Sorghum – Garlic Blended Diet Prophylactically Fed to Experimental Rats Prevent the Establishment of trypanosomes in the T. b brucei- Infected Animals

M. H. Garba¹,²*, A. Abubakar², A. Abdulkadir², Y. Garba³, M. Inuwa⁴, L. M. Hafsa⁵, B. J. Lekene¹ and S. Abubakar¹

¹Department of Animal Production Technology, Biochemistry and Nutrition Unit, Federal College of Wildlife Management, PMB 268, New Bussa, Niger State, Nigeria.
²Department of Biochemistry, Malaria and Trypanosomiasis Research Unit, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria.
³Department of Biological Sciences, Federal College of Education, Kontagora, Niger State, Nigeria.
⁴Department of Animal Production Technology, Veterinary Clinic Unit, Federal College of Wildlife Management, PMB 268, New Bussa, Niger State, Nigeria.
⁵Department of Basic Sciences, Biological Sciences Unit, Federal College of Wildlife Management, PMB 268, New Bussa, Niger State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between all authors. Author MHG designed the study, wrote the protocol and supervised the work. Authors LMH, BJL and SA carried out all laboratories work and performed the statistical analysis. Author MI managed the analyses of the study. Author YG wrote the first draft of the manuscript. Authors A. Abubakar and AA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/24356
Editor(s): (1) Carmen Lucia de Oliveira Petkowicz, Federal University of Parana, Curitiba, Parana, Brazil.
Reviewers: (1) Uttara Singh, Bundelkhand University, India. (2) Cimanga Kanyanga, University of Antwerpen, Belgium. (3) James Adams, University of Southern California, USA.
Complete Peer review History: http://sciencedomain.org/review-history/14201

Received 16th January 2016
Accepted 4th March 2016
Published 15th April 2016

ABSTRACT

Aim: This research work set to investigate the effect of feeding a mixture of two functional foods with a view to ascertain their therapeutic effect against Trypanosoma brucei brucei in challenged rats.
**Experimental Design:** Complete randomized clinical trial design was used in the experiment. The rats were blocked for sex and grouped into A, B, C, D, E, F, G, H, I, and J respectively. Each group consists of three (3) rats.

**Place and Duration of the Study:** The research work was conducted in the Biochemistry and Nutrition laboratory in the Department of Animal Production Technology, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. Four weeks feeding trial was employed in the cause of the experiment.

**Methodology:** A total of 30 albino Wister rats were randomly grouped into ten (10) treatments each containing 3 rats (i.e. A- J). To groups A- E, feed containing graded level of inclusion of blended Sorghum- Garlic (10:1) at 5, 10, 15, 20, and 25% level was fed to them. Group F, were fed the conventional feed only (i.e. negative control). Group G, were fed solely with the pulverised *sorghum bicolor* (without Garlic added to it). Group H, were fed with conventional feed supplemented with garlic only. Another group of three rats, (i.e. group I) were fed with the supplemented diet with the average inclusion rate of 15% for 3 days prior to infection. To the final group (i.e. group J), as in group I but the feeding period prior to infection was extended to 7 days. Parasitaemia was thereafter monitored on two days interval while the effects of non prophylactic feeding (25%) and seven days prophylactic feeding (at 12.5%) of the experimental diet on the haematological parameters of rats was also determined.

**Results:** Seven days prophylactic feeding of the experimental animals with 12.5% inclusion of the sorghum-garlic prior to their infection with the parasites proves to be effective against the establishment and subsequent proliferation of the parasites. Also, significant difference ($P = 0.05$) in the haematological parameters was observed between the group prophylactically fed the sorghum-garlic supplemented diet for seven days period at 12.5% inclusion and the group fed at 25% inclusion and infected just a day after.

**Conclusion:** This study demonstrate the potency of prophylactic feeding of sorghum-garlic (functional foods) as nutraceutical against trypanosomiasis and as a haematopoietic agent.

### Keywords: Sorghum; garlic; trypanosome; nutraceutical; Haematopoietic agent.

### 1. INTRODUCTION

According to the World Health Organization (WHO) human African trypanosomiasis (HAT) is one of the deadliest disease found on the earth [1,2]. No vaccine is developed, and there is no commercial interest in developing new drugs [3]. Three of the four main drugs (i.e Suramin (1916); Pentamidine (1937); Melarsoprol (1946)) used in treatment were registered before 1950. The most recent drug (i.e Efflornithine (1977)) was initially developed as an anti-cancer drug, only by coincidence found to be effective against HAT. HAT is a disease which the affects poorest of the poor [4]. Despite the fact that man is the natural mammalian host for trypanosomes, studies on animals revealed the involvement of numerous wild animals such as non-human primates, reptiles, antelopes, and wild bovid in its transmission and sustenance [5]. Both are usually transmitted by the bite of an infected tsetse fly and are more common in rural areas [6]. Detection of infection is via finding the parasite in a blood smear or in the fluid of lymph node of the suspected individual [7].

Animal African trypanosomiasis (AAT) is a serious and delabidating disease of livestock caused by protozoan parasite of the genus *Trypanosoma* and transmitted cyclically by tsetse (*Glossina*) and mechanically by other hematophagous flies such as *Tabanus, Haomatopota, Stomoxys and chrysoys* [8]. Two tsetse transmitted parasites, *T. brucei gambiense* and *T. brucei rhodesiense*, cause human African trypanosomiasis (HAT)/sleeping sickness, which affects both humans and animals [1]. The remaining tsetse-transmitted trypanosomes primarily affect animals and cause African animal trypanosomiasis (AAT). The most important species in this disease are *Trypanosoma congolense, T. vivax* and *T. brucei brucei*. Other species such as *T. simiae* and *T. godfreyi* can also cause AAT.

It is major obstacle to livestock production on the African continent as it prevents full use of land to feed the rapidly increasing population. The limitations due to tsetse and trypanosome problems continue to frustrate efforts and prevent progress in the livestock and crop production thus contributing to hunger, poverty and the suffering of entire communities in Africa [9]. This disease is therefore a serious impediment to agricultural and economic advancement in the affected areas [10]. The economic losses due to
African continents with approximately 7 million acres of fertile land spread across 36 countries on the continent of Africa only 10 million cattles are found within the tsetse belt due to the presence of tsetse flies [12]. Added to the economic losses is the tsetse infestation of about 10 million km² of the land being suitable for mixed agriculture if this disease was controlled [11]. Out of 165 million cattle found in Africa only 10 million are found within the tsetse belt due to the disease constraint and these are the lowest in the world [13,14]. This devastating disease vastly and directly affects the milk and meat productivity of animals, reduce the birth rate and increase the abortion rate as well as mortality rate which cumulatively affect the herd size and herd composition [12,15].

A nutraceutical is referred to as “any substance that is a food or a part of a food that provide medical or health benefits; including the prevention and treatment of diseases” [16]. Today, researchers have identified hundreds of compounds from both plants and animals sources with functional qualities, and they continue to make new discoveries surrounding the complex benefits of phytochemicals (non-nutritive plant chemicals that have protective or disease preventive properties) in foods. In Japan, England and other countries, nutraceuticals already have become part of the dietary landscape. Due to risk of toxicity or adverse effect of drug, consumers are turning massively to food supplements to improve health where pharmaceutical fails. This resulted in a worldwide nutraceutical revolution [17,18]. Over 470 nutraceuticals and functional food products are presently available with documented health benefits [19]. Many of these new products that are being promoted to treat various diseases find their origin in the plant kingdom [19]. This is an obvious choice as many plants produce secondary compounds such as alkaloids, saponins, terpenoids, tannins, phenols etc to protect themselves from infections and these constituents may be useful in the management of human infection. Many of the phyto-medicines are the typical examples. The old proverb “an apple a day will keep the doctor away” is now replaced by “a nutraceutical a day may keep the doctor away.”

Grain sorghum (Sorghum bicolor L. Moench;) ranks fifth in worldwide production among cereal crops after wheat, rice corn and barley [20,21]. Sorghum is used for human consumption and animal production [20] as well as industrial products such as alcohol [22]. Over 55% of sorghum grain produced in the world is used for human consumption and about 33% of grain used in animal nutrition [23]. Recent research has demonstrated that the consumption of whole grains reduces the risk of some major human diseases due to the dietary fibre and phytochemicals, which are mainly concentrated in the bran, such diseases are cardiovascular disorders, neuron degeneration, cancer, diabetes and hypercholesterolemia as well as being involved with the process of ageing [24,25]. Sorghum is a rich source of various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols and policosanols [26]. The phytochemicals have potential to significantly impact human and animal health. Sorghum grain has 95 to 98% of the nutritional value of maize; vitamin content for corn and sorghum is similar but sorghum has a higher mineral content than maize [27]. Its rich antioxidant properties makes Sorghum grain to have a lot of nutritional benefits [28]. It is higher in protein (11.5 to 16.5%) and calories than several other grains [29]. One hundred gram (100 g) of sorghum was found to contain 143 g of carbohydrate, 12 g of dietary fibre, and would provide 47% of the recommended daily value for iron based on a 2,000 calorie intake [30]. The same 100 g of sorghum contains 325 calories and has 10.8 mg of protein, 0 mg of sugar, 3.1 mg of fat, 6.0 mg of fibre and 0 mg of cholesterol. Vitamins and minerals such as: B₁, B₂, B₃, calcium (Ca) recommended dietary allowance (RDA) of 5.4%, potassium (K) 19% RDA, iron (Fe) 47% RDA, phosphorus (P) 55%, and sodium (Na) 0.5% RDA were found to be contained in sorghum [30].

*Allium sativum*, commonly known as garlic, is a species in the onion genus, *Allium*. Its close relatives include the onion, shallot, leek, chive, [31,32]. With a history of human use of over 7,000 years, garlic is native to central Asia, [33] and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. A member of the Liliaceae family, garlic (*Allium sativum*) is a cultivated food highly regarded throughout the world. Originally from Central Asia, garlic is one of the earliest of cultivated plants [34]. In 1997, garlic was the most widely used natural supplement in US households. Garlic was also shown to be used more than...
twice as much as any other natural supplement [35].

That sorghum contained and in sufficient proportion daily requirement allowance (RDA) of most of the vitamins, minerals and other macromolecules required for all the physiological activities required to sustain life. Its consumption was revealed to strengthens the immune system, helps in the elimination of toxic waste from the body, increases endurance, assists in blood cells building, boost appetite, relieves diarrhoea, aids rapid recovery, stimulates free flow of blood and lowers cholesterol levels [36]. Sorghum is also reported to contain varied array of phenolic compounds such as simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids (flavanols, flavones, flavanones, isoflavones and anthocyanins), chalcones, aurones (hispidol), hydroxycoumarins, lignans, hydroxystilbenes and polyflavans (proanthocyanidins and prodeoxyanthocyanidins) [37,38]. The main thrust of this work is therefore to investigate the possible synergistic activities of the various phytochemicals from these plant samples (i.e.Sorghum and garlic) with a view to come up with a possible functional food(s) that is easily obtainable, affordable, accessible and above all commonly available in the areas mostly ravaged by this dilapidating, and neglected “orphaned” disease of the tropics.

2. MATERIALS AND METHODS

2.1 Samples Collection and Preparation

Ten kilogram (10 Kg) of red variety of *Sorghum bicolor* along with 1000 g (1 Kg) of *Allium sativum* (Garlic variety) were purchased from the Sabo/Wawa Market in Borgu Local Government Area of Niger State, Nigeria. The sorghum was soaked for 2-3 days until it begins to sprout. It was then drained and allowed to dry at room temperature until the moisture content is about 10-15%. One thousand gram (1000 g) of garlic was crushed using pestle and mortar and then mixed with the dried sorghum in the ratio 10:1. The mixed sample was then pulverised and sieved through to obtained fine powder. Conventional rat feed was obtained from the sales point within Minna metropolis in Niger State. Graded level inclusion of the Sorghum-garlic blend at 5, 10, 15, 20, and 25% was prepared.

2.2 Trypanosomes Used

The breed of the parasite used in this work is *T. b. brucei* which affects both man and domestic animals. The strain of the parasites was obtained from the Nigeria Institute of Trypanosomiasis Research (NITR), Kaduna field station, Kaduna State. The parasite was maintained in our Laboratory by serial passage to healthy rats.

2.3 Experimental Animals

A total of 30 Albino Wister rats weighing between 137 – 152 g were purchased from Food and Nutrition Dept. of the Niger State Polytechnic, Zungeru, Niger State, Nigeria. They were acclimatized for seven (7) days in the Biochemistry and Nutrition Laboratory of Animal Production Technology Department, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. and used in the experiment. The animals were grouped into ten (10) treatments each containing 3 rats (i.e. A- J).

2.4 Feeding Experiment and Parasitaemia

From the mean of daily consumption of conventional feed determined (i.e.168g±8.6), 5% (8.4 g), 10% (16.8 g), 15% (25.2 g), 20% (33.6 g) and 25% (42 g) of the conventional feed was replaced with the sorghum-garlic blend which was administered to groups A- E respectively. Group F, were fed 12.5% of the conventional feed substituted with the pulverized sorghum only (21 g). Group G, were fed 12.5% the conventional feed substituted with of garlic only (21 g). Group H, were fed 15% of the conventional feed substituted with sorghum-garlic blend (25.2 g) for three (3) days prior to infection, Group I were fed 15% as in Group H but the feeding period prior to infection was extended to seven (7) days. Group J received 100% conventional feed and served as negative control. All the feeding experiment lasted for -21 days. In all the treatments and control, the monitoring of parasitaemia was simultaneously carried out fortnightly according to the “rapid matching” method of Herbert and Lumsden, [38]. All the animals in groups A-G were infected a day after the feeding trial commenced, while those in groups H and I were infected on the 3rd and 7th day after the feeding with the substituted sorghum-garlic has commenced. Groups J served as negative control.

The infection was carried out as thus: Blood was collected by cardiac puncture with ethylenediaminetetraacetic (EDTA) acid-coated syringe from a heavily infected mouse and immediately diluted with physiological saline to serve as the inoculums. Healthy mice were infected intraperitoneally (i.p.) with 0.02 ml of the
diluted blood containing $1 \times 10^6$ trypanosomes. Monitoring of parasitaemia was done every 48 hr by microscopic examination of blood sample taken from the tail of infected rats pre-sterilised with Methylated spirit [39].

2.5 Determination of Hematological Parameters

The haematological components including haemoglobin, haematocrite, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), granulocyte count (GRA), lymphocytes, platelete count, mean platelete volume (MPV), Platelatecrit and platelete distribution weight (PDW) were determined in the group prophylactically fed at 12.5% inclusion for 7 days prior to infection in which clearance was achieved and a group fed at 25% inclusion and infected 24hrs later, using the automated haematological analyzer Sysmex kx21, as reported by Bashir et al. [40].

2.6 Statistical Analysis

Data obtained in this study was subjected to a one-way analysis of variance (ANOVA) to derive mean values of parasitaemia which was compared with least significant difference.

3. RESULTS AND DISCUSSION

The prophylactic benefits of food plants are being investigated for potential use as novel medicinal remedies due to the presence of pharmacologically active compounds. The result from this study clearly indicates that feeding rats with diets mixed with Garlic- Sorghum (as functional foods) non prophylactically at the inclusion level of 5-25% does not exert any trypanocidal nor trypanostatic effect in all the groups fed the functional food (Fig. 1). If not for Nok et al. and Nose, et al. [41,42] that reported on the in-vitro trypanocidal effect of garlic, there has not been any previous report on the efficacy of prophylactic feeding of either the sorghum or garlic against trypanosomes, but both due to their high phenolic contents, are known to portray some strong antioxidant activities [43-46]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals [47]. An interesting observation made is the inability of the parasites to survive beyond two days In vivo in animals fed the mixed diet at 15% inclusion of garlic-sorghum blend seven days prior to their infection (Fig. 3) whereas, those fed for three days prior to infection had the trypanosomes inoculated into them growing normally and eventually causing the havoc and all the characteristic pathologies it is known to cause but at period a bit longer than the negative control (Fig. 2). Feeding garlic (only) and Sorghum (only) as functional foods at 12.5% inclusion level separately (Fig. 1) does not also prevent or stop the growth and the subsequent multiplication of the trypanosomes in the infected animals.

The possible reason that can be attributed to the efficacy observed when the experimental animals were prophylactically fed sorghum-garlic blended diet is, while most plants, including other cereals, are lacking 3-deoxyanthocyanidins, e.g. apigeninidins and luteolinidins, sorghum is unique in containing a relatively high level of these compounds [48,49]. Sorghum grain is rich in health promoting phytochemicals: phenolic acids, sterols, policosanols, and anthocyanins. The most abundant anthocyanins in sorghum grain are 3-deoxyanthocyanidins, e.g. apigeninidin and luteolinidin [48,50,51]. The red color of the grain’s pericarp is essentially due to the presence of 3-deoxyanthocyanidins [51,52] and Tzu-Chieh, et al. [53] demonstrated the effective therapeutic effect of sorghum proanthocyanidins against human melanoma. The binding of proanthocyanidins with proteins participates in their antibacterial activity [54,55]. Proanthocyanidins have been shown to inhibit the growth of human immunodeficiency virus 1 (HIV-1), influenza virus, and herpes simplex virus by blocking their entry in the host cells [56,57]. The mechanism of proanthocyanidins toxicity against microbes is related to inhibition of hydrolytic enzymes, interactions to inactivate microbial adhesions and cell envelope transport proteins, and non-specific interaction with carbohydrates [58]. Among sorghum flavonols, the flavan-4-ols have particular therapeutic interest because of their antitumor activity [59,60,61]. Flavan-4- ols revealed strong host mediated antitumor activity, which is due to the enhancement of immune response of the host animals through the actions on tumor cells and some immunocytes [62,63]. The anti cancer activities of 3-deoxy anthocyanidins in sorghum pericarp against the HL-60 cell line, derived from a patient with acute leukemia, and HepG2 is an adherent cell line derived from liver tissues of a hepatocarcinoma patient has been well elaborated by [64]. While on one hand the green, dry “folds” in the center of the garlic clove are
especially pungent. The sulfur compound allicin, produced by crushing or chewing fresh garlic, produces other sulfur compounds: ajoene, allyl polysulfides (Diallylsulphides, Diallyldisulphides and Triallyltrisulphides) and vinylthiin. The presence of S-allylcysteine has also been observed in garlic. Despite the fact that Garlic is known for causing bad breath (halitosis) [65], as well as causing sweat to have a pungent “garlicky” smell, which is caused by allyl methyl sulfide (AMS) [66]. AMS is a volatile liquid which is absorbed into the blood during the metabolism of garlic-derived sulfur compounds; from the blood it travels to the lungs and from there to the mouth, causing bad breath; and skin, where it is exuded through skin pores [67,68]. Garlic has been used for thousands of years as a remedy for many different ailments, including intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, and symptoms of aging. Modern research indicates that garlic may help improve heart health in a number of different ways. It is a blood thinner that helps to lower both high blood pressure and blood triglycerides. The anti-arthritis property of garlic is due to diallyl sulphide and thiacremonone [69]. Garlic also has anti-inflammatory properties. Several population studies also show an association between an increased intake of garlic and a reduced risk of certain cancers, including colon, stomach, esophagus, pancreas, and breast cancer. [70,71]. Garlic’s anti-cancer properties are due to the allyl sulphides it contains [72]. One special interesting area of research on garlic and cancer prevention involves meat cooked at high temperatures. Heterocyclic amines (HCAs) are cancer-related substances that can form when meat comes into contact with a high-temperature cooking surface (400°F/204°C or higher). One such HCA is called PhiP (2-amino-1-methyl-6-phenylimidazopyridine). PhiP is thought to be one reason for the increased incidence of breast cancer among women who eat large quantities of meat because it is rapidly transformed into DNA-damaging compounds. Diallyl sulfide (DAS), one of the many sulfur-containing compounds in garlic, has been shown to inhibit the transformation of PhiP into carcinogens [73,74]. DAS blocks this transformation by decreasing the production of the liver enzymes (the Phase I enzymes CYP1A1, CYP1A2 and CYP1B1) that transform PhiP into activated DNA-damaging enzymes CYP1A1, CYP1A2, and CYP1B1) that transform PhiP into activated DNA-damaging enzymes. Additionally, garlic is a triple threat against infections, offering antibacterial, antiviral, and antifungal properties. Garlic has even been found to be effective at killing antibiotic-resistant bacteria, including MRSA. Observation has been made that virtually most of the drugs with trypanocidal activities were observed to also display some cytotoxic (Anti cancer) and antitumor activities; it can be seen that Anthocyanins, Pro anthocyanidins and 3-deoxyanthocyanidins contained in sorghum and the diallyl sulphide found in garlic inhibits the transformation of PhiP (a type of compound that has been associated with increased incidence of breast cancer) into carcinogen. The probable interaction of these compounds in sorghum and garlic might synergistically impair with one or more of the survival pathways of the trypanosome as observed (Fig. 3). Also, the positive immunomodulatory effect observed as a result of sustained feeding of the experimental animals (for seven days) with the sorghum-garlic (as functional foods) can clearly be seen from the Haematological parameters in (Table 1) which might be an additional attribute to the ability of the experimental animals to resist the infection by the parasites.

Currently, most antiparasitic drugs are considered orphan drugs with the main exception of antimalarials. Economic considerations of the pharmaceutical industry outweigh all others, because of the very low return of the developmental costs. Therefore, it is necessary to find alternative and cheaper ways to approach the treatment of trypanosomal disease [6]. This could be achieved by looking into the possibilities of utilising one or combination more than one functional food substances commonly available, affordable and accessible with nutraceutical properties that could serve the same purpose. Sorghum contains large quantities of phenolics and other compounds of use in human foods to prevent deterioration of health. Different phenols with varying properties exist, but relatively little effort to demonstrate the potential of these compounds in human health has been made. Of all the cereals, sorghum has the potential to be bred specifically to produce high levels of different phenols that can be easily concentrated by simple processes. These special sorghums have reasonable grain yields and agronomic characteristics that make them productive and trials to demonstrate their health promoting properties is required [75].
Fig. 1. Trypanocidal effect of various inclusion level of sorghum-garlic blend in the conventional feed

Fig. 2. Prophylactic activity of the functional food fed for three days prior to infection
8

Fig. 3. Prophylactic activity of the functional food fed for seven days prior to infection

Table 1. Effects of Non prophylactic feeding and 7-days prophylactic feeding of the experimental diet on haematological parameters of rats

| Parameters                  | Control rats (fed solely conventional feed) | Fed 12.5% inclusion 7 days prior to infection | Non prophylactic 25% inclusion |
|-----------------------------|---------------------------------------------|---------------------------------------------|--------------------------------|
| WBC (x 10^9 /L)             | 3.71±1.11a                                  | 6.58±1.18a                                  | 5.29±0.90a                     |
| Granulocytes (%)            | 0.80±0.70a                                  | 0.71±0.24a                                  | 2.59±0.80b                     |
| Lymphocytes (x 10^9 /L)     | 2.58±0.60a                                  | 4.50±0.58b                                  | 3.86±0.79b                     |
| MCTC (x10^3/L)              | 0.21±0.08a                                  | 0.12±0.005a                                 | 0.28±0.37b                     |
| RBC(x10^12/L)               | 2.69±0.10a                                  | 2.89±0.05a                                  | 0.48±0.77b                     |
| Haemoglobin (g/L)           | 96.23±1.20a                                 | 94.78±1.85a                                 | 95.00±5.03a                    |
| MCH (pg)                    | 29.96±1.59a                                 | 31.50±0.35a                                 | 29.67±1.31a                    |
| MCHC (g/L)                  | 388.33±2.74a                                | 434.87±3.60a                                | 399.00±2.98a                   |
| MCV (fL)                    | 99.23±1.20b                                 | 94.93±1.85b                                 | 73.33±0.33b                    |
| RCDW-cv (fL)                | 41.13±0.08a                                 | 36.23±3.16a                                 | 41.43±0.93a                    |
| RCDW-sd (fL)                | 18.00±0.36a                                 | 15.97±0.11a                                 | 16.00±0.36a                    |
| PC (x 109/L)                | 336.78±36.08a                               | 806.65±6.83a                                | 759.00±26.50c                  |
| MPV (fL)                    | 9.00±0.09a                                  | 9.79±0.35a                                  | 13.01±0.30b                    |
| Plateletcrit (L/L)          | 0.64±0.15a                                  | 1.48±0.15a                                  | 1.78±0.17b                     |
| PDW (%)                     | 19.46±0.41a                                 | 18.59±0.03a                                 | 26.23±1.11b                    |

LY: Lymphocytes; MCTC: Mid cell total count; RCDW-cv: Red cell width coefficient of variation; RCDW-sd: Red cell width standard deviation; PC: Platelet count; Values are mean±SEM of 5 determinations. The values along the same row with different superscripts are significantly different (P = 0.05)

4. CONCLUSION

Result obtained from this studies reveals the nutraceutic effect against trypanosome infection and also haematopoietic potentials of sorghum-garlic blend when incorporated in the diet at a determined inclusion level (12.5%). This finding therefore, provide a cheap and readily available disease management alternative for those in the tse tse fly infested areas, those migrating across the tse tse infested belt or those in areas ravaged by civil unrest/wars(e.g south Sudan, Congo DR and Central Africa republic) whose environment has been re-colonised by the tse tse flies.
ETHICAL APPROVAL

Authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organisation (W.H.O) Media Centre (March, 2014). Fact sheet N° 259: Trypanosomiasis, Human African (Sleeping sickness). (Retrieved 25 Nov., 2015).
2. Frost L. Dental management of the tropical disease human African trypanosomiasis: an unusual case of pseudobulbar palsy. Br. Dent. J. 2011;8:210(1):13-6.
3. Esterhuizen J, Njiru B, Vale GA, Lehane MJ, Torr SJ. Vegetation and the importance of insecticide-treated target siting for control of Glossina fuscipes fuscipes. PLoSNegl Trop Dis. 2011;5(9):e1336.
4. Ngoyi DM. Shortening of the post-treatment follow-up in gambiense human African trypanosomiasis. Antwerpen. 2010;33(8):430-7
5. Mbaya A, Kumshe H, Nwosu CO. The mechanism of anaemia in trypanosomiasis: A review. In: Silverberg S, editor. Anemia. Croatia: In Tech. 2012;269-82.
6. Equitable and affordable health coverage against neglected tropical diseases is achievable (W.H.O). Geneva, 19 February 2015 – The third WHO report on neglected tropical diseases. Fact sheet N°259 Updated May 2015.
7. Kennedy PG. Clinically features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). Lancet Neurology 2013;12(2):186-94. DOI: 10.1016/S1474-4422(12)70296-X PMID: 23260189
8. Baldacchino F, Muenworn V, Desquesnes M, Desoli F, Charoenviriyaphap T, Duvallet G. Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): A review. Parasite. 2013;20:26. Epub 2013 Aug 29.
9. Shimelis M, Mekonnen A, Abebe F. Study on the prevalence of major trypanosomes affecting bovine in tsetse infested Asosa District of Benishangul Gumuz Regional State, Western Ethiopia. Global Veterinaria 2011;7(4):330-336.
10. Janvan DA, Brice R. New insights in the interactions between African trypanosomes and tsetse flies. Front Cell Infect Microbiol. 2013;3:63. (Published online 2013 Oct 16). DOI: 10.3389/fcimb.2013.00063 PMCID: PMC3797390
11. Van den Bossche P, de La Rocque S, Hendrickx G, Bouyer J, Lu L. A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. Trends Parasitol. 2010;26(5):236-43. DOI: 10.1016/j.pt.2010.02.010 Epub 2010 Mar 19.
12. Mersha C, Chemirew A, Basaznew B. Haematopathology and haematological parametric alterations in indigenous cattle due to trypanosomiasis. Global Veterinary. 2012;9(5):546-551.
13. Shaw AP, Cecchi G, Wint GR, Mattioli RC, Robinson TP. Prev Vet Med. 2014;113(2):197-210. (Epub 2013 Nov 7).
14. Zewdu S, Getachew T, Hagos A. Farmers’ perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. BMC Vet Res. 2013;9:214. (Published online 2013 Oct 20) DOI: 10.1186/1746-6148-9-214 PMCID: PMC4015653
15. Basaznew B, Kebede W, Mersha C. Occurrence and identification of bovine trypanosomiasis in Genji District, Western Ethiopia. Acta Parasitologica Globalis. 2012;3(3):38-42.
16. Alissa EM, Fern GA. Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. J. Nutri. Metab. 2012;569486. DOI: 10.1155/2012/569486
17. Global Industry Analysts, Inc. (GIA). Global Nutraceuticals Market to Cross US$243 Billion; 2015. Available: http://www.strategyr.com/Nutraceuticals Market Report.asp (Accessed on 13/10/2015)

18. Palaniselvam K, Mashitah MY, Gaanty PM, Solachuddin JAI, Ilavenil S, Nathanamurugaraj G. Nutraceuticals as potential therapeutic agents for colon cancer: A review. Acta Pharmaceutica Sinica B. 2014;4(3):173–181.

19. Lipi D, Eshani B, Utpal R, Runu C. Role of nutraceuticals in human health. J Food Sci Technol. 2012;49(2):173–183. (Published online 2011 Feb 26) DOI: 10.1007/s13197-011-0269-4 PMCID: PMC3550857

20. Selle PH, Cadogan DJ, Li X, Bryden WL. Implication of sorghum in broiler chicken nutrition. Animal Feed Science and Technology. 2010;156:57-74.

21. Wong JH, Tsang L, Nick C, Jaswinder S, Jeffery FP, William HV, William JH, Jeff DW, Peggy GL, Bob BB. Digestibility of protein and starch from sorghum (Sorghum bicolor) is linked to biochemical and structural features of grain endosperm. Journal of Cereal Science. 2009;49:73-82.

22. Shawrang P, Sadeghi AA, Behgar M, Zarestahi H, Shahhoseini G. Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. Food Chemistry. 2011;125:376-379.

23. Gangaish B. Kharif crops. NISCAIR (CSIR). Web Publication, New Delhi; 2007.

24. Grundy SM, Cleeman JI, Merz, CN, Brewer HB, Clark JR, Hunninghake LT, Pasternak DB, Smith RC, SC Jr, Stone NJ. Circulation. 2004;110:227-39.

25. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. J. Agric. Food Chem. 2004;52:4026-37.

26. Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem. 2002;50:6182-6187.

27. Balota M. Sorghum (Sorghum vulgare L.) marketability. Grain colour and relationship to feed values. Virginia Cooperative Extension; 2012. Available: www.ext.vt.edu

28. Green C. Sorghum grain nutrition; 2012. Available: www.ehow.com

29. Asha VB, Geetha K, Sheela K, Dhanapal GN. Nutritional composition of sorghum and moth bean incorporated traditional recipies. J. Hum. Ecol. 2005;7(3):201-203.

30. Thompson L. Sorghum Grain Nutrition; 2010. Available: www.livestrong.com

31. Block E, Garlic and other alliums: The lore and the science. Royal Society of Chemistry; 2010. ISBN 0-85404-190-7

32. AllergyNet — Allergy Advisor Find”. Allallergy.net. (Retrieved Sept., 14, 2015)

33. Ensminger AH. Foods & nutrition encyclopedia. CRC Press. 1994;1:750. ISBN 0-8493-8980-1

34. Londhe VP, Gavasane AT, Nipate SS, Bandawane DD, Chaudhari PD. Role of garlic (Allium sativum) in various diseases: An overview. Journal of Pharmaceutical Research and Opinion. 2011;1:129-34.

35. Bathaei FS, Akhondzadeh S. Cardiovascular effects of Allium sativum (Garlic): An evidence based review. Journal of Tehran University Heart Centre. 2008;1:5-10.

36. Adegbola AJ, Awagu EF, Kamaldeen OS, Kashetu RO. Sorghum: Most under-utilized grain of the semi-arid Africa. Journal of Agricultural Science. 2013;3(4):147-153. ISSN 2276-7118.

37. Liu S, Jiang S, Wu S. Tannins inhibits HIV-1 entry by targeting gp41. Acta Pharma. 2004;25:213-218.

38. Chung T, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit. Rev. Food Sci. Nutr. 1998;38: 421–464.

39. Herbert W, Lumsden WH. Trypanosoma brucei: A rapid “matching” method for estimating the host’s parasitemia. Experimental Parasitology. 1976;40:427-431.

40. Bashir L, Shittu KO, Abubakar AN, Garba MH, Sani S, Prince CO. Haematopoetic effect of methanol extract of Nigerian honey bee (Apis mellifera) propolis in mice. Journal of Coastal Life Medicine. 2015;3(8):648-651.

41. Nok AJ, Williams S, Onyenekwe PC. Allium sativum induced death of African trypanosomes. Parasitology Research. 1996;82:634.
42. Nose M, Koide T, Morikawa K, Inoue M, Ogihara Y, Yabu Y, Ohta N. Formation of reactive oxygen intermediates might be involved in the trypanocidal activity of gallic acid. Biol. Pharm. Bull. 1998;21:583.

43. Dicko MH, Gruppen H, Alfred ST, Alphons GV, Willem JH, Van Berkel. Phenolic compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology Review. 2006;(1):21-38.

44. Maha RG. Efficacy of Allium sativum (garlic) against experimental cryptosporidiosis. Alexandria Journal of Medicine. 2012;48:59–66.

45. Tao Z, Cui-Li Z, Fu-Yong S, Xiu-Lan Z, Li-Hua Y, Zhen-Ping Z, Ke-Qin X. The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress" Biochimica et Biophysica Acta (BBA) - General Subjects. 2013;1830(10):4848–4859. (Accessed October 17th 2013)

46. Jung-Hye S, Ji HR, Min JK, Cho RH, Jaehee H, Dawon K. Short-term heating reduces the anti-inflammatory effects of fresh raw garlic extracts on the LPS-induced production of NO and pro-inflammatory cytokines by downregulating allicin activity in RAW 264.7 macrophages. Food and Chemical Toxicology. 2013;58:545–551. (Accessed October 17th 2015)

47. Bors W, Michel C, Stetmaier K. Structure-activity relationships governing antioxidant capacities of plant polyphenols. Methods Enzymol. 2001;335:166-181.

48. Awika JM, Rooney LW, Waniska RD. Properties of 3-deoxyanthocyanins from sorghum. J. Agric. Food. Chem. 2004b;52:4388-4394.

49. Dicko MH, Gruppen H, Traoré AS, van Berkel WJH, Voragen AGJ. Evaluation of the effect of germination on content of phenolic compounds and antioxidant activities in sorghum varieties. J. Agric. Food Chem. 2005a;53:2581-2588.

50. Guarjardo-Flores D, Rooney LW. (Patents) Publication number: WO 2011066301, Application type: Application, Application Number: PCT/US2010/057956, Publication Date: 2010, Published as: US20110151093; 2011.

51. Ali K, Meredith AW, Thomas HR. Techniques for analysis of plant phenolic compounds. A review. Molecules. 2013;18:2328-2375. DOI: 10.3390/molecules18022328 ISSN: 1420-3049 Available:www.mdpi.com/journal/molecules

52. Yuye W, Xianran L, Wenwen X, Chenguang Z, Zhongwei L, Yun W, Jiarui L, Satchidanand P, Dustan DR, Guihua B, Ming LW, Harold NT, Scott RB, Mitchell RT, Tesfaye TT, Jiaoming Y. Presence of tannins in sorghum grains is conditioned by different natural alleles of Tannin1. Proc Natl Acad Sci U S A. 2012;109(26):10281–10286. (Published online 2012 Jun 13) DOI: 10.1073/pnas.1201700109 PMCID: PMC3387071

53. Tzu-C, Tung-Ti C, Ming-Jen F, Cheng-Chun L, and Yu-Chian CC. In silico insight into potent of anthocyanin regulation of FKBP52 to prevent alzheimer’s disease. Evidence-Based Complementary and Alternative Medicine. 2014;(2014):Article ID 450592:20 Available:http://dx.doi.org/10.1155/2014/450592

54. Rossi VE, MP, Lagiou P, Franchi M, Ferraroni M, Decarli A, Zucchetta A, Serraino D, Dal Maso L, Negri E, La Vecchia C. Proanthocyanidins and other flavonoids M in relation to endometrial cancer risk: A case–control study in Italy. British Journal of Cancer. 2013;109:1914–1920. DOI: 10.1038/bjc.2013.447 Available:www.bjcancer.com (Published online 6 August 2013)

55. Leandro MR, Pablo AC, Johana ED, Mariano EF. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front Microbiol. 2014;5:118. (Published online 2014 Mar 27) DOI: 10.3389/fmicb.2014.00118 PMCID: PMC3973907

56. Min HL, Bog-Hieu L, Ji-Youn J, Doo-Sung C, Kyung-Tack K, Changsun C. Antiviral effect of korean red ginseng extract and ginsenosides on murine norovirus and feline calicivirus as surrogates for human norovirus. Journal of Ginseng Res. 2011;35(4):429–435. DOI: 10.5142/jgr.2011.35.4.429 PMCID: PMC3659551
57. Emanuel H, Carmen H, Alice H, Alicja S, Ulrich W, Christina E, Stephan L, Oliver P. Antiviral activity of Ladania 067, an extract from wild black currant leaves against influenza A virus in vitro and in vivo, Front Microbiol. 2014;5:171.
(Published online 2014 Apr 22)
DOI: 10.3389/fmicb.2014.00171
PMCID: PMC4001074

58. Cowan MM. Plants products as antimicrobial agents. Clin. Microbiol. Rev. 1999;12:564-582.

59. Romagnolo DF, Selmin OI. Flavonoids and cancer prevention: A review of the evidence. J Nutr Gerontol Geriatr. 2012;31(3):206–38.
DOI: 10.1080/21551197.2012.702534
PMID 2288839

60. González CA, Sala N, Rokkas T. Gastric cancer: Epidemiologic aspects. Helicobacter. 2013;18(Supplement 1):34–38.
DOI: 10.1111/hel.12082
PMID 24011243

61. Abdur -Rauf RK, Muslim R, Haroon K, Samreen P, Vincenzo DF, Francesco M, Nicola M. Suppression of inflammatory response by chrysin, a flavone isolated from Potentilla evestita Th. Wolf. In silico predictive study its mechanistic effect Flototerapia. 2015;103.
DOI: 10.1016/j.floto.2015.03.019

62. Young-Mi G, Sang-Moo K, James RR, Michael O, Dean PJ. Increased inflammatory signaling and lethality of influenza H1N1 by nuclear thioredoxin1. PLoS One. 2011;6(4):e18918.
(Published online 2011 Apr 15)
DOI: 10.1371/journal.pone.0018918
PMCID: PMC3078150

63. Woo HD, Kim J. Dietary flavonoid intake and smoking-related cancer risk: A meta-analysis. PLoS ONE. 2013;8(9):e75604. Bibcode: 2013PLoSO...875604W.
DOI: 10.1371/journal.pone.0075604
PMID 2377962.
PMID 24069431

64. Chun-Hat S, Siu-On S, Ricky N, Elaine W, Lawrence CM. Chiu, Ivan KC, Clive L. Quantitative analysis of anticancer 3-deoxyanthocyanidins in infected sorghum seedlings. J. Agric. Food Chem. 2007;55: 254-259.

65. Iris T, Bad breath: Causes and treatment. My Health News Daily Contributor. 2014;03:36pm ET.
(Assessed on 04/11/2015)

66. Barzantny H, Brune I, Tauch A. Molecular basis of human body odour formation: Insights deduced from corynebacterial genome sequences. Int J Cosmet Sci. 2012;34(1):2-11.
(Epub 2011 Jul 25)

67. Troccaz M, Starkenmann C, Niclass Y, van de Waal M, Clark AJ. 3-Methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. Chem Biodivers. 2004;1(7):1022-35.

68. James AG, Austin CJ, Cox DS, Taylor D, Calvert R. Microbiological and biochemical origins of human axillary odour. FEMS Microbiol Ecol. 2013;83(3):527-40.
(Epub 2012 Dec 31)

69. Siegel G, Michel F, Ploch M, Rodriguez M, Malmsten M. Inhibition of arteriosclerotic plaque development by garlic. Wien Med Wochenschr. 2004;154(21-22):515-22. PMID 15638070

70. Tilli CM, Stavast-Kooy AJ, Vuerstaek JD, Thissen MR, Krekels GA, Ramaekers FC, Neumann HA. The garlic-derived organosulfur component ajoene decreases basal cell carcinoma tumor size by inducing apoptosis. Arch Dermatol Res. 2003;295(3):17-23.

71. Rodrigo A, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L, and Ortuño-Sahagún D. Immunomodulation and anti-inflammatory effects of garlic compounds. J. Immunol Res. 2015;2015: 401630. Published online 2015 Apr 19.
DOI: 10.1155/2015/401630
PMCID: PMC4471560

72. Schäfer G, Kaschula CH. The immunomodulation and anti-inflammatory effects of garlic organosulfur compounds in cancer chemoprevention. Anticancer Agents Med Chem. 2014;14(2):239-40.

73. Wilson CL, Aboyade-Cole A, Darling-Reed S, Thomas RD. Poster Presentations, Session A, Abstract 2543: A30 Diallyl Sulfide Antagonizes PhIP Induced Alterations in the Expression of Phase I and Phase II Metabolizing Enzymes in Human Breast Epithelial Cells. Presented
at the American Association for Cancer Research's Frontiers in Cancer Prevention Research meeting in Baltimore, MD; 2005.

74. Shin HA, Cha YY, Park MS, et al. Diallyl sulfide induces growth inhibition and apoptosis of anaplastic thyroid cancer cells by mitochondrial signaling pathway. Oral Oncol. 2010;46(4):e15-8.

75. Dykes Linda, Rooney LW. Sorghum and millet phenols and antioxidants (A Review). Journal of Cereal Science. 2006;44:236–251.