouse with oral gavage. Then, any sign of over toxicity and/ or mortality were observed for 24 hours with special emphasis to the first 4 hours. As no death or over toxicity was observed within 24 hours, and followed for 14 days to assess delayed toxicity of the solvent fractions. The mice were observed for any potential signs of acute toxicity such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation and/or mortality (OECD, 2001).

Mortality:

The mortality rate of infected mice was monitored during the period of the experiment according to the number of mice that died (Oche et al., 2016).

Determination of MST:

Survival time was recorded to observe the effect of the extract of *Vernonia amygdalina* on the survival of *P. berghei* infected mice. The mice were fed ad libitum and observed for about 29 days. Any death that occurred during this period was noted for each mouse in the treatment and control groups to determine the MST (Bantish et al., 2014).

MST= Sum of survival time for all mice in group (in days)

Total numbers of mice in that group

Body weight:

The body weight of each mouse was taken using a weighing balance before inoculation of parasite and after treatment (Bhat, Surolia, 2001).

Blood sample collection for *plasmodium bergheiparasiteamia* screening:

After 72 hr of infection, blood samples were collected from the infected mice to check for the presence of the parasites. Pricking the tip of the tails of the mice cause blood to flow which was collected on slides and smeared to make a thin blood film and dried under the shade for about 10 minutes and was fixed with methanol for 5 minutes. After the methanol dried completely, the blood was stained with Giemsa stain for 45 minutes and rinsed with water which was taken for microscopic examination after drying.

Immersion oil was added to the stained slides and each of the slides were viewed under the X100 objective lens. The *Plasmodium berghei* were seen in the infected blood as spherical curved or cluttered images in the stained samples. Parasitaemia count was done on Giemsa positive bodies which represents the parasitized red blood cells. Parasitaemia count was done and recorded 72 hours after infection and daily throughout the period of administration (Oche et al., 2016).

Parasite Inoculation:

Chloroquine-sensitive *P. berghei* (ANKA strain) obtained from animal house in the University of Jos maintained by subsequent passage of blood frominfected mouse to a healthy one every 5 days was used for the experiment. The anti-Plasmodial activity of *Vernonia amygdalina* was assessed. 21 male and female mice were randomly divided into 7 groups of three mice in each. Blood from a donor mouse was used to infect test mice. *Plasmodium berghei* parasitized erythrocytes were obtained from the tail of the donor mice and were diluted with 0.9 % normal saline. Mice were inoculated intraperitoneally with 0.5 mL blood suspension on day 0 and were monitored for manifestation of parasitaemia for 72 hour without treatment. The mice were randomly divided into 6 groups of three (3) mice per group and treated for 5 consecutive days with daily doses of the extracts (200, 300 and 400 mg/kg per body weight) and standard antimalarial drug (Chloroquine, 25 mg/kg per body weight) by oral route (Challand, Willcox, 2009).

Parasitaemia Count:

Parasitaemia count was carried out on group 1, 2, 3, 4, and 7 daily using light microscope. Blood was collected from the tail of the mice and dropped on a clean grease free microscopic slide. A cover-slip was used to make a thin film and allowed to air dry. After drying, the film was fixed with a methanol for five minutes, air-dried and stained with Giemsa stain for 45 minutes, and left to air-dry. After drying, drop of immersion oil was placed on the film, the microscope was connected to a light source and parasitaemia count was done using X100 (oil immersion) objective Lens (Bhat, Surolia, 2001).

% parasite = Total number of parasitize cells ×10

Total RBCs number

Packed cell volume (PCV):

Blood samples were collected from the infected mice to check the effect of the extract *Vernonia amygdalina* on the blood level of *P. berghei* infected mice. Pricking the tip of the tails of the mouse causes blood to flow which was collected on heparinized capillary tube. The tube was sealed at one end with sealant and centrifuged in a haematocrit centrifuge for 5 minutes at 10000 RPM. At the end of centrifugation, the height of the packed red cells is recorded as a percentage of the total blood cell and plasma column. This was done using a haematocrit reader.

Unit of measurement; it is expressed as % of the blood (SI unit are L/L). The conversion formula to SI unit is as follows

% ÷ 100=L/L

Packed cell volume (PCV), n- The measure of the ration of the volume occupied by red blood cells to the volume of the whole blood, expressed as fraction. Note; the term “haematocrit” has been, and is often, used for this quantity (Baird, 2013).

PCV = Measure of the ration of the volume occupied by red blood cells × %

Volume of the whole blood

3. Results

Acute oral toxicity of extract:

The extract of the leaves of *V. amygdalina* did not cause mortality in mice at the level of 2000 mg/kg b. wt and 5000 mg/kg b. wt. The extract and its fractions did not induce any sign of over toxicity such as loss of appetite, hair erection, lacrimation, tremors, convulsions and salivation during the 14 days of observation. Based on the acute toxicity study, the LD50 of extract of the leaves of *V. Amygdalina* were found to be greater than 5000 mg/kg b. wt, indicating their wide safety margin. The present result is in line with the finding of Adiukwu, Amon, Nambatya (2012). Who reported that *V. Amygdalina* caused no clinical signs of toxicity at doses between 2000 and 5000 mg/kg b. wt /day for 14 consecutive days and that of Anoka et al. (2013) who reported the absence of signs of over toxicity or adverse toxicological effects at all tested dose also found no toxic effect of extracts of *V. Amygdalina in vivo* on rats. Generally, if LD50 value of the test chemical is more than three times the minimum effective dose, the substance is considered to be a good candidate for further studies in vivo assays. The LD50 has also been used for classification of chemicals. Based on WHO hazard classification system, the extract of the leaves *V. amygdalina*, to which the LD50 was greater than 5000 mg/kg b. wt, are designated as "unlikely to be hazardous." Therefore, the extract of the plant is considered to be safe at the tested doses. The aforementioned descriptive toxicological studies also support the finding (OECD, 2001).

Differences in body weight:

Effects of ethanolic leaf extract of *Vernonia amygdalina* on the body weight of albino mice infected with *Plasmodium berghei* were shown in Figure 1. The weight of the groups treated with 200 mg/kg b. wt extract, 300 mg/kg b. wt extract, 400 mg/kg b. wt, and infected/untreated reduced by 9.3 g, 4.5 g, 7.7 g and 6.0 g and 10.5 g respectively. Whereas the weight of the groups treated with 25mg/kg bwt extract and uninfected and untreated increased by 6.1 g and 3 g respectively.

Packed cell volume:

Effects of ethanol leaf extract of *Vernonia amygdalina* on packed cell volume of *Plasmodium berghei* infected mice were shown in Figure 2. The percentage packed cell volume of the groups treated with 200 mg/kg b. wt extract, 300 mg/kg b. wt extract, 400 mg/kg b. wt extract 30 %, 19 %, 11 % and untreated reduce by 15 %, on day 11, while 25 mg/kg b. Wt chloroquine increase by 8 % and neutral increase by 3 % and also treated/uninfected increase by 2 %.

Parasitaemia count:

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with ethanolic leaf extract of are shown in Figure 3. The average daily parasitaemia of infected mice treated with 300 mg/kg b. wt of ethanolic leaf extract of *Vernonia amygdalina* extract and 25 mg/kg b. wt of chloroquine significantly ($P < 0.05$) reduced when compared with negative control group. However, there is no significant ($P > 0.05$) difference in the level of parasitaemia in 200 mg/kg b. wt and 400 mg/kg b. Wt.

Mortality rate post infection:

The death rate of the experimental albino mice was monitored daily after the inoculation of *Plasmodium berghei*. Groups 1, 2, 3, and 4 lived up to day 19, 24, 22, and 15 respectively meanwhile groups 5, 6, 7 lived beyond the experimental period as shown in Table 2.

Table 1. Phytochemical profile of the ethanolic leaf extract of *Vernonia amygdalina:*

PHYTOCHEMICAL	RESULT
Alkaloid	+
Flavonoids	+
Tanins	+
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	+
Carbohydrate	-

Phenol	+
Resins	+

KEY:- = absent + = present

Table 2. Mode of survival time (mst)

GROUPS	SURVIVAL RATES
200 mg/kg b. wt	19 days
300 mg/k b. wt	24 days
400 mg/kg b. wt	22 days
Infected	15 days
Uninfected	Lived beyond experimental period
Uninfected/treated	Lived beyond experimental period
25 mg/kg b. wt of chloroquine	Lived beyond experimental period

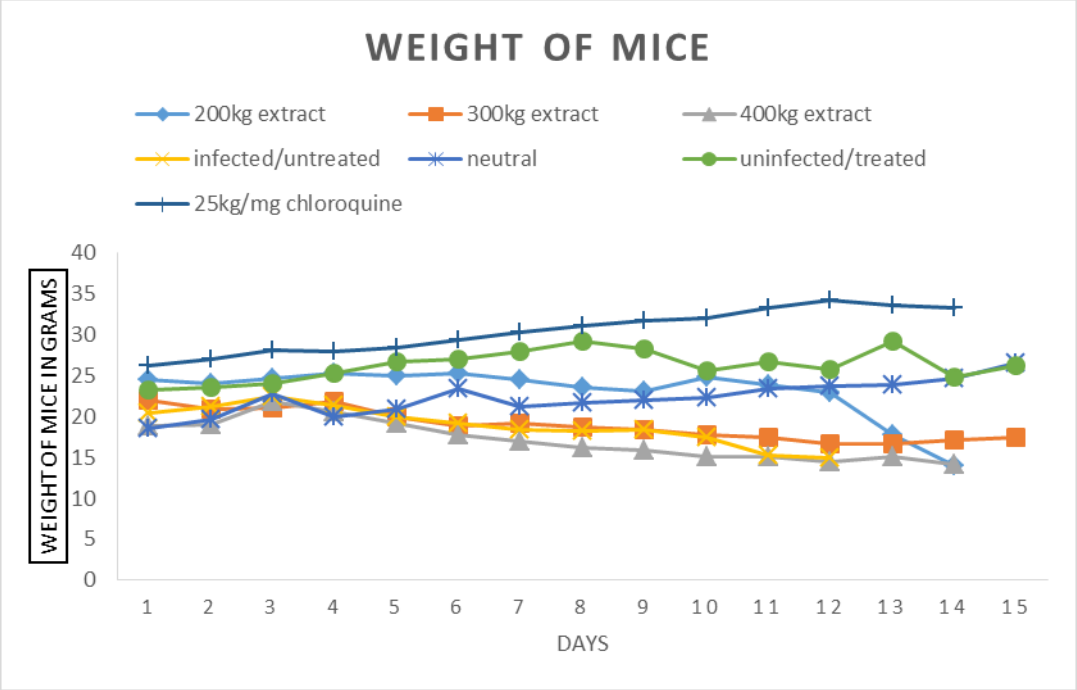

Fig. 1. The average mean of weight of mice

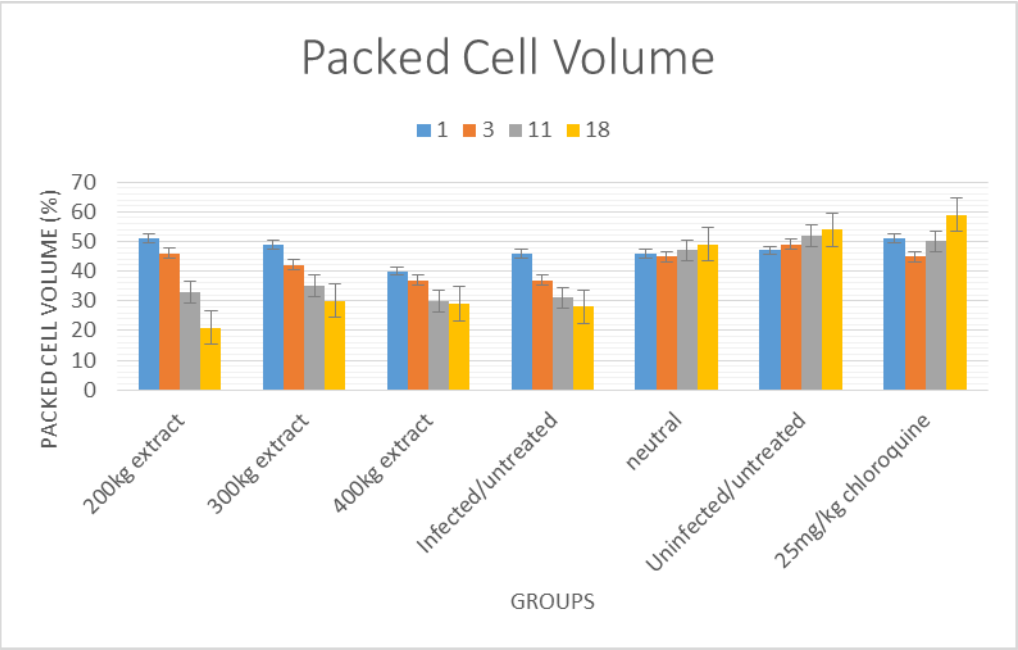

Fig. 2. Effect of the extract of *Vernonia Amygdalina* on packed cell volume of experimental mice

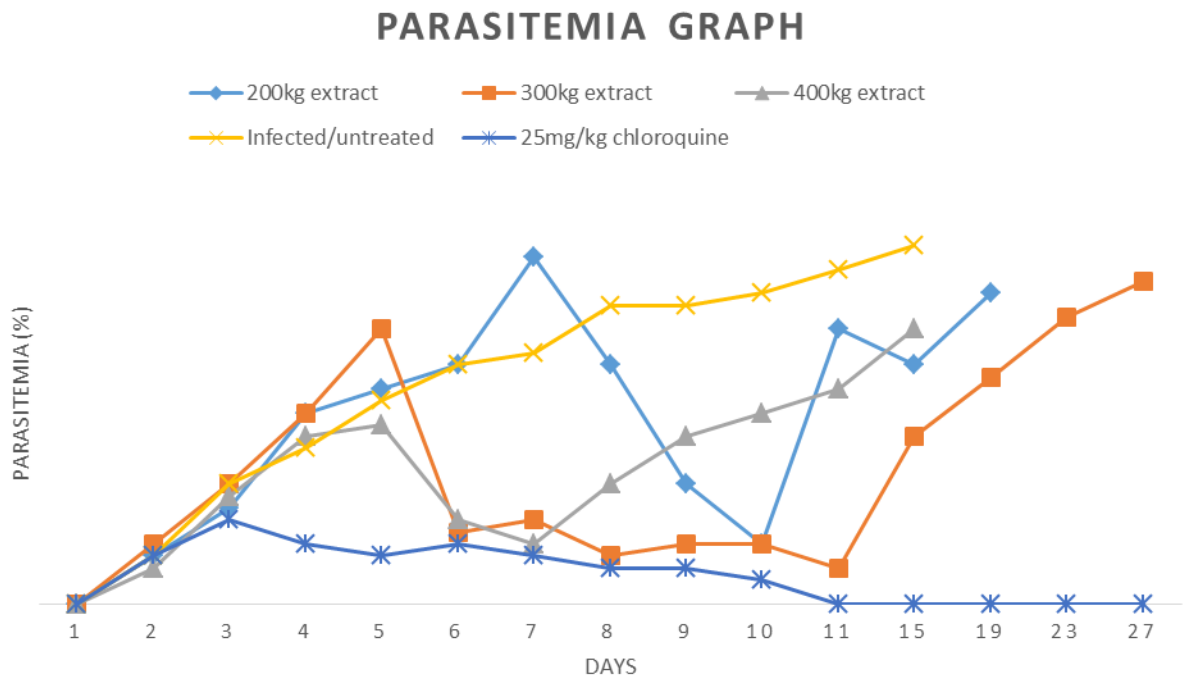

Fig. 3. The effect of the administration of the aqueous extract of *Vernonia Amygdalina* on the parasiteamia of experimental mice

4. Conclusion

Man in solving its numerous medical challenges have for ages depend on his immediate environment taking advantages of nature's provisions of its beauty for life and survival. They have learnt to depend on plants and in some cases animals in providing solutions to the myriad of their health problems (Oliver, 1960). However, the increasing use of plants for the therapeutic and medicinal use warrants an adequate scientific investigation to confirm the suitability of plants or otherwise for the purpose for which they are used. Hence, the purpose of this research which was to

investigate the *in vivo* anti-Plasmodial activity of the ethanolic leaf extract of *Vernonia amygdalina* in experimental mice. The ethanolic leaf extract of *Vernonia amygdalina* was extracted using absolute ethanol and the extract was further subjected to *in vivo* anti-Plasmodial studies compared to chloroquine, a standard anti-malaria drug which were all found to possess dose dependent anti-Plasmodial activities against the *Plasmodium berghei* species of the malaria parasite in experimental mice. The various dosage concentration of 200 mg/kg. bwt, 300 mg/kg. bwt and 400 mg/kg per body weight all showed curative properties of the ethanolic leaf extract of *Vernoniaamygdalina*and chloroquine in varying proportions. However, the 25 mg/kg b. wt of chloroquine and the 300 mg/kg b. wt dosage concentration of the ethanolic leaf extract of *Vernonia amygdalina* showed more curative activities compared to the dosage concentration of 200 mg/kg b. wt and 400 mg/kg per body weight. Anaemia, body weight loss and body temperature reduction are the general features of malaria infected mice. So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice (Bantish et al., 2014). This research study presented that the 200 mg/kg b. wt of the ethanolic leaf extract of *Vernonia amygdalina* significantly prevented weight loss associated with increase in parasitemia level the phytochemical screening of the ethanolic leaf extract of *Vernonia amygdalina* showed that the leaf contains useful phytochemicals which contributed to its anti-plasmodial activities in experimental mice. The presence of alkaloids, flavonoids, tannins,terpenes and steroids, phenol, resins, and cardiac glycosides in the ethanolic extract attributes terpenes and steroids, phenol, resins, and cardiac glycosides in the ethanolic extract attributes this anti-plasmodial activities of the ethanolic leaf extract of *Vernonia amygdalina*. Flavonoids have been reported to have exhibited significant in vitro antimalarial activity against *P. falciparum*. This could justify the antimalarial activities exhibited by the plant extract since flavonoids was found to be present in the results of the phytochemical screening.

At the varying concentrations of the ethanolic extract's dosage, the leaf of *Vernonia amygdalina* showed varying degrees of treatment of the malaria parasite. Therefore, this shows that *Vernonia amygdalina* has both curative and suppressive activities since at lower concentrations, the parasite load was seen to reduce from the initial load before administration and at higher concentrations, the parasite was seen to reduce to the lowest level indicating that if treatment period is extended concentration is administered, the parasite will be cleared completely, thus, the curative activities of the *Vernonia amygdalina* leaf extract.

This study shows that the presence of alkaloids, flavonoids, tannins, terpenes and steroids, balsam, phenol, resins, and cardiac glycosides in the ethanolic leaf extract of *Vernonia amygdalina* has been shown to possess anti-plasmodial activities which can be developed into Anti-Malarial Combination Therapy (ACT) could help in dealing with the malaria cases. The result of this study also verifies the folk use of *Vernonia amygdalina* leaf for the treatment of malaria, and other infectious diseases that affects the health of people leaving in the tropical areas. Even though the study was carried out using the ethanolic leaf extract of *Vernonia amygdalina* on *Plasmodium berghei* species, this has given new insight into developing anti-malarial drugs that have high efficacy against the resistant strain of *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malariae* that affect humans.

5. Recommendations

Since the ethanolic leaf extract of *Vernonia amygdalina* showed high levels of anti-plasmodial activities, further work on *Vernonia amygdalina* is required on this promising plant since most of the folks in the rural areas have put this plant into use for several years. Development of an appropriate anti-malarial drug using this plant will also help in treatment of malaria since the present Anti-Malarial Combination Therapy are faced with the problem of resistance of the malaria parasites due to constant exposure and subsequent mutation of the mosquito parasite to adapt to the active compounds present in the malaria drugs and hence its resistance to the drug.

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Some Diuretic Plants in Traditional Medicine of Turkey (A Review)

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Abstract

Ethnobotanic is a branch of botanic science that examines human-plant relationships, and it is one of the sciences that deals with the purposes of which people use plants and has attracted more attention in recent years. Recording of ethnobotanical information; it is important that this information is not lost in time, such as those concerned, relevant disciplines and national economy and cultural wealth. This work is a small review research some plants used as diuretic in Turkey. Besides sentetic diuretic drug used in Turkey, herbal methods still remain to be used. Because of some side effects of drugs, herbal medicines used commonly in Turkey and in the world.

The fact that 75 % of natural compounds of herbal origin used in the treatment were discovered as a result of researching traditional folk remedies. For this reason traditional medicine apllications, researches and issues remains up-to-date as always. In this study, some plants (186 taxa) which were used as diuretic in traditional medicine of Turkey to contribute related studies. The main families according to the number of taxa are as follows; Asteraceae 21 taxa, Fabaceae 19 taxa, Rosaceae 18 taxa, Lamiaceae 13 taxa and others. In addition 42 plant taxa were detected from Çelikhan (Adıyaman-Turkey) provinces which have potential diuretic property.

Keywords: diuretic plants, ethnobotany, Turkey, traditional medicine.

1. Introduction

Ethnobotanic can be described as examining human-plant relationships or “the way local people use plants to meet their needs”. Ethnobotany has actually started with human history, since the beginning of human history, plants have been made by people for food, medicine, various tools and equipment, paint, fuel, substance has been used for different purposes such as feed (Ghorbani, 2004). These important ethnobotanic informations unfortunately has been lost, because it has not been recorded in time and correctly. It is important to record this information, which takes centuries and is very valuable to be obtained and to transfer it to the next generations, in terms of relevance of the information, related disciplines, national economy and cultural wealth. Japan there is more demand for herbal preparations as official drug. Humans use many plant taxa in food and medicine; these plant species contribute significantly to food and health, especially in developing countries. It is also estimated that the traditional and modern medicine uses more than 50000 plant taxa. The availability of natural resources threatens the revenue from the wild harvest, health and welfare of the people who depend on them (Macía, 2005). By definition, diuretic drugs are bring about an increase in urinary volume as well as in the electrolyte output. Due to this they are used to regulate both volume and

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composition of the milieu interiéur in different affections like high blood pressure, heart failure, nephrotic syndromes among other indications.

Herbal medicines are currently in demand and their popularity is rising day by day. The use of natural medicinal and aromatic plants is becoming popular due to toxicity and adverse effects of allopathic drugs. This led to sudden increase in the number of herbal drug manufactures (Verma, Singh, 2008). In modern day to day practice diuretics can be used as a first line therapy in hypertensive patients. Herbal medicines are in great demand in the developed as well as in the developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs. However, the number of studies about diüretic plants are limited and we recommend that further studies to be conducted to confirm reported activities. Such evidence is needed to provide scientific confidence to development of future medicines and treatments and treatment guidelines.

This review research is about some plant taxa used as diuretics in Turkey ethnobotany and some potential diuretic plants of Çelikhan (Adıyaman-Turkey) provinces. This study is expected to contribute to the literature on the subject.

2. Materials and methods

In this study, the literature and the plant species determine around Çelikhan (Adıyaman) were used. The family name, scientific name, local name, used part and usage patterns of the detected plants are presented in Table 1. Within the scope of this study, 186 taxa were determined from the literature. When plant taxa are systematically evaluated; Asteraceae 21 taxa, Fabaceae 19 taxa, Rosaceae 18 taxa, and Lamiaceae 13 taxa were the first rank of the determined plants on family basis. The plants in general; fruits, aboveground parts, leaves, seeds and other parts are used. The general use of plants is in the form of infusion and decoction. In addition as a result of field studies from Çelikhan (Adıyaman-Turkey) provinces in the 2019–2020 years 42 plant taxa (have potential diuretic effects) were collected from their habitat, dried herbarium technics, identified and being preserved in Adıyaman University, Pharmacy Faculty herbarium.

Table 1. Plants used as diüretic in Turkey ethnobotany

Family	Botanical name	Local name	Part used	Usage
Pinaceae	*Juniperus oxycedrus*	Ardıç	Fruits	Fruits are drunk like water decoction tea (Köse et al., 2005)
Urticaceae	*Urtica dioica*	Isırgan	Seeds and Fresh leaves	Tea is made (Koçyiğit, Özhatay, 2006)
Asparagaceae	*Asparagus officinalis*	Kuşkonmaz	Root	Decoction is used (Kara, 2006)
Fabaceae	*Glycyrrhiza glabra*	Meyan	Root	Decoction is used (Kara, 2006)
Brassicaceae	*Armoracia rusticana*	Bayır turpu	Leaves and roots	The roots are boiled, the leaves are boiled (Kara, 2006)
Malvaceae	*Malva neglecta*	Ebegümeci	Herba	Boil and drink water (Elçi et al., 2006)
Poaceae	*Zea mays*	Mısır	Styluses	Used in decoction (Tuzlacı, Tolon., 2001)
Equisatecaea	*Equisetum telmateia*	Kırkkilit otu	Herba	Used in decoction (Tuzlacı, Tolon., 2001)
Brassicaceae	*Raphanus sativus*	Kara turp	Root	It is ground and eaten raw (Pirenoi et al., 2005)
Cucurbitaceae	*Cucurbita maxima*	Kabak	Fresh fruits	Cooked. Externally rubbed into the navel (Pirenoi et al., 2005)
Poaceae	*Hordeum vulgare*	Arpa	Seeds	Decoction (Pirenoi et al., 2005)
Rosaceae	*Prunus armeniaca*	Kayısı	Dried fruits	Decoction (Pirenoi et al., 2005)
Caprifoliaceae	*Sambucus nigra*	Kara mürver	Flowers, leaves and roots	Decoction (Koçyiğit, Özhatay, 2006)
Caprifoliaceae	*Sambucus ebulus*	Mürver	Flowers, leaves and roots	Decoction (Ecevit and Özhatay, 2006)
Poaceae	*Cynodon dactylon*	Ayrık otu	Herba	Decoction (Koçyiğit, Özhatay, 2006)

Cucurbitaceae	*Ecballium elaterium*	Acıkelek	Fruits	It is used by squeezing fruit juice (Koçyiğit, Özhatay, 2006)
Equisetaceae	*Equisetum ramosissimum*	Kilitotu	Herba	Decoction (Koçyiğit, Özhatay, 2006)
Lamiaceae	*Mentha longifolia*	Eşek nanesi	Herba	Infusion (Koçyiğit, Özhatay, 2006)
Rhamnaceae	*Paliurus spina-chiristi*	Karaçalı	Fruits and roots	Decoction (Koçyiğit, Özhatay, 2006)
Platanaceae	*Platanus orientalis*	Çınar	Fruits	Infusion (Koçyiğit, Özhatay, 2006)
Rosaceae	*Sorbus aucuparia*	Üvez	Fruits	Fruits are eaten (Koçyiğit, Özhatay, 2006)
Boraginaceae	*Anchusa pusilla*	Sığırdili	Roots	Decoction (Sezik et al., 1992)
Rosaceae	*Mespilus germanica*	Muşmula	Leaves and fruits	Leaves decoction fruits are eaten (Sezik et al., 1992)
Rosaceae	*Origanum vulgare*	Mercanköşk	Leaf	Infusion (Sezik et al., 1992)
Rosaceae	*Prunus avivum*	Kiraz çöpü	Fruit stalks	Decoction (Fujita et al., 1995)
Brassicaceae	*Cardaria draba*	Diğnik	Flowers	Infusion (Tuzlacı, Erol, 1999)
Rosaceae	*Cerasus avium*	Kiraz	Fruit stalks	Decoction (Tuzlacı, Erol, 1999)
Asteracea	*Doronicum orientale*	Doğu kaplanotu	Leaf	Decoction(Tuzlacı, Erol, 1999)
Rosaceae	*Rubus sanctus*	Böğürtlen	Fruits	Eaten (Tuzlacı, Erol, 1999)
Poaceae	*Sorghum halepense*	Kanyaşı	Rhizomes	Decoction or infusion (Tuzlacı, Erol, 1999)
Aspleniaceae	*Asplenium adiantum-nigrum*	Karabacak	Leaf and herba	Decoction (Tuzlacı, Eryaşar, 2001)
Ericaceae	*Erica arborea*	Funda	Flower brackets	Decoction (Tuzlacı, Eryaşar, 2001)
Apiaceae	*Petroselinum crispum*	Maydonoz	Herba	Decoction (Gozüm, Ünsal, 2004)
Rosaceae	*Cerasus vulgaris*	Vişne	Cherry stems	Fruit is eaten, syrup and jam are made (Bağcı et al., 2006)
Rosaceae	*Rubus canescens*	Böğürtlen	Root and leaves	The root part is boiled. The leaves are crushed and porridge is made (Bağcı et al., 2006)
Apiaceae	*Foeniculum vulgare*	Rezene	Seeds, leaves and roots	Seed is popped. Leaf and root are boiled (Bağcı et al., 2006)
Caryophyllaceae	*Vaccaria pyramidata*	İnek sabun otu	Roots are used	Drink two glasses a day in decoction (6 %) (Çakılcıoğlu et al., 2007)
Anacardiaceae	*Pistacia terebinthus* subsp. *palaestina*	Menengiç	Dried ripe fruits	Coffee is made or eaten (Çakılcıoğlu et al., 2007)
Rosaceae	*Crataegus monogyna* subsp. *monogyna*	Alıç	Flowers	Tea is made from flowers (Çakılcıoğlu et al., 2007)
Convolvulaceae	*Convolvulus arvensis*	Tarla sarmaşığı	Flowers	Infusion (Kültür, 2007)
Lamiaceae	*Teucrium chamaedrys* subsp. *chamaedrys*	Kısacık mahmut	Leaves	Decoction (Kültür, 2007)
Fabaceae	*Ononis spinosa*	Kayışkıran	Roots	Decoction (Kültür, 2007)
Zygophyllaceae	*Tribulus terrestris*	Çoban çökerten, Demir hindi	Flowers	Decoction (Kültür, 2007)
Ericaceae	*Calluna vulgaris*	Süpürge otu	Leaves and flowers	Decoction (Yeşilada, 2008)
Geraniaceae	*Geranium pratense*	Çayır Turnagagası	Flowers	Infusion (Yeşilada, 2008)
Asteraceae	*Achillea millefolium* subsp. *millefolium*	Civan perçemi	Flowers and leaves	Infusion (Ugulu et al., 2009)

Asteraceae	*Cichorium intybus*	Hindiba	Its roots and flowers	Decoction (Ugulu et al., 2009)
Asteraceae	*Scolymus hispanicus*	Şevketibostan	Seeds	Decoction (Ugulu et al., 2009)
Asteraceae	*Tussilago farfara*	Öksürük otu	Leaf	Decoction (Ugulu et al., 2009)
Boraginaceae	*Borago officinalis*	Hodan	Flowers and leaves	Infusion (Ugulu et al., 2009)
Brassicaceae	*Lepidium sativum*	Tere	Leaves	Infusion (Ugulu et al., 2009)
Caryophyllaceae	*Saponaria officinalis*	Sabun otu	Leaves	Decoction (Ugulu et al., 2009)
Fabaceae	*Lupinus angistifolius*	Yahudi baklası	Fruits	Fruits are consumed by boiling in water (Ugulu et al., 2009)
Fabaceae	*Spartium junceum*	Katırtırnağı	Leaves	Decoction (Ugulu et al., 2009)
Lamiaceae	*Lamium album*	Ballıbaba	Leaves	Decoction (Ugulu et al., 2009)
Lamiaceae	*Thymus vulgaris*	Kekik	Flowers and leaves	Essential oil is used (Ugulu et al., 2009)
Rosaceae	*Rubus idaeus*	Ağaç çileği, Ahududu	Fresh fruits	Fresh fruits are eaten (Ugulu et al., 2009)
Ulmaceae	*Celtis australis*	Çitlembik	Leaves	Decoction (Ugulu et al., 2009)
Violaceae	*Viola tricolor*	Hercai meneke	Flowers	Infusion (Ugulu et al., 2009)
Rosaceae	*Pyrus elaeagnifolia* subsp. *elaeagnifolia*	Yaban armudu	Fruits	Infusion (Çakılcıoglu, Türkoğlu., 2009)
Primulaceae	*Cylamen coum*	Siklamen	Tubers	Decoction (Yaldız et al., 2010)
Thymelaeaceae	*Daphne pontica*	Karadeniz defnesi	Leaves	Decoction (Yaldız et al., 2010)
Asteraceae	*Achillea aleppica* subsp. *aleppica*	Yılan çiçeği	Flowers and leaves	Infusion and decoction (Çakilcioglu, Türkoğlu, 2010)
Asteraceae	*Bellis perennis*	Koyungözü papatya	Flowers	Decoction (Çakilcioglu, Türkoğlu, 2010)
Asteraceae	*Scorzonera semicana*	Yemlik	Flowers and leaves	Infusion and decoction (Çakilcioglu, Türkoğlu, 2010)
Boraginaceae	*Echium italicum*	Engerek otu	Flowers and leaves	Decoction (Çakilcioglu, Türkoğlu, 2010)
Asteraceae	*Achillea biebersteinii*	Ormaderen	Capitulate	Decoction (Altundağ, Öztürk, 2011)
Rosaceae	*Agrimonia eupatoria*	Koyunotu	Roots	Decoction (Altundağ, Öztürk, 2011)
Lamiaceae	*Ajuga chamaepitys*	Mayasıl otu	Above ground	Decoction (Altundağ, Öztürk, 2011)
Malvaceae	*Alcea setosa*	Hatmi	Leaves	Infusion (Altundağ, Öztürk, 2011)
Rosaceae	*Alchemilla pseudocartalinica*	Yıldıznişanı	Leaves	Infusion (Altundağ, Öztürk, 2011)
Resedaceae	*Reseda lutea* var. *lutea*	Muhabbet çiçeği	Roots	Decoction (Çakilcioglu, Türkoğlu, 2010)
Rosaceae	*Agrimonia eupatoria*	Koyun otu	Leaves and fiowers	Infusion (Çakilcioglu, Türkoğlu, 2010)
Rubiacea	*Galium aparine*	Yapışkan otu	Leaves and flowers	Decoction (Çakilcioglu, Türkoğlu, 2010)
Rosaceae	*Pyrus elaeagnifolia* subsp. *elaeagnifolia*	Yaban armudu	Fruits	Infusion (Çakilcioglu, Türkoğlu, 2010)
Poaceae	*Arundo donax*	Kargı	Rhizome	Decoction (Gücel and Arundo, 2010)
Brassicaceae	*Capsella bursa-pastoris*	Çoban çantası	Flowers and leaves	The juice of the leaf is squeezed and drunk (Toksoy et al., 2010)
Juglandaceae	*Juglans regia*	Ceviz	Leaves	Infusion (Toksoy e al., 2010)
Lamiaceae	*Ocimum basilicum*	Reyhan	Leaves and flowers	Infusion (Toksoy et al., 2010)

Lamiaceae	*Lavandula angustifolia*	Lavanta	Flowers	Infusion (Toksoy et al., 2010)
Fabaceae	*Cerationa siliqua*	Keçiboynuzu	Leaves	Decoction (Toksoy et al., 2010)
Fabaceae	*Glycyrrhiza glabra*	Meyan	Herba and roots	Powder and infusion (Toksoy et al., 2010)
Myristicaceae	*Myristica fragrans*	Hindistan cevi	Seeds	Powder and decoction (Toksoy et al., 2010)
Pedaliaceae	*Sesamum indicum*	Susam	Fixed oil	Powder and decoction (Toksoy et al., 2010)
Rosaceae	*Rosa canina*	Kuşburnu	Fruits and leaves	Infusion (Toksoy et al., 2010)
Ranunclaceae	*Nigella sativa*	Çörekotu	Seeds	Infusion and powder (Toksoy et al., 2010)
Hypolepidaceae	*Pteridium aquilinum*	Eğrelti	Roots	Infusion (Bulut, 2011)
Polygonaceae	*Rumex crispus*	Labada	Leaves	Infusion (Bulut, 2011)
Lamiaceae	*Salvia cryptantha*	Sarı şabla	Leaves and flowers	Infusion (Tuzlacı, 2011)
Liliaceae	*Allium scorodoprassum*	İt soğanı	Onions	It is eaten raw or cooked (Altundağ, Öztürk, 2011)
Ranunculaceae	*Anemone albana* subsp. *armena*	Dağ lalesi	Leaves	Infusion (Altundağ, Öztürk, 2011)
Asteraceae	*Anthemis nobilis*	Sarı papatya	Flowers	Infusion (Altundağ, Öztürk, 2011)
Asteraceae	*Artemisia abrotanum*	Pelin	Above ground	Infusion (Altundağ, Öztürk, 2011)
Asteraceae	*Artemisia chamaemellifolia*	Yavşan	Flowers and herba	Decoction (Altundağ, Öztürk, 2011)
Brassicaceae	*Barbarea vulgaris*	Su teresi	Leaves	Infusion (Altundağ, Öztürk, 2011)
Chenopodiaceae	*Chenopodium album* subsp. *album* var. *album*	Unluca	Above ground	Decoction (Altundağ, Öztürk, 2011)
Cyperaceae	*Cyperus rotundus*	Topalak	Roots	Infusion(Altundağ, Öztürk, 2011)
Fabaceae	*Glycyrrhiza echinata*	Dikenli meyan	Rhizome	Decoction (Altundağ, Öztürk, 2011)
Asteraceae	*Helichrysum plicatum* subsp. *plicatum*	Herdem taze	Above ground	Infusion (Altundağ, Öztürk, 2011)
Fabaceae	*Lotus corniculatus* subsp. *corniculatus*	Gazelboynuzu	Above ground	Decoction (Altundağ, Öztürk, 2011)
Asteraceae	*Achillea millefolium* subsp. *pannonica*	Civanperçemi	Aerial parts	Decoction (Kılıç, 2016)
Dipsacaceae	*Scabiosa argentea*	Uyuz otu	Roots	Decoction(Çakilcioglu, Türkoğlu, 2010)
Fabaceae	*Ononis spinosa* subsp. *leiosperma*	Kayışkıran	Roots	Decoction (Çakilcioglu, Türkoğlu, 2010)
Poaceae	*Agropyron repens*	Ayrık otu	Rhizome	Decoction (Çakilcioglu, Türkoğlu, 2010)
Poacea	*Avena sativa*	Yulaf	Rhizome	Decoction (Çakilcioglu, Türkoğlu, 2010)
Polygonaceae	*Polygonum cognatum*	Madımak	Leaves	Infusion (Çakilcioglu, Türkoğlu, 2010)
Rhamnaceae	*Rhamnus catharticus*	Cehri	Peeled shells	Decoction (Altundağ, Öztürk., 2011)

Rosaceae	*Sanguisorba minor* subsp. *minor*	Çayır düğmesi	Above ground	Decoction (Altundağ, Öztürk., 2011)
Asteraceae	*Tanacetum balsamita*	Marsuvan otu	Above ground	Infusion (Kızılarslan, Özhatay, 2012)
Ranunculaceae	*Thalictrum minus* var. *minus*	Karakatran otu	Above ground	Porridge is used (Kızılarslan, Özhatay, 2012)
Zygophyllacea	*Tribulus terrestris*	Deveçökerten	Above ground	Decoction (Kızılarslan, Özhatay, 2012)
Papaveraceae	*Chelidonium majus*	Temreotu	Latex and air particles	Infusion (Kızılarslan, Özhatay, 2012)
Asteraceae	*Filago vulgaris*	Kuzaotu	Aerial parts	Decoction (Kızılarslan, Özhatay, 2012)
Asteraceae	*Petasites hybridus*	Ayikulagi	Leaves	It is used in the form of heating (Kızılarslan, Özhatay, 2012)
Rosaceae	*Prunus spinosa* subsp. *dasyhylla*	Çakalerigi,	Fruits	Eaten raw (Kızılarslan, Özhatay, 2012)
Apiaceae	*Torilis arvensis* subsp. *arvensis*	Derecikotu	Aerial parts	Porridge is used (Kızılarslan, Özhatay, 2012)
Hypericaceae	*Hypericum perforatum*	Kantaron	Flowers	It is used by applying infusion and oil externally to the skin (Kızılarslan, Özhatay, 2012)
Cornaceae	*Cornus mas*	Kızılcık kirazı	Fruits	Decoction (Demirci, Özhatay, 2012)
Berberidaceae	*Berberis vulgaris*	Karamuk	Fruits	Eaten raw (Tetik et al., 2013)
Boraginaceae	*Anchusa azurea* var. *azurea*	Fısır, sormuk	Leaves	Infusion (Tetik et al., 2013)
Caryophyllaceae	*Telephium imperati* subsp. *orientale*	Zulzula	Leaves	Porridge is used (Tetik et al., 2013)
Geraniaceae	*Erodium cicutarium*	Çoban değneği	Aerial parts	Infusion (Tetik et al., 2013)
Plantaginaceae	*Plantago major* subsp. *major*	Cevahız, doğnık	Leaves	Decoction (Tetik et al., 2013)
Violaceae	*Viola odorata*	Kokulu menekşe	Flower and leaves	Infusion (Tetik et al., 2013)
Caryophyllaceae	*Gypsophila arrostii* var. *nebulosa*	Çöven	Roots and rhizome	Infusion (Akbulut, Bayramoğlu, 2013)
Ericaceae	*Erica arborea*	Funda	Flowers	Infusion (Akbulut, Bayramoğlu, 2013)
Fabaceae	*Ceratonia siliqua*	Keçiboynuzu	Fruits	Decoction (Akbulut, Bayramoğlu, 2013)
Lamiaceae	*Rosmarinus officinalis*	Biberiye	Leaves	Infusion (Akbulut, Bayramoğlu, 2013)
Liliaceae	*Colchicum speciosum*	Acı çiğdem	Seeds and onions	Consumed fresh (Akbulut, Bayramoğlu, 2013)
Oleaceae	*Olea europaea*	Zeytin	Leaves	Infusion (Akbulut, Bayramoğlu, 2013)
Papilionaceae	*Anagyris foetida*	Keçi gevişi	Fruits	It is eaten raw (Arıcan, Genç Ecevit, 2013)
Capparaceae	*Capparis spinosa*	Kebere	Bud and fruits	Pickled gherkins (Arıcan, Genç Ecevit, 2013)
Chenopodiacea	*Chenopodium album* subsp. *album*	Selmı, Sılmastık	Aerial parts	Boiled (Polat et al., 2013)
Equisetaceae	*Equisetum ramosissimum*	-	Aerial parts	Decoction (Polat et al., 2013)
Adiantaceae	*Adiantum capillus-veneris*	İshalotu	Aerial parts	Infusion (Kilic, Bağcı, 2013)
Brassicaceae	*Nasturtium officinale*	Su teresi	Aerial parts	Cooked (Kilic, Bağcı, 2013)
Dipsacaceae	*Scabiosa argentea*	Uyuz otu	Aerial parts	Decoction (Kilic, Bağcı, 2013)

Fabaceae	*Melilotus officinalis*	Yonca	Aerial parts	Decoction (Kilic, Bağcı, 2013)
Poaceae	*Panicum miliaceum*	Darı	Leaves	Decoction (Akaydın et al., 2013)
Fabaceae	*Lathyrus digitatus*	Efenk otu	Aerial parts	Decoction (Akyol, Altan, 2013)
Rubiaceae	*Galium aparine*	Yoğurt otu	Above ground	As an infusion and ointment (Gül, 2014)
Lamiaceae	*Mentha pulegium*	Filiskin, yarpuz	Leaves	Infusion (Ugulu et al., 2009)
L iliaceae	*Asphodelus aestivus*	Çiriş otu	Tubers	Infusion (Ugulu et al., 2009)
Malvaceae	*Althaea officinalis*	Hatmi	Leaves	Decoction (Ugulu et al., 2009)
Malvaceae	*Malva sylvestris*	Ebegümeci	Leaves	Infusion (Ugulu et al., 2009)
Myrtaceae	*Eugenia caryophllata*	Karanfil ağacı	Buds	Infusion [(Ugulu et al., 2009)
Solanaceae	*Physalis alkekengi*	Güvey feneri	Fruits	Infusion (Gül, 2014)
Ericaceae	*Rhododendron ponticum*	Mor çiçekli orman gülü	Leaves	Decoction (Gül, 2014)
Tiliaceae	*Tilia rubra*	Ihlamur	Flowers and leaves	Infusion and decoction (Gül, 2014)
Fabaceae	*Lupinus albus*	Acı bakla	Flowers and seeds	Decoction (Hergenç, 2015)
Ericaceae	*Arctostaphylos uva-ursi*	Ayı üzümü	Leaves and fruits	Decoction (Hergenç, 2015)
Adoxaceae	*Viburnum opulus*	Gilaburu	Fruits	Juice is removed (Korkmaz et al., 2015)
Lythraceae	*Punica granatum*	Nar	Flowers	Infusion (Korkmaz et al., 2015)
Zingiberaceae	*Curcuma longa*	Zerdeçal	Roots and rhizome	Paste and infusion (Korkmaz et al., 2015)
Zingiberaceae	*Zingiber officinale*	Zencefil	Root and leaves	Paste and infusion (Korkmaz et al., 2015)
Cucurbitaceae	*Cucumis melo*	Kavun	Fruit and seeds	Fruit kernels are crushed (Akan, Sade Bakır, 2015)
Fabaceae	*Cassia angustifolia*	Sinameki	Leaves	Decoction (Akan, Sade Bakır, 2015)
Verbenaceae	*Vitex agnus-castus*	Hayıt	Flowers and leaves	Decoction (Akan, Sade Bakır, 2015)
Boraginaceae	*Trachystemon orientalis*	Galdirek	Aerial parts	Infusion (Polat et al., 2015)
Fabaceae	*Lathyrus sativus*	Burçak, mürdümük	Seeds	Infusion (Özdemir, Alpınar., 2015)
Lamiaceae	*Marrubium globosum*	Amel otu	Aerial parts	Infusion (Özdemir, Alpınar., 2015)
Zygophyllaceae	*Peganum harmala*	Üzerlik	Seeds and roots	Decoction (Özdemir, Alpınar., 2015)
Asteraceae	*Cyanus segetum*	Dağ karanfili	Flowers and leaves	Infusion (Sargin et al., 2015)
Asteraceae	*Onopordum myriacanthum*	Eşek kengeri	Aerial parts	Infusion (Sargin et al., 2015)
Papaveraceae	*Papaver argemone*	Gelincik	Leaf, seed, Flower	Infusion (Sargin et al., 2015)
Asterace	*Senecio vulgaris*	Kanarya otu	Air particles, seeds	Infusion (Sargin et al., 2015)
Caprifoliaceae	*Lonicera etrusca* var. *etrusca*	Hanımeli	Leaves and fruits	Infusion (Güler et al., 2015)
Adianthaceae	*Adianthum capillus-veneris*	Ishalotu	Aerial parts	Infusion (Kılıç, Bagci, 2013)
Euphorbiaceae	*Mercurialis annua*	Sultan otu	Fruits	Infusion (Güler et al., 2015)
Fabaceae	*Cicer arietinum*	Nohut	Fruits	Eat(Güler et al., 2015)
Fabaceae	*Vicia faba*	Bakla	Fruits	Eat (Güler et al., 2015)
Vitaceae	*Vitis vinifera*	Üzüm	Fruits	Eat (Güler et al., 2015)

Solanaceae	*Capsicum annuum*	Kırmızı biber	Fruits	It is eaten dried (Akgül et al., 2016)
İridaceae	*Crocus sativus*	Safran	Flowers	Infusion (Akgül et al., 2016)
Lamiaceae	*Satureja* sp.	Kekik	Flowers and dried leaves	Infusion (Akgül et al., 2016)
Lauraceae	*Persea gratissima*	Avokado	Leaf	Decoction (Korkmaz et al., 2016)
Fabaceae	*Onobrychis stenostachya*	Körülgen	Above ground	Decoction (Altundağ, Öztürk., 2011)
Fabaceae	*Onobrychis transcaucasica*	Gorulgan	Above ground	Decoction (Altundağ, Öztürk., 2011)
Fabaceae	*Ononis spinosa* subsp. *leiosperma*	Kayışkıran	Above ground	Decoction (Altundağ, Öztürk., 2011)
Portulacaceae	*Portulaca oleracea*	Semizotu	Above ground	Decoction (Altundağ, Öztürk., 2011)
Brassicaceae	*Raphanus raphanistrum*	Turp	Above ground	It is eaten raw (Altundağ, Öztürk., 2011)
Lamiaceae	*Stachys lavandulifolia* var. *lavandulifolia*	Hava parçacıkları	Air particles	Decoction (Bulut et al., 2016)
Caryophyllaceae	*Silene vulgaris*	Yapışkan otu	Leaf	Decoction(Akdag, Dogu, 2016)
Liliaceae	*Muscari macrocarpum*	Dağ sümbülü	Onions	Decoction (Kayıran et al., 2017)
Asparagaceae	*Ornithagalum narbonense*	Akbaldır	Leaf	Decoction (Kayıran et al., 2017)
Liliaceae	*Urgenia maritima*	Ada soğanı	Onions	Decoction (Kayıran et al., 2017)

3. Results and discussion

Many indigenous drugs have been claimed to have diuretic effect in traditional medicine or in ethnobotany, but they were not properly investigated. Naturally occurring diuretics plant taxa are more safe and efficacious. Several plant derived chemical entities have proved to be more efficacious and safe. It remains for the modern scientists to give scientific validation for the plants claimed for therapeutic activity to make use of herbal potential in a more productive way. In this review paper, validation of these plant taxa which were classical literature as diuretic, and further, they are economically feasible, safe and efficacious and will be beneficial for further studies and clinical trials.

In this study, 186 plant taxa were identified from the literature of Turkey traditional medicine. In addition, as a result of field studies from Çelikhan (Adıyaman-Turkey) provinces in the 2019–2020 years 42 plant taxa which potential diuretic effects like plant taxa in Table 1 (Juniperus oxycedrus subsp. oxycedrus, Malva neglecta, Zea mays, Hordeum vulgare, Prunus armeniaca, Equisetum ramosissimum, Mentha longifolia var. longifolia, Platanus orientalis, Rubus sanctus, Rubus canescens, Vaccaria pyramidata, Crataegus monogyna subsp. monogyna, Convolvulus arvensis, Teucrium chamaedrys subsp. chamaedrys, Achillea millefolium subsp. millefolium, Cichorium intybus, Saponaria officinalis, Lamium album, Althaea officinalis, Pyrus elaeagnifolia subsp. elaeagnifolia, Helichrysum plicatum subsp. plicatum, Bellis perennis, Echium italicum, Rumex crispus, Reseda lutea var. lutea, Capsella bursa-pastoris, Juglans regia, Rosa canina, Anthemis nobilis, Ononis spinosa subsp. leiosperma, Portulaca oleracea, Sanguisorba minor subsp. minor, Anchusa azurea var. azurea, Plantago major subsp. major, Viola odorata, Chenopodium album subsp. album, Adiantum capillus-veneris, Melilotus officinalis, Vitis vinifera, Stachys lavandulifolia var. lavandulifolia, Silene vulgaris var. vulgaris, Ornithagalum narbonense).

Due to the active substances they contain, these plants used for diuretic purposes should be careful both in terms of usage, dosage and preparation. It is necessary to pay attention to the way plants are collected from nature and the time of harvest, as well as the cultivation of cultivated plants. It is possible that the livestock activities carried out in the region with the unconscious and large collection of plants will narrow the living space of many taxa and affect the number of individuals. By comparing the traditional knowledge obtained with ethnobotanical researches with the existing scientific literature, more universal and more effective results can be obtained.

4. Conclusion

Many indigenous drugs have been claimed to have diuretic effect in traditional medicine and ethnobotany but they were not properly investigated. Naturally occurring diuretics and potential diuretic plants are more safe and efficacious. It remains for the modern scientists to give scientific validation for the herbs claimed for therapeutic activity to make use of herbal potential in a more productive way. This brief review study shows the richness potential diuretic plants of Turkey and Çelikhan (Adıyaman-Turkey) provinces. It can be concluded that in their habitats there are so many plants which possess potent diuretic activity. Herbal medications are free from or very low side effects unlike the allopathic medicines. The current review provides an overview of knowledge adjoining the plant taxa used as diuretics.

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Antiplasmodial Activity of Methanolic Extract of *Achyranthes aspera* Shoot against *Plasmodium berghei* Infection in Albino Mice

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Abstract

Malaria remains one of the diseases that lack satisfactory treatment worldwide and continued to pose serious challenges with rapid spread of resistant parasite. Achyranthes aspera leaf, stem, and root extracts were found to exhibit antiplasmodial activities. The shoot has little or not been investigated despite its vital chemicals. Based on this, the study attempted to investigate antiplasmodial activity of methanol extract Achyranthes aspera shoot against Plasmodium berghei infection in mice. Achyranthes aspera shoot was sliced, air-dried, then pulverized into powdered form then extracted with methanol, filtered and concentrated with rotary evaporator at 40°C. The crude extract obtained was subjected to phytochemical analysis followed by acute toxicity study. Antiplasmodial effect of the extract was investigated in Swiss albino mice infected with $1x10^7$ Plasmodium berghei (NK-65) strain intrperitonealy. Eight groups of 5 mice were used; group 1: normal control, group 2: infected mice and untreated, group 3&4: infected mice + standard drugs, group 5-7: infected mice + extracts (200 mg/kg, 400 mg/kg, 600 mg/kg body wt.), group 8: infected mice + vehicle. During the experiment, parasitemia levels and PCV were monitored. Body weight and temperature were also measured.

The study found that the plant shoot contains vital phytochemicals (alkaloids, saponins, phenols and flavonoids) and is safe with LD_{50} greater than 5000 mg/kg body weight of extract of mice. Treating Plasmodium berghei infected mice with methanol extract of the plant shoot displayed remarkable effect as evidenced by the reduction in their parasitemia levels and increase survival rate in a manner comparable to chloroquine and artemisinin. The methanol extract of A. aspera is safe, possess vital phytochemicals and exhibited antiplasmodial activity in mice. As it is a good antimalarial candidate, further research should characterize the active component(s) and their mode of action.

Keywords: *Achyranthes aspera*, shoot, phytochemicals, antimalarial, *Plasmodium berghei*, Albino mice.

1. Introduction

Malaria still remains one of the diseases in which its treatment is not satisfactory worldwide and continued to pose serious challenges to people from both rural and urban communities in Nigeria and other African nations (Nguta et al., 2010). In 2019, it was estimated to have affected 219 million people, where 435,000 have died from the disease (WHO, 2018). Sub-Saharan Africa

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was reported to account for about 94 % of the malaria case out of which 25 % is from Nigeria (WHO, 2019).

The disease is mainly caused by *Plasmodium falciparum* and *P. vivax*, which have become increasingly resistant to available antimalarial drugs (Muregi et al., 2003; Qais et al., 2011). This has pushed some people to resort heavily in the use of herbal claimed medicinal plants' preparations as alternative treatment to meet their primary health care needs, to be able to overcome the enormous and persisting challenges of resistance, and adverse side effects such as cardiotoxicity associated with available antimalarial drugs (White, 2007; Qais et al., 2011). These research have been geared toward finding effective and safer agent mainly from plants since, medicinal plants have been a promising source for discovery and development of novel drugs. This have yielded a positive results in the past recent years where principle or active component in some of the recent drugs of choice against malaria infection such as artemisinin was isolated from plant known as *Artemisia annua* (Katuura et al., 2007).

Achyranthes aspera is a perennial shrub, popularly known as "pricky chaff flower" which belongs to the *Amaranthaceae* family, and a habitat of Asia, South Americ and Africa (Jain et al., 2006). In Nigeria, it is known locally as "Kiban Katangare" (Hausa), "Ndefiat" (Mwaghavwul) and "Kigye tukusan" (Ron). The plant is used by traditional medicine practitioners in the treatment of fever, particularly malaria fever, dysentery, asthma, hypertension and diabetes (Girach, Khan, 1992). Scientific studies reveal that Achyranthes aspera contained numerous active constituents with several medicinal properties. Among the important component are; ecdysterone, achyranthine, betaine, pentatriacontane, 6-pentatriacontanone, hexatriacontane and triacontane which were identified from leaves, seeds and roots parts of *Achyranthes aspera* (Tang, Eisenbrand, 1992).

The antimalarial effect of Achyranthes aspera has been studied by several researchers. The antiplasmodial studies started with the use of rodent models that have been validated through the identification of several antimalarials of Mefloquine and Artemisinin. In view of their proven use in the production of treatment outcome for human infection, thus these models remain a standard part of drug discovery and development pathway (Fidock et al., 2004). The study conducted by (Inbaneson et al., 2012) on the antiplasmodial activity of the leaf, stem, and root extracts of Achyranthes aspera in vitro reports that plants were effective against *Plasmodium falciparum* with IC_{50} values between 50 and 100 µg/ml. Following literature reports on antimalarial activity of *Achyranthes aspera* reveals that, the shoot part of the plant has little or not been investigated despite its vital chemicals and antimicrobial activity (Parvenu, 2018). Based on this, the current study attempted to investigate antiplasmodial activity of methanolic extract of *Achyranthes aspera* shoot against *Plasmodium berghei* infection in mice model.

2. Materials and methods

Plant collection and identification

Fresh *Achyranthes aspera* shoot were collected from Nchiya in Mangu Local Government Area of Plateau State, Nigeria. It was authenticated at the Herbarium Unit of the Forestry Research Institute, Jos, Plateau State, Nigeria.

Chemicals

All chemicals and solvents used were of analytical grade procured from Sigma Aldrich, USA.

Experimental Animals

A total of Thirty five Swiss albino mice of both sexes with age range of about 6 to 8 weeks were purchased from the Animal House Unit of Pharmacology Department, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State Nigeria. They were kept in standard clean cages with 12/12 h light/dark photoperiod, and fed with animal's feeds (ECWA – Vital feeds, Jos) and tap water *ad-libitum*. The study was approved by the Institute of Animal Ethical Committee as regulated by the board for the purpose of control and supervision of experimental animals. Ethics approval and consent to participate was obtained with approval number (UJ/FPS/F17-00379). The mice were handled with humane care and quarantine for two weeks before used for the experiment.

Plant Extraction

The plant shoot was washed with tap water, air-dried under shade for a period of 7 days, then pulverized using mortar and pestle into powdered form, and was extracted with methanol as described by (Yared et al., 2012). Exactly, 80 g of powdered plant shoot was mixed with 300 ml of methanol and kept at room temperature (25^{o}C) for 72 hours with intermittent shaking using shaker

at 12,000 rpm. It was then filtered through a cheese cloth and concentrated using rotary evaporator at 40°C then finally air dried. Portion of the crude methanol extract obtained was put in an air-tight sterilized container and kept at 4°C in a refrigerator until used.

Phytochemical screening

Preliminary phytochemical analysis of methanolic extract of Achyranthes aspera shoot was done following method described by (Harbone, 1984).

Acute toxicity

Acute toxicity was performed according to guidelines of Organization of Economic Corporation and Development 425 (OECD, 2001). Briefly, three groups of five female mice each were weight and administered orally with 2000 mg/kg and 5000 mg/kg bwt^{-1} of mouse of the aqueous extract in a single dose using oral intubation tube. Mice were deprived of food for 3 hours prior to dosing. After each extract dose administration, observation was done at 30 min interval for 4 hours then there after 24 hours for any behavioral change or death. The animals were observed for 14 days for various sign of toxicity including hair erection, diarrhoea and mortality.

Parasites and inoculation

Chloroquine sensitive *Plasmodium berghei* (NK-65) strain obtained from National Institute of Medical Research (NIMR), University of Ibadan, Oyo State, Nigeria was used. The parasites were maintained in vivo by serial passage of blood from infected mice to non-infected ones on weekly basis. Inoculation was done by intraperitoneal injection of mice with $1x10^7$ *Plasmodium berghei* (NK-65) strain infected erythrocytes. Infection was then allowed to be established for 72 hours and observed by collecting blood from their tails and used for parasitemia determination as described by Ryley, Peters, (1970).

Experimental Animals and Grouping

A total of forty mice were used for the study which were randomly divided into eight groups of five mice each; seven groups were mice infected with *Plasmodium berhgei*, while one group is non-infected mice which served as normal control. Treatment with plant extract at three different doses lasted for about five days as follows; after the experimentation mice were not euthaniced, but the survival of each mouse recorded.

- Group 1 (Normal control): Received normal feed and distilled water only;
- Group 2 (Negative Control): Infected Mice Untreated;
- Group 3 (Standard drug control 1): Infected Mice Treated with 10 mg/kg $b.wt^{-1}$ chloroquine;
- Group 4 (standard drug control 2): Infected Mice Treated with 10 mg/kg $b.wt^{-1}$ Artemisinin (ACT);
- Group 5 Infected Mice + 200 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 6 Infected Mice + 400 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 7 Infected Mice + 600 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 8 Infected Mice + 0.2 ml of 2 % tween 80.

Determination of Parasitemia, Mean Survival Time and PCV

The standard method of (Ryley, Peters, 1970) was followed to evaluate curative activity. Standard inoculation of $1x10^7$ *P. berghei* parasitized red blood cells was injected intrapertoneally into mice on the first day. After 72 hr, mice were divided into eight groups of five mice in each group. Different doses of methanolic extract of A. aspera (200, 400 and 600 mg/kg/day) were administered orally to these groups. Chloroquine (10 mg/kg/day) and Artemisinin (25 mg/kg/day) were given to the two positive control groups and only distilled water to the negative control group. The extract and drugs were given once daily for 5 days. Parasitemia levels were monitored daily from each mice group. Blood was collected by tail rub, smeared on microscopic slides, fixed with absolute methanol and stained with 10 % giemsa for 10 mins, then rinse with water. Immersion oil was added to the stained slide where the smeared parasitized red blood cells were examined under x100 magnification using Olympus microscope (Olympus-CH). Three different fields on each slide were examined to calculate the average parasitemia (Ural et al., 2014).

Parasitemia was calculated by using the formula below:

$$\% \text{ Parasiteamia} = \frac{\text{Numberofparasitized RBC}}{\text{total numberofRBC}} x100 \quad (12).$$

The mean survival time for each group was determined by finding the average survival time (days) of mice (post-inoculation) over a period of 30 days (D0–D29) as a principle compound that prolonged survival time beyond 12 days is regarded as active (Ural et al., 2014; Mulisa et al., 2018).

The Packed Cell Volume was measured by taking blood from the mouse tail with heparinized mircro-hematocrit tubes to ¾ heights and sealed with sealing clay. The tubes were centrifuged with sealed ends outwards for 5 mins at 12,000 rpm. PCV was determined using micro-hematocrit reader (Hawskley and Sons, England) and calculated Gilmour, Skyle, 1951.

$$PCV = \frac{\text{Volme of erythrocytes in a given volume of blood}}{\text{total blood volume}}$$

Determination of the Body weight and rectal body temperature of mice: The body weights of mice were measured using sensitive weighing balance (Ohaus, USA) on day 0 before infection and day 8 in the curative test. The average body weight was compared with control group over time in each group as described by (Dikasso et al., 2001). The rectal body temperature of mice in all groups were measured using clinical digital thermometer, on day 0 before infection and days 8 in the curative test to see the effect of extract or rectal body temperature. The mean body temperature was compared with control group over time in each group.

Statistical analysis

Data from the experiment were expressed as mean ± Standard Error of Mean (SEM). Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan's multiple range test (DMRT) (Duncan, 1957). Significant difference was accepted at $P < 0.05$.

3. Results

Yield and Phytochemical Content of Methanolic extract of *Achyranthes aspera* shoot

The yield of the methanolic extract of *Achyranthes aspera* shoot is about 12.5 %. The phytochemicals identified were alkaloids, tannins, saponins, balsam, and phenols.

Acute toxicity of the Methanolic extract of Achyranthes aspera Shoot

No mortality was recorded during the toxicity study neither was there any adverse effect in the behavior of mice administered both extract doses. The LD_{50}value was therefore considered to be more than 5000 mg/kg body weight for oral administration of methanolic extract of Achyranthes aspera Shoot in mice.

Effects of Methanolic extract of Achyranthes aspera shoot on PCV of Infected Mice

The result of PCV of mice infected with *P. berghei* following treatment with methanolic extract of Achyranthes aspera shoot is shown in Figure 1. There was a fluctuation in PCV levels of untreated mice and those treated with the plant extract and standard drugs particularly at day 6 of the experiment but were still within normal value. Packed cell volume of normal control mice increased gradually through-out the experimental period.

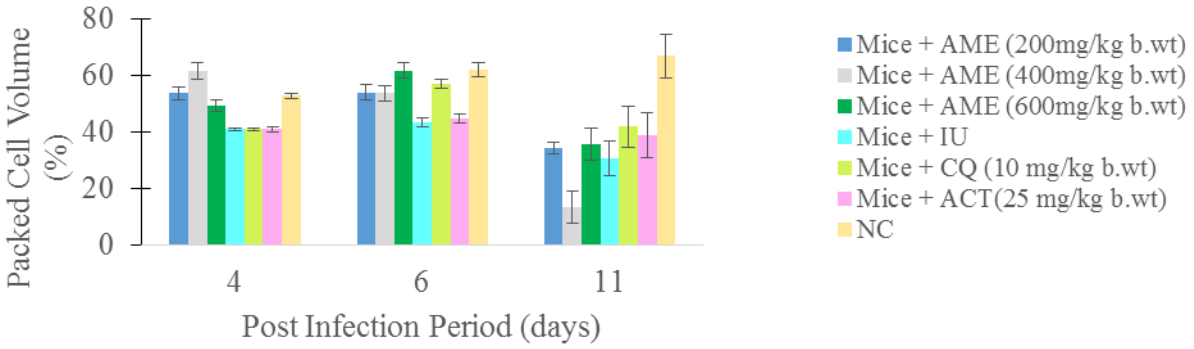

Fig. 1. Change in Packed Cell Volume of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

Effect of Methanolic extract of *Achyranthes aspera* Shoot on Temperature of Infected Mice

The change in temperature of mice infected with *P. berghei* following treatment with methanolic extract of *Achyranthes aspera* shoot is presented in Figure 2. The study showed no significant changes in temperature of infected mice treated when compared to the untreated and normal control mice.

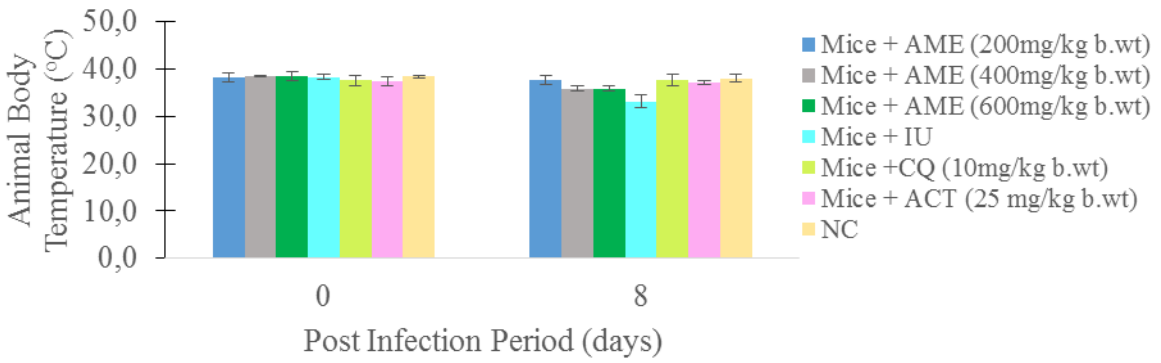

Fig. 2. Change in Body Temperature of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg)

Effect of Methanolic extract of Achyranthes aspera Shoot on Weights of Infected Mice

The result of body weights of mice infected with *P. berghei* following Treatment with methanolic extract of Achyranthes aspera Shoot is shown in Figure 3. There was gradual increased in weight by infected mice following treatment with plant extract as was in the normal control. But, slight loss in body weight was recorded by mice administered the standard drugs compared to those not treated.

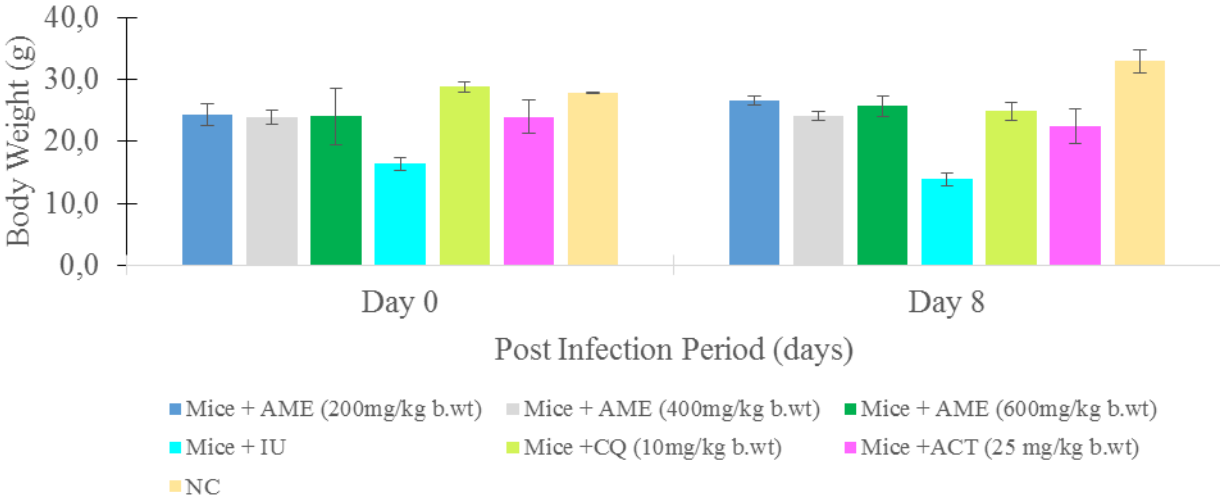

Fig. 3. Change in Body Weight of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

Antiplasmodial Effect of Methanolic extract of Achyranthes aspera Shoot in Infected Mice

The antiplasmodial activity of methanolic extract of *Achyranthes aspera* is presented in Figure 4. The result showed a gradual reduction in parasitemia levels in mice treated with methanol extract, Chloroquine and Artimisinin compared with the untreated mice. The reduction of parasitemia by methanol extract of the plant shoot was in a dose dependent manner where higher reduction in parasitemia was recorded in mice that were administered 600 mg/kg body wt. plant shoot extract.

The result of mean survival rate of infected mice is presented in Figure 5. The study recorded high survival rate of mice treated with Chloroquine followed by those that received Artimisinin and 600 mg/kg dose of the methanol extract of *Achyranthes aspera* shoot.

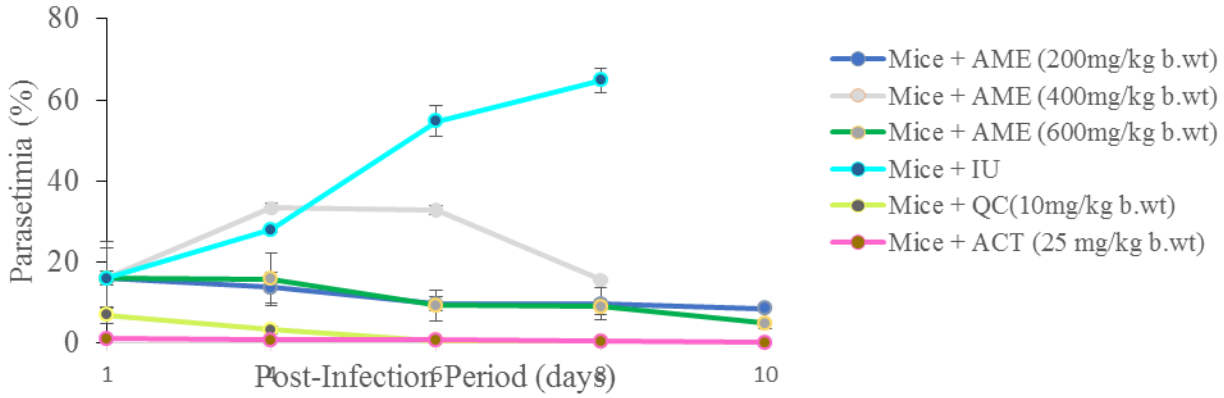

Fig. 4. Change in Parasitemia Levels in Mice Infected with Plamodium berhei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

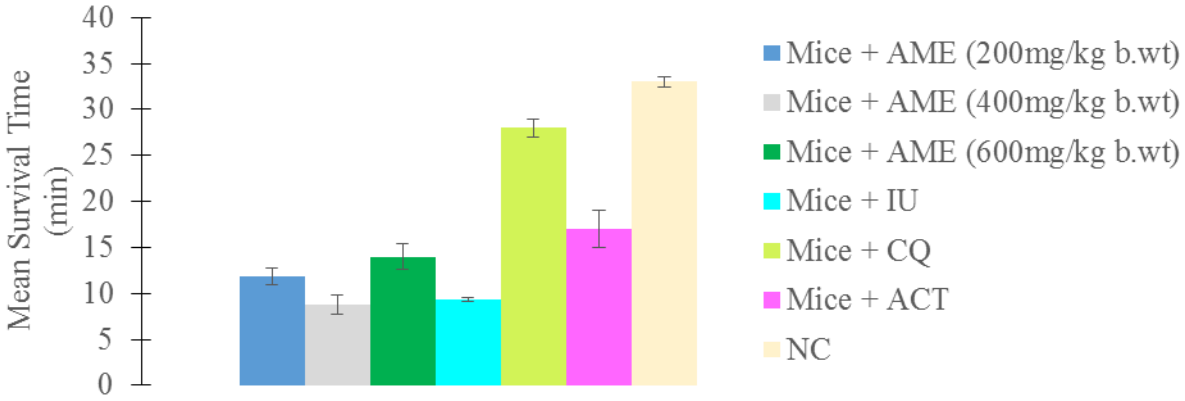

Fig. 5. Survival Rate of Mice Infected with *Plasmodium berghei* following Treatment with Methanolic Extract of *Achyranthes aspera* Shoot

Mice + AME = Infected mice treated with *Achyranthes aspera* shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artemisinin (25 mg/kg).

4. Discussion

In this study, methanolic extract of the shoot part of the plant contain vital phytochemicals like alkaloids, saponins, phenols and flavonoid. The alkaloids, phenols and saponins identified in methanolic extracts of *Achyranthes aspera* shoot in the study are in-line with the findings reported in a studies conducted by Sharma, Chandhary (2015) and Tiwari et al. (2018) on methanolic and ethanolic leaf extract of *Achyranthes aspera*. Alkaloids were reported to exert antimalarial activity by blocking protein synthesis in *P. falciparum* (Liu et al., 2017), likewise phenols and tannins are reported to exhibit antimalarial activities (Soh et al., 2012). Phenols have been reported in several plants as the major pharmacological contributors, thereby suggesting that same chemical compounds might be likely the key player for the vast medicinal potentials of *Achyranthes aspera*. Saponins are other important type of bioactive chemical constituents which are involved in plant disease resistance due to their anti-microbial activity, which found to be associated with vast biological activities (Anyasor et al., 2010).

The methanolic extract of *A. aspera* shoot was studied for in vivo antiplasmodial activity in order to ensure its safety and efficacy in various doses according to (Chandel, Bagai, 2010). Mice infected with parsasite die within 7-10 days of infection in normal cause of infection in the present study, treating *Plasmodium berghei* infected mice with methanolic extract, showed a significant effect as evidence by the reduction in their parasitemia level and increased in survival rate in manner comparable to chloroquine and artemisnin treated drugs. This may be due to the phytochemicals identified and the reduced pathologic effects of the extract. The anitmalarial activity observed may be due to these constituents, the methanolic extract is highly effective and activity could be due to synergistic effect of these secondary metabolites. In vitro studies revealed that the methanolic extract of *Uvariopsis congolana* leaves (*Amaranthaceae*) displayed good activity (4.57±076 µ/ml against *P. falciparum* (W_2) strain in vitro (Hilou et al., 2006).

The decrease in PCV levels after the administration of the extract could be due to the loss or destruction of red blood cell. This may be explained by the fact that, since, the *Plasmodium* parasite is localized in the cells and treatment may likely involve lysing cells which after clearing the parasites, the cells gradually divide and replenish resulting in PCV fluctuation. Similar scenario have been reported in a studies conducted by Yang et al., 2005 with *Dodonae angustipholia*, Bantie et al. (2014) with *Croton macrostachyus* extracts, chloroform leaf extract and diethyl ether leaf extracts of *Eucalyptus cameldulensis* (Ishaya et al., 2019a; Ishaya et al., 2019b). Changes in body weight and rectal temperature of infected mice observed in the study are associated with general features of malaria disease (Langhorne et al., 2017). However, treating malaria with antimalarial agents may reverse the changed, extract prevented weight loss at all dose associated with *P. berghei* infection, and is an indication of ameliorative potentials of the plant extract on the aneamia induced by the malaria infection. This could also be due to the presence of appetite enhancing and immune modulatory component(s) in the extract (Fenthahun et al., 2017). The insignificant changes observed in the rectal temperature could be as a result of increase in tropical metabolite rate thus indicating potential active components(s) responsible for this effect were likely found in a good concentration that have antimalarial activities in the extract.

5. Conclusion

The study confirmed methanolic extract of *Achyranthes aspera* shoot, to possess vital phytochemicals. Extract is safe as no mortality was reported and possessed good antiplasmodial activity. Further research to explore the active compound and mode of action in recommended.

- Conflict of Interest

The authors declare that they have no competing interest

- Acknowledgements

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- Ethics and consent approval to participate:

The study was approved by the Institute of Animal Ethical Committee, University of Jos. Ethical clearance obtained with approval number (UJ/FPS/F17-00379).

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Resultant Effect of Early Endogenous Thermal Acclimatization on Performance of Heat-Stressed Broiler Finishers on Different Levels of Dietary Protein

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Abstract

The guarantee of a naturally stable thermal environment that will keep poultry birds within the zone of maximum comfort all year round in tropical and sub-tropical regions in the 21st century cannot be established. Therefore, it becomes necessary to look into stabilizing internal environment of vulnerable chicks, with the sole aim of re-exhibiting their masked potentials for fast growth and high yield. Crude protein (CP), though essential for growth and repair, dissipates more heat load than other nutrients during metabolism. Hence, it becomes imperative to investigate the effect of early endogenous thermal acclimatization on performance of heat-stressed broilers (HSB) at finisher phase. Using a total of 288, one day-old Arbor Acre broiler chicks, birds were randomly allotted to four dietary treatments (T1 – 21 % CP; T2 – 19 % CP; T3 – 17 % CP; and T4 – 15 % CP) at finisher phase with six replicate groups in a completely randomized design in order to evaluate the resultant effect of a 2 %, 4 % and 6 % reduction in dietary CP. Data were analysed using descriptive, ANOVA, regression and correlation statistics. Low crude protein diets improved performance of HSB at finisher phase. However, a 2 % dietary CP reduction (19 % CP) best enhanced performance of HSB at finisher phase. Some parameters that were supposed to strongly correlate were not as a result of the influence of early endogenous manipulation. Endogenous heat load reduction through dietary crude protein and electrolyte inter-play have resulted in a proportionate increase in feed intake, body weight gain, protein efficiency ratio and enhanced feed conversion ratio in HSB at finisher phase. The resultant effect of early endogenous thermal acclimatization on performance of heat-stressed broiler chickens at finisher phase has been beneficial.

Keywords: broiler finishers, crude protein, endogenous heat, heat stress, resultant effect.

1. Introduction

The extent of adaptation to heat stress conditions by poultry birds is dependent on the enhancing strategies adopted during thermal conditioning in chicks. Popoola et al. (2020a) affirmed that tropical regions with characteristic high environmental temperatures face more

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difficulty raising fast-growing meat type chickens because birds` health, performance and meat quality are adversely affected. Decuypere et al. (2001) also opined that adaptation to heat stress conditions can be enhanced by thermal conditioning in chicks, without impairing performance, as chicks regulate body temperature during early post-hatch periods more with proportionate increase in age (Debonne et al., 2008). However, the metabolism of crude protein ingested by chicks results in a greater increase in heat production than carbohydrates or fats and results in increased body temperature (Musharaf, Latshaw, 1999). A reduction of crude protein in rations fed to broilers under heat stress condition, with adequate amount of essential amino acids may improve performance (Zaman et al., 2008). Yahav (2000) suggested that early thermal manipulations in poultry may be useful in tropical regions with more vulnerability to heat stress, when the chicks` body temperature and feedback regulatory mechanisms are immature. Nichelmann, Tzschentke (2002) stated that early post-hatch period is more important where major developmental and physiological processes occur. Although, chicks anatomically seem complete post-hatch, yet some systems such as digestive, immune, and thermoregulatory systems need further development and maturation for optimum performance. Soleimani et al. (2012) opined that living organisms respond to thermal stressors by synthesizing a group of highly conserved proteins known as heat shock proteins as they function in modifying physiological stress response and stress tolerance acquisition. Tan et al. (2010) noted that heat stress during early post-hatch periods may result in greater adaptability to thermal stress even when endogenous heat production is higher. Since birds must maintain internal body temperature despite thermal oscillation, they do so at the expense of production, by diverting nutritionally beneficial molecules to homeostatic adjustments. The level of heat tolerance in poultry is dependent on the activation of heat loss mechanism and the ability to reduce the endogenous heat production (Nichelmann, Tzschentke, 2002). Also, Popoola et al. (2020b) noted that ideal DEB is prerequisite to blood acid-base balance and reduced incidences of hemodilution in heat-stressed broilers, and if supplied in adequate amount, there would be a substantial positive feedback. Azad et al. (2010) noted that heat stress affects metabolic processes, causing oxidative damage to skeletal muscles with impairment of functional properties of meat. Leterrier et al. (2009) noted that the growth of broilers have been improved by genetic selection. However, some visceral organs have not been genetically modified alongside traits of most importance, thereby creating a negligence in effective capacity of cardiovascular and respiratory systems for hyperventilation and heat loss. Yahav, McMurtry (2001) reported that pre-starter exposure of chicks to heat stress enabled them to withstand heat challenge at finisher phase when metabolic processes increased, with subsequent reduction in mortality as De Basilio et al. (2003) reported that chickens that did not survive heat challenge at 34 days of age had higher body temperatures of about 0.6 °C prior to heat challenge than those of the survivors. Although, the beneficial effects of early thermal-conditioning are known, yet the resultant effect of an internal adjustment of heat load in fast-growing broiler chickens on performance and protein utilization and the extent of deviation from the standard effect upon reduction of dietary crude protein have not been clearly established in heat-stressed broiler chickens and it is necessary as Popoola et al. (2020c) opined that poultry meat and feed quality deterioration; and loss of customers` preference to chicken meat exposed to heat stress conditions may persist in the 21st century poultry farming systems (Popoola, 2020).

2. Materials and methods

The study was carried out at the Teaching and Research Farm, University of Ibadan, Nigeria, after the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee, through the Agricultural Biochemistry and Nutrition Unit of the Department of Animal Science. A total of 288, one day-old Arbor Acre broiler chicks with initial body weight of 41±3g were randomly allotted to four dietary treatments (CP, %: 23, 21, 19, 17) at pre-starter phase and 21, 19, 17 and 15 % CP at finisher phase with six replicate groups in a completely randomized design in order to evaluate the resultant effect of a 2 %, 4 % and 6 % reduction in dietary CP, and early endogenous thermal acclimatization on performance of heat-stressed broiler chickens at finisher phase. Feed grade potassium chloride and sodium bicarbonate, and the inherent potassium, sodium and chloride ions in feed ingredients were the electrolyte sources computed for determining the aggregate DEB using the equations derived by Popoola, Iyayi (2018). The derived equations were based on assumptions opined by Popoola et al. (2020c) for an ideal DEB, affirming

that not more than 30 to 140 mEq/kg DEB are required from mineral sources, with about 115 to 210 mEq/kg DEB obtainable from feed ingredients.

The derived equation of DEB is $\sum DEB = \sum (Na^+ + K^+) - \sum Cl^-$ ...[y] [c]..... (1)

Where [y] = mineral sources

and [c] = other macro ions (Ca, Mg, P, S etc) held constant.

$\sum DEB = \iota DEB + \varepsilon DEB$ ..... (2)

Where $\sum$ DEB = Aggregate DEB; ι DEB = Inherent DEB in rations and ε DEB = DEB in Electrolyte sources.

Feed intake was determined by giving a known quantity of feed to the birds and subtracting the left over for a given period from the quantity supplied. This difference was divided by the number of birds in a replicate group to estimate the feed intake per bird. Body weight gain of birds was determined by subtracting the initial weight for each week from the final weights with the aid of sensitive weighing scale.

The percentage deviation from the standard effect was calculated as described by the authors using the formula

$$\delta\ ^{\%} = \frac{(\dot{S}^{\hat{e}} - \hat{A}^{\hat{e}})}{\dot{S}^{\hat{e}}} \times 100$$

Where $\delta\ ^{\%}$ = percentage deviation from the standard effect

$\dot{S}^{\hat{e}}$ = standard mean effect

$\hat{A}^{\hat{e}}$ = Actual mean effect for individual treatments

and the resultant effect (R) was calculated for each parameter as the difference between the initial and final standard deviations from the standard effect as

$R = {}^{f}\delta_{Tn} - {}^{i}\delta_{Tn}$

Where ${}^{f}\delta_{Tn}$ = final percentage deviation from the standard effect for each treatment

${}^{i}\delta_{Tn}$ = initial percentage deviation from the standard effect for each treatment

A total of 32 pens were used in this study in order to measure water intake in heat-stressed broiler chicks as described by Popoola et al. (2019). Maximum and minimum average ambient temperature and relative humidity were monitored on a daily basis using a digital hygro-thermometer. Rectal temperature was measured in the morning (06:00-08:00 h) and afternoon (13:00-15:00 h), with the use of a digital rectal probe. Two birds per pen with body weight closest to the class mean weight were identified for body temperature measurement. Proximate analysis of the feeds was determined according to AOAC (2005) procedure. Assay was conducted for sodium and potassium (Flame spectrophotometer), and chloride (titration) in diets fed to broiler chickens at different phases of growth (Lacroix et al., 1970). Data obtained were subjected to descriptive statistics, analysis of variance using SAS (2012) package, regression and correlation statistics. Means for treatments in the analysis of variance were compared using Duncan Multiple range test and all statement of significance were based on probability level of 0.05.

3. Results

Table 1 shows the chemical analyses of diets fed to heat-stressed broiler chickens at finisher phase. Although, the diets differed significantly in CP, yet they all met the optimum dietary electrolyte balance requirement for heat-stressed broiler finishers.

Table 2 shows the performance of heat-stressed broiler chickens fed diets with varying crude protein at finisher phase. Feed intake (FI) was highest ($P < 0.05$) in heat-stressed birds on 21 % CP (1777.33) compared to other dietary treatments. The lowest ($P < 0.05$) FI was observed in birds on 15 % CP (1462.08) and did not differ significantly from 17 % CP (1485.58). The body weight gain (BWG) observed in birds on 21 % CP (866.08) and 19 % CP (869.08) were similar and significantly ($P < 0.05$) higher compared to 17 % CP (757.50) and 15 % CP (623.08). However, the lowest ($P < 0.05$) BWG was observed in birds on 15 % CP. Birds on 15 % CP (2.35) had the highest ($P < 0.05$) FCR value compared to other dietary treatments. However, birds on 19 % CP (1.87) had the lowest FCR value at finisher phase. Gain to feed ratio was significantly ($P < 0.05$) higher in birds on 19 % CP (0.54) compared to 21 % (0.49) and 15 % CP (0.43), but did not differ significantly from 17 % CP (0.51). Protein intake (PI) was highest ($P < 0.05$) in birds on 21 % CP (373.24) and decreased significantly with decreasing dietary CP. However, protein efficiency ratio (PER) was significantly ($P < 0.05$) higher in birds on 19 % CP (2.83), 17 % CP (2.99) and 15 % CP (2.85) compared to 21 %

CP (2.32) at finisher phase. Water intake values observed in heat-stressed birds on 21 % (849.34), 19 % CP (851.03), and 17 % CP (838.39) were similar and significantly ($P < 0.05$) higher compared to 15 % CP (723.01).

Table 1. Analysed nutrients in diets fed to heat-stressed broiler chickens at finisher phase

Nutrients	21 % CP	19 % CP	17 % CP	15 % CP
Crude protein (%)	20.89	18.83	17.20	15.33
ME, kcal/kg	3038.90	3046.63	3083.08	3087.65
Ether extract (%)	3.84	3.80	3.81	3.78
Crude fibre (%)	3.49	3.28	3.08	2.93
Calcium (%)	1.03	0.99	1.02	1.02
Total phosphorus (%)	0.74	0.69	0.67	0.65
NPP (%)	0.43	0.42	0.41	0.40
Ca:NPP	2.39	2.36	2.49	2.55
Sodium (%)	0.23	0.27	0.31	0.35
Potassium (%)	1.15	1.13	1.07	1.05
Magnesium (%)	0.17	0.16	0.16	0.15
Chlorine (%)	0.54	0.58	0.58	0.63

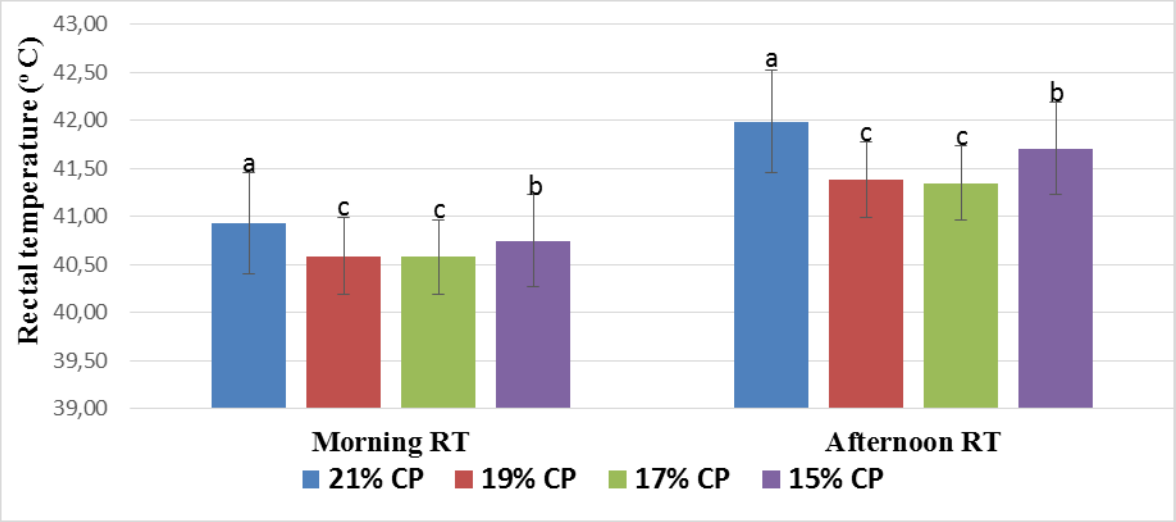
ME – Metabolizable energy, NPP – Non-phytate phosphorus, Ca – Calcium, CP – Crude protein

Table 2. Performance of heat-stressed broilers chickens fed diets with varying crude protein at finisher phase

Dietary treatments	FI (g/bird)	BWG (g/bird)	FCR (g/g)	Gain: Feed (g/g)	Protein Intake (g/bird)	PER (g/g)	Water intake (mL /bird/day)
21 % CP	1777.33^{a}	866.08^{a}	2.11^{b}	0.49^{b}	373.24^{a}	2.32^{b}	849.34^{a}
19 % CP	1617.83^{b}	869.08^{a}	1.87^{c}	0.54^{a}	307.39^{b}	2.83^{a}	851.03^{a}
17 % CP	1485.58^{c}	757.50^{b}	1.98^{bc}	0.51^{ab}	252.55^{c}	2.99^{a}	838.39^{a}
15 % CP	1462.08^{c}	623.08^{c}	2.35^{a}	0.43^{c}	219.31^{d}	2.85^{a}	723.01^{b}
SEM	24.63	40.57	0.10	0.02	4.25	0.12	36.21
P Value	0.00	0.00	0.02	0.02	0.00	0.01	0.05

^{abcd} Means of treatments along a column with different superscripts differed significantly ($P < 0.05$) using DMRT. FI – Feed intake, BWG – Body weight gain, FCR – Feed conversion ratio, PER – Protein efficiency ratio.

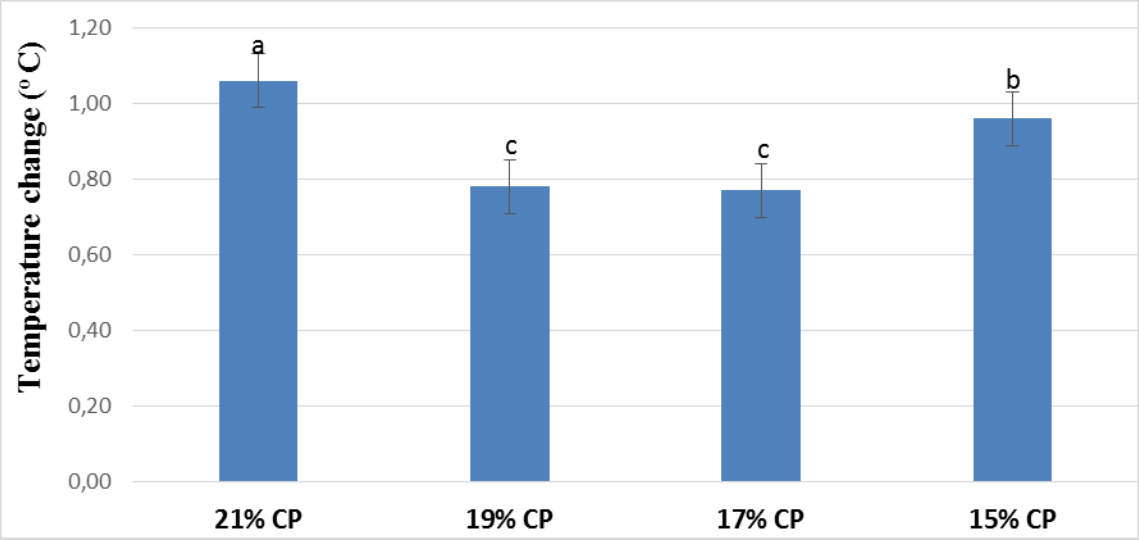
Figure 1 shows the rectal temperatures (RT) of heat-stressed broiler chickens at finisher phase. Higher ($P < 0.05$) morning RT was observed in birds on 21 % CP compared to other dietary treatments. However, birds on 19 % CP and 17 % CP had the lowest ($P < 0.05$) morning RT. Similar trend was observed in afternoon RT of heat-stressed birds on varying levels of dietary CP, though, it was at a much more elevated temperature range.



Bar means of treatments with different superscripts differed significantly (P < 0.05) using DMRT. RT – Rectal temperature; CP – Crude protein

Fig. 1. Rectal temperature of heat-stressed broiler chickens on different levels of dietary protein at finisher phase

Figure 2 shows the temperature change in heat-stressed broiler chickens on different levels of dietary crude protein at finisher phase. It was observed that birds on 21 % CP (1.06) had the highest (P < 0.05) fluctuation in body temperature compared to other treatments. However, birds on 19 % CP (0.78) and 17 % CP (0.77) had the lowest (P < 0.05) body temperature change at finisher phase.

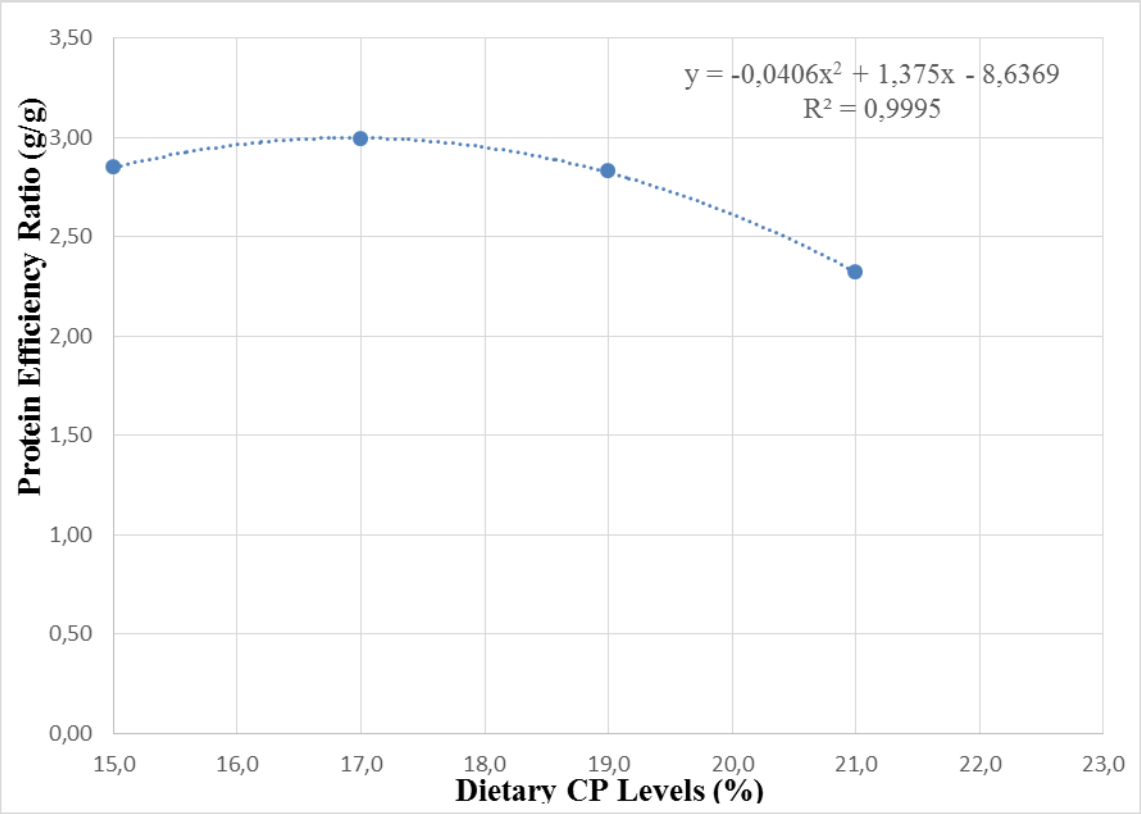


Bar means of treatments with different superscripts differed significantly (P < 0.05) using DMRT. CP – Crude protein

Fig. 2. Temperature change in heat-stressed broiler chickens on different levels of dietary protein at finisher phase

Figure 3 revealed the relationship between varying levels of dietary crude protein and protein efficiency ratio in heat-stressed broiler chickens at finisher phase. An optimum level of dietary CP was observed at 17 % in heat stressed birds and decreases significantly with increasing dietary CP

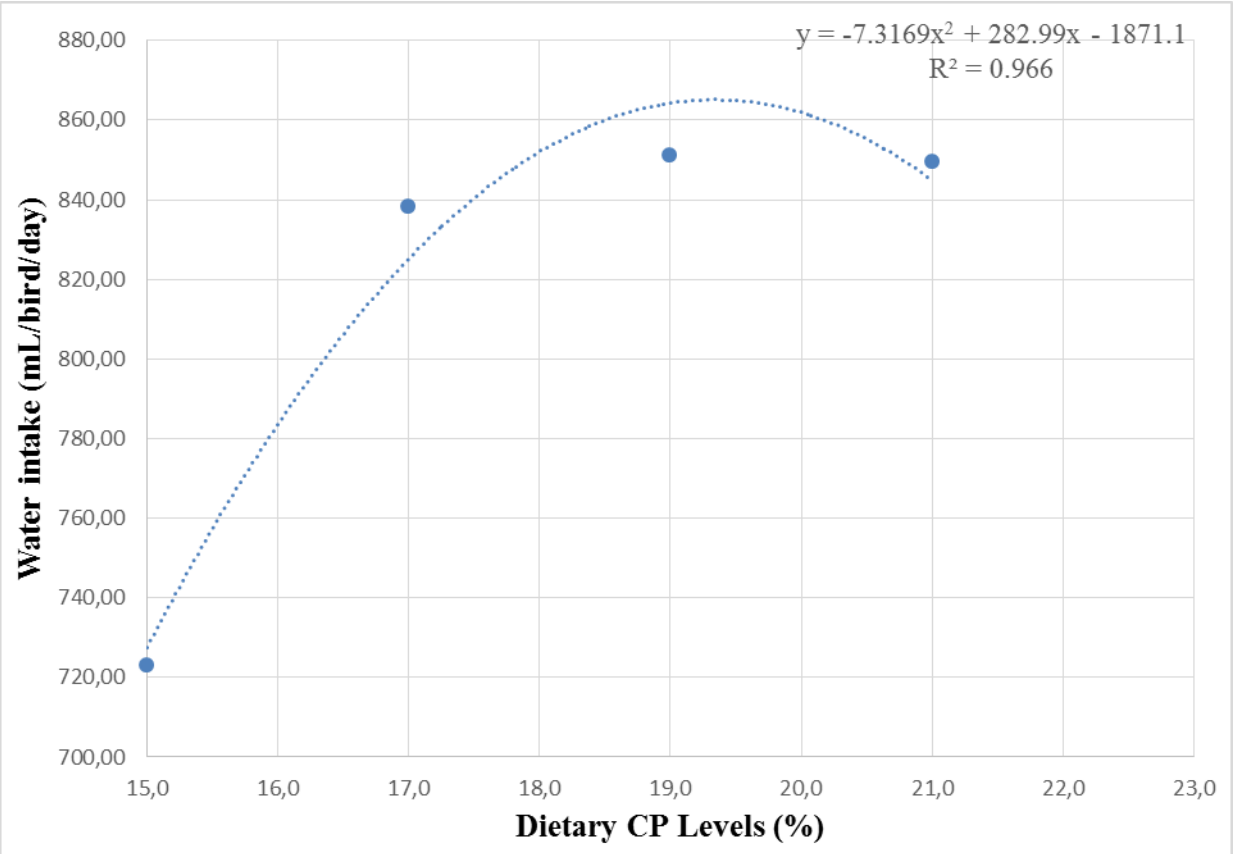
under heat stress condition. The R^2 value (0.99) indicated that about 99 % of the observed changes in PER were as a result of dietary crude protein levels.



CP – Crude protein

Fig. 3. Relationship between dietary crude protein levels and protein efficiency ratio in heat-stressed broiler chickens at finisher phase

Figure 4 revealed the relationship between varying levels of dietary crude protein and water intake in heat-stressed broiler chickens at finisher phase. An optimum level of dietary CP was observed at 19.5 % in heat stressed birds and decreases significantly with increasing dietary CP under heat stress condition. The R^2 value (0.97) indicated that about 97 % of the observed changes in water intake of heat-stressed birds at finisher phase were as a result of dietary crude protein levels.



CP – Crude protein

Fig. 4. Relationship between dietary crude protein levels and water intake of eat-stressed broiler chickens at finisher phase

Table 3 revealed the percentage deviation from the standard effect and resultant effect of early endogenous thermal acclimatization on performance of heat-stressed broiler finishers. At pre-starter phase, a 2 % decrease in dietary CP resulted in a 4.45 % reduction in FI, while a 4 % dietary CP reduction resulted in 8.09 % reduction in FI. However, a 6 % dietary CP reduction resulted in about 19.90 % reduction in FI at pre-starter phase. A 2 % dietary CP reduction resulted in a 15.50 % reduction in BWG in pre-starter chicks, and 4 % dietary CP reduction resulted in about 13.52 % reduction in BWG, while 6 % reduction in dietary CP resulted in about 31.11 % reduction in BWG in heat-stressed pre-starter broiler chicks. A 13.61 % increase in FCR was observed in birds on 2 % dietary CP reduction, while those on 4 % dietary CP reduction had about 6.12 % increase in FCR. However, those on 6 % dietary CP reduction had about 17.01 % increase in FCR, at pre-starter phase.

Gain: feed ratio (feed efficiency) of pre-starter chicks on 2 % dietary CP reduction resulted in about 11.76 % reduction, while a 4 % dietary CP reduction resulted in about 5.88 % reduction in feed efficiency. However, a 6 % reduction in dietary CP resulted in about 13.24 % reduction in feed efficiency. A 2 % decrease in dietary CP resulted in about 12.76 % reduction in protein intake, while a 4 % decrease in dietary CP resulted in about 24.08 % reduction in PI. However, a 6 % decrease in dietary CP resulted in about 40.80 % decrease in PI at pre-starter phase. The PER value observed from a 2 % dietary CP reduction was about 1.48 % lower than the standard effect. However, a 4 % dietary CP reduction resulted in about 15.43 % increase in PER, while a 6 % dietary CP reduction resulted in about 23.15 % increase in PER of heat-stressed pre-starter chicks. A 2 % dietary CP reduction also resulted in about 9.87 % reduction in WI compared to the standard effect, while a 4 % dietary CP reduction resulted in about 1.51 % reduction in WI. However, a 6 % reduction in dietary CP resulted in about 9.20 % reduction in WI.

At finisher phase, a 2 % reduction in dietary CP resulted in about 8.97 % reduction in FI, while a 4 % and 6 % reduction in dietary CP resulted in 16.42 % and 17.74 % reduction in feed intake, respectively. A 2 % reduction in dietary CP resulted in about 0.35 % increase in BWG, while

4 % and 6 % dietary CP reduction resulted in 12.54 % and 28.06 % reduction in BWG, respectively. For FCR, a 2 % dietary CP reduction resulted in about 11.37 % reduction in FCR, while a 4 % reduction resulted in about 6.16 % decrease in FCR. However, a 6 % dietary CP reduction resulted in about 11.37 % increase in FCR value of heat-stressed broiler chickens at finisher phase. Feed efficiency in heat-stressed broiler chickens on a 2 % dietary CP reduction resulted in about 10.20 % increase compared to the standard effect. A 2 % reduction in dietary CP resulted in 17.64 % decrease in PI, while a 4 % and 6 % dietary CP reduction resulted in about 32.34 % and 41.24 % decrease in PI, respectively. A 2 % dietary CP reduction resulted in 21.98 % increase in PER, while a 4 % and 6 % reduction in dietary CP resulted in 28.88 % and 22.84 % increase at finisher phase, respectively.

A 2 % reduction in dietary CP at finisher phase resulted in 0.19 % increase in WI compared to the standard effect. A 4 % and 6 % dietary CP reduction resulted in about 1.29 % and 14.87 % decrease in WI of heat-stressed broiler chickens at finisher phase.

Resultant effect

Early endogenous thermal acclimatization resulted in about 4.52 % increase in FI of birds on a 2 % dietary CP reduction, while a 4 % reduction in dietary CP resulted in about 8.32 % increase in FI. However, a 6 % dietary CP reduction resulted in about 2.17 % reduction in FI. Early endogenous thermal acclimatization resulted in about 15.85 % increase in BWG at finisher phase, in birds on 2 % dietary CP reduction, while about 0.98 % and 3.05 % increase in BWG were observed in broiler finishers on 4 % and 6 % dietary CP reduction, respectively. Early endogenous thermal acclimatization resulted in about 24.98 %, 12.28 % and 5.63 % improvement in FCR of birds on 2 %, 4 %, and 6 % dietary CP reduction. Feed efficiency was improved at finisher phase in birds on 2 %, 4 % and 6 % dietary CP reduction in the order: 21.97 %, 9.96 % and 0.99 %, respectively. It was observed that early endogenous thermal acclimatization resulted in about 4.88 %, 8.26 % and 0.44 % increase in protein intake of heat-stressed broiler chickens at finisher phase. Early endogenous heat acclimatization resulted in about 23.47 %, 13.45 % and 0.30 % improvement in PER of heat-stressed birds on 2 %, 4 % and 6 % dietary CP reduction, respectively. Early endogenous thermal acclimatization resulted in about 10.07 % and 0.22 % reduction in WI of heat-stressed birds on 2 % and 4 % dietary CP reduction, respectively. However, birds on 6 % dietary CP reduction resulted in about 5.67 % increase in WI.

Table 3. Percentage Deviation from the standard effect and resultant effect of early endogenous thermal acclimatization on performance of heat-stressed broiler finishers

Phase	Treatments	δ % FI	δ % BWG	δ % FCR	δ % Gain :feed	δ % PI	δ % PER	δ % WI
Pre-starter	T1	0	0	0	0	0	0	0
	T2	4.45	15.50	-13.61	11.76	12.76	1.48	9.88
	T3	8.09	13.52	-6.12	5.88	24.08	-15.43	1.51
	T4	19.91	31.11	-17.01	13.24	40.80	-23.15	9.20
Finisher phase	T1	0	0	0	0	0	0	0
	T2	8.97	-0.35	11.37	-10.20	17.64	-21.98	-0.19
	T3	16.42	12.54	6.16	-4.08	32.34	-28.88	1.29
	T4	17.74	28.06	-11.37	12.24	41.24	-22.84	14.87
Resultant effect (R)	T1	0	0	0	0	0	0	0
	T2	4.52	-15.85	24.98	-21.97	4.88	-23.47	-10.08
	T3	8.32	-0.98	12.28	-9.96	8.26	-13.45	-0.22
	T4	-2.17	-3.05	5.63	-0.99	0.44	0.30	5.67

δ % – Percentage deviation from the standard effect; FI- Feed intake, BWG-Body weight gain, FCR-Feed conversion ratio, PI-Protein intake, PER- Protein efficiency ratio, WI-Water intake.

Table 4 shows the correlation between performance parameters and protein intake of heat-stressed broiler chickens at finisher phase. It was observed that feed intake had a strong and positive correlation with PI ($r = 0.99$; $P < 0.01$). Although, FI was also positively correlated to BWG

(r = 0.81), gain: feed (r = 0.35), water intake (r = 0.62), morning RT (r = 0.65), afternoon RT (r = 0.60) and TC (r = 0.53), yet no significant relationship was found. The BWG was positively correlated to PI (r = 0.88), but was not significant. The FCR was negatively correlated to PI (r = -0.39) and their relationship was not strong. A negative, but weak relationship existed between gain: feed and rectal temperatures of heat-stressed birds at finisher phase. The PI was positively correlated to WI (r = 0.73), morning RT (r = 0.55), afternoon RT (r = 0.49) and TC (r = 0.41), and were not significant. The PER was negatively correlated to PI (r =-0.85), morning RT (r = -0.91), afternoon RT (r = -0.88), and TC (r = -0.83). The WI is positively correlated to BWG (r = 0.93) in heat-stressed broiler chickens at finisher phase, though not significant. Morning RT had a strong and positive correlation with afternoon RT (r = 0.99; P < 0.01) and TC (r = 0.99; P < 0.01). The TC was negatively correlated to PER (r = -0.83; P > 0.05).

Table 4. Correlation between performance parameters and protein intake of heat-stressed broiler chickens at finisher phase

Parameters	FI	BWG	FCR	Gain: feed	PI	PER	WI	Morning RT	Afternoon RT	TC
FI		0.81^{ns}	-0.27^{ns}	0.35^{ns}	0.99^{*}	-0.91^{ns}	0.62^{ns}	0.65^{ns}	0.60^{ns}	0.53^{ns}
BWG	0.81^{ns}		-0.78^{ns}	0.83^{ns}	0.88^{ns}	-0.49^{ns}	0.93^{ns}	0.09^{ns}	0.02^{ns}	-0.07^{ns}
FCR	-0.27^{ns}	-0.78^{ns}		-0.99^{**}	-0.39^{ns}	-0.15^{ns}	-0.88^{ns}	0.55^{ns}	0.60^{ns}	0.67^{ns}
Gain: feed	0.35^{ns}	0.83^{ns}	-0.99^{**}		0.47^{ns}	0.07^{ns}	0.90^{ns}	-0.48^{ns}	-0.54^{ns}	-0.61^{ns}
PI	0.99^{*}	0.88^{ns}	-0.39^{ns}	0.47^{ns}		-0.85^{ns}	0.73^{ns}	0.55^{ns}	0.49^{ns}	0.41^{ns}
PER	-0.91^{ns}	-0.49^{ns}	-0.15^{ns}	0.07^{ns}	-0.85^{ns}		-0.28^{ns}	-0.91^{ns}	-0.88^{ns}	-0.83^{ns}
WI	0.62^{ns}	0.93^{ns}	-0.88^{ns}	0.90^{ns}	0.73^{ns}	-0.28^{ns}		-0.11^{ns}	-0.19^{ns}	-0.28^{ns}
Morning RT	0.65^{ns}	0.09^{ns}	0.55^{ns}	-0.48^{ns}	0.55^{ns}	-0.91^{ns}	-0.11^{ns}		0.99^{**}	0.99^{*}
Afternoon RT	0.60^{ns}	0.02^{ns}	0.60^{ns}	-0.54^{ns}	0.49^{ns}	-0.88^{ns}	-0.19^{ns}	0.99^{**}		0.99^{**}
TC	0.53^{ns}	-0.07^{ns}	0.67^{ns}	-0.61^{ns}	0.41^{ns}	-0.83^{ns}	-0.28^{ns}	0.99^{*}	0.99^{**}	

*P < 0.05; ** P < 0.01, ns – not significant; FI – Feed intake, BWG – Body weight gain, FCR – Feed conversion ratio, PER – Protein efficiency ratio, WI – Water intake, RT – Rectal temperature, TC – Temperature change.

4. Discussion

Early endogenous heat manipulation has resulted in enhanced broiler performance at finisher phase, even in the face of thermal oscillation as observed in present study. Heat-stressed birds are characterized by a sharp neglect of feed or reduction in marginal feed intake. However, endogenous heat load reduction through dietary crude protein and electrolyte inter-play have resulted in a proportionate increase in feed intake at finisher phase where the effect of thermal stress is more felt due to increased metabolic processes and feathering. Contrarily, birds on a 6 % reduction in dietary CP from the standard exhibited about 2.17 % reduction in feed intake at finisher phase and this is symbolic to a clinical organism struggling to ensure thermal equilibrium. The result of present study is consistent with the report of Si et al. (2004) who also noted reduced appetite in birds fed low-CP diets. From current study, a 2 % reduction in dietary CP at pre-starter phase resulted in about 15.8 % increase in BWG. Early endogenous thermal manipulation resulted in about 25 %, 12 % and 5 % improvement in FCR for heat-stressed birds on 2 %, 4 % and 6 % dietary CP reduction as against the standard. Present findings were consistent with the assertions of Yahav, Hurwitz (1996) who noted that broiler chicks exposed to high temperatures at pre-starter period maintained higher rate of viability when challenged with higher temperatures at finisher phase, and concluded that such adaptive responses were due to previous exposure to heat stress. The results of present study were consistent with the assertions of Oliveira et al. (2006), who affirmed that performance decline in broilers under heat stress conditions resulted from the inability of birds to expel excess endogenous heat load. Current findings also contradicted the report of Cheng et al. (1997) who noted that the provision of higher crude protein in diets of heat-stressed broilers, in attempt to compensate for low appetite, is detrimental to production

parameters. However, contrasting reports were documented by Temim et al. (2000), affirming that low-CP diets are not beneficial to poultry under heat stress conditions. Present findings have proven that extremely low CP diets are not indeed beneficial to heat-stressed broiler chickens at finisher phase as it appears that a dietary crude protein threshold exists for heat-stressed broiler chickens. Similar reports by Awad et al. (2014) affirmed present findings, as they reported a decrease in performance of broilers fed diets with low CP, even when all essential amino acid requirements are met. However, Popoola et al. (2020d) hypothesized that extremely low CP diets are unbeneficial to broilers in a state of thermal comfort, but when heat-stressed, these low CP diets become beneficial in reducing endogenous heat contribution and increasing the efficiency of nutrients. The results of present study are consistent with the reports of Buyse et al. (1992) who noted reduced growth performance in response to increased heat production in male broiler chickens, while affirming that the heat increment in broilers fed a lower protein diet was as a result of elevated plasma triiodothyronine (T3) concentration, which may consequently increase heat production. From current study, water intake was reduced in heat-stressed broiler chickens on reduced dietary CP, indicating a decline in overall heat load. This feedback showcases a significant relationship between dietary CP levels and water intake trend in heat-stress broilers. Soleimani et al. (2012) reported that performance of broiler chickens was negatively affected by dietary CP level reduction at both starter and finisher phases, regardless of feed-grade amino acid manipulation. Although, Aftab et al. (2006) established that the reduction of dietary CP will reduce both essential and non-essential amino acids, and could alter the balance of such in low-CP diets, with an aftermath on feed intake as the amount of ingested free amino acids into the blood stream increases in birds on low CP diets and may affect the balance of plasma amino acid profile. Waldroup (2007) noted that the reduced performance in broilers on low-CP diets could be associated with insufficient nitrogen for non-essential amino acid synthesis, because chickens on standard levels of dietary protein can synthesize the non-essential amino acids from excess essential amino acids, but when absent or in low supply, synthesis is reduced and performance decline is aggravated. Ali, Hossain (2010) stated that heat stress in broilers can be reduced by early-age thermal conditioning, as it is a sensitive process of induction of thermo-tolerant traits in immature neonatal chicks at an early age, by developing the hypothalamus, which is the thermo-regulatory centre, and has been proven to be the most appropriate age to exploit and induce thermotolerance in broiler chickens (Yahav et al., 2005).

5. Conclusion

Dietary CP levels of 17 % and 19 % enhanced protein efficiency ratio in heat-stressed broiler chickens at finisher phase. Low crude protein diets improved performance of heat-stressed broiler chickens at finisher phase. However, a 2 % dietary CP reduction (19 % CP) best enhanced performance of heat-stressed broiler chickens at finisher phase. Some parameters that were supposed to strongly correlate were not as a result of the influence of early endogenous manipulation. Endogenous heat load reduction through dietary crude protein and electrolyte inter-play have resulted in a proportionate increase in feed intake, body weight gain, protein efficiency ratio and enhanced feed conversion ratio in heat-stressed broiler chickens at finisher phase. The resultant effect of early endogenous thermal acclimatization on performance of heat-stressed broiler chickens at finisher phase has been beneficial.

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Conflicts of Interest

The authors declare no conflicts of interest as regarding the publication of this paper.

Ethical Approval

The study received the ethical approval of the Institutional Animal Care and Use Committee, through the Agricultural Biochemistry and Nutrition Unit of the Department of Animal Science, University of Ibadan, Nigeria.

Contributorship

I.O. Popoola designed, implemented and analyzed data; O.R. Popoola and I.O. Popoola drafted the manuscript. I.F. Olaleru, I.O. Busari, F.J. Oluwadele, O.M. Ojeniyi and Q.T. Alegbejo reviewed, contributed and approved the final draft of the manuscript.

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