IN VIVO SAFETY OF DICHLOROMETHANE-METHANOLIC EXTRACTS OF Allium sativum L. IN SWISS ALBINO MICE

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May, 2017
DECLARATION

I, Duncan Maina Kariuki, duly declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution.

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We hereby confirm that the work reported in this thesis was carried out by the candidate under our supervision

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DEDICATION

This thesis is hereby dedicated to my family for their selfless sacrifices towards my education.
ACKNOWLEDGEMENTS

I am greatly indebted to Kenyatta University for giving me an opportunity to further my education and get to be a member of the Alumni. My supervisors Dr. Mathew Ngugi and Dr. David Mburu tremendous guidance, inspiration and valuable support have not only made the completion of this research study possible but have left me a great sense of fulfillment and achievement which will continue to influence my work. Your valuable mentorship is greatly cherished.

I also owe gratitude to the following people for their enormous support. Thanks to the entire staff of Biochemistry and Biotechnology department for all the assistance. The following people deserve special mention, Daniel Gitonga and James Adino for offering me with technical assistance.

To my family, I say thank you so much for your understanding and perseverance when I was absent from home in the course of my work. I greatly appreciate you for the emotional support, inspiration and encouragement for me to soldier on throughout this endeavor.

Above all I most sincerely thank the Almighty God our father for giving me strength, good health and sound mind to accomplish this project. From him is all knowledge, understanding, power and wisdom, glory be to his holy name.

Lastly, to all who contributed to the success of my work mentioned or not, may God bless you in a mighty way.
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<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>ALA</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ASA</td>
<td>Aspartate aminotransferase</td>
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<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
</tr>
<tr>
<td>CL</td>
<td>Cutaneous leishmaniasis</td>
</tr>
<tr>
<td>DADS</td>
<td>Diallyl-di-sulfide</td>
</tr>
<tr>
<td>DAS</td>
<td>Diallyl-sulfide</td>
</tr>
<tr>
<td>DBILI</td>
<td>Direct bilirubin</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>DPX</td>
<td>Dibutyl phthalate in xylene</td>
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<tr>
<td>EDTA</td>
<td>Diethylene-diamine-tetra-acetic acid</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organization Statistical Databases</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma-glutamyltransferase</td>
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<tr>
<td>GRA</td>
<td>Granulocytes</td>
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<tr>
<td>HCT</td>
<td>Hematocrit</td>
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<tr>
<td>HB</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>LYM</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>LD50</td>
<td>Lethal dose</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean corpuscular hemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean cell volume</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean platelets volume</td>
</tr>
<tr>
<td>MON</td>
<td>Monocytes</td>
</tr>
<tr>
<td>OECD</td>
<td>Organization for Economic Co-operation and Development</td>
</tr>
<tr>
<td>PDW</td>
<td>Mean platelets width</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>TBILI</td>
<td>Total bilirubin</td>
</tr>
<tr>
<td>WBC’s</td>
<td>White blood cells</td>
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</table>
The indiscriminate use of *A. sativum* L. in treatment of various diseases can pose a great danger to various body functions. It results to toxicity within the body system e.g. in both dogs and cats, *A. sativum* L. has been associated with oxidative hemolysis and Heinz body formation leading to anemia and methemoglobinemia. Therefore, assessment of hematological and biochemical parameters of this plant extract can be used to determine its safety and toxic margins. Just like other terrestrial plants, *A. sativum* L. has a variety ethnopharmacological uses and has been exploited by both local and international individuals in search of various remedies for various diseases. Although, *A. sativum* is widely known to have various curative properties, there has been no scientific data documented about its safety on hematological and biochemical parameters. The body weight of both treated and control mice were recorded before, during and at the end of the experiment. Blood samples were collected from experimental and control groups on 29th day for biochemical and hematological profiles and were analysed using an auto-analyser. The results of this study showed that DCM-MeOH extract of *A. sativum* L. induced significant increase in the levels of red blood cell, hemoglobin and hematocrit across the 100, 500, 1000 and 2000mg/kgbw dose levels (p<0.05). However, the red blood cell indices (MC, MCH and MCHC) did not show a significant change at all the dose levels (p>0.05). The total and differential white blood cell counts also increased significantly at all dose levels (p<0.05). However, the platelets and the related parameters did not have any significant change at all the dose levels during this study period (p>0.05). The DCM-MeOH extract of *A. sativum* L. caused a significant increase in the levels of liver functions profiles across the 100, 500 and 2000mg/kgbw dose levels (p<0.05). The kidney functions parameter levels also increased significantly at all the dose levels (p<0.05). Qualitative phytochemical screening confirmed the presence of various phytochemicals which included alkaloids, flavonoids, steroids, saponins, cardiac glycosides and phenolics. These phytochemicals are understood to play a major role in gene expression, erythropoietin stimulatory, thrombopoietic stimulatory, immune-stimulatory and enzyme activities. Flavonoids, cardiac glycosides and alkaloids are also responsible in the increase in the RBC count through their antioxidant properties. Conversely, an increase in saponins may result to a decrease in red blood cell indices (MCV, MCH and MCHC). It was therefore concluded that the plant extract, subject to various stipulated assays, is safe at particular doses as indicated by changes in hematological parameters. On the other hand, the study also shows that plant extract is not safe at high doses as indicated by change in the liver and kidney parameters which showed hepatotoxicity and nephrotoxicity respectively. It was recommended that dosages above 1000mg/kgbw of *A. sativum* L. were unsafe and therefore should not be used. Moreover, it was suggested that there was need to carryout chronic *in vivo* safety study of the extract in order to compare the outcomes. Additionally, it was further suggested that similar study should be pursued using an alternative route of extract administration.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Garlic also known as A. sativum L. has been one of the oldest cultivated plants worldwide. It has been used in a variety of ways as food, spice and conventional medicine for over 4000 years (Milner, 1996; Rivlin, 2001). The garlic plant has been included in about 22 therapeutic formulations which make it an effective remedy for a variety of ailments including heart problems, headache, bites, tumors and also worms (Block, 1985).

Garlic stands as the second most utilized supplement behind Echinacea. According to the US Food and Drug Administration survey of 900 people, it was found out that approximately 17% of the population utilized a garlic supplement in the preceding 12 months (Timbo et al., 2006). This plant has been appreciated for its unique functions such as anti-microbial (Johnson and Vaughn, 1969), hypolipidemic (Bordia et al., 1975), anticarcinogenic (Hussain et al., 1990), anti-viral, anti-bacterial, antifungal (Amer et al., 1980), anti-atherosclerotic (Bordia and Verma, 1980) and antioxidant (Banerjee et al., 2003) capacities. There are also additional functions that garlic has been reported to have including anti-atherosclerotic and anti-cancer properties.

Garlic was involved in the pasteurization of milk due to its antibacterial properties as was reported by the famous chemist and microbiologist Louis Pasteur (Block, 1985). In
the past centuries in India, garlic was used as antiseptic lotion for wound washing and ulcers. Moreover, soldiers of war during the World War II were treated with garlic in order to increase wound healing (Essman, 1984). Garlic tea which had been taken for a long time in China was known to combat ailments such as fever, dysentery, cholera, and headache. In addition, rural Japan uses a soup containing garlic (miso-soup) as a remedy for common cold with headache, fever and sore throat as well (Sato and Miyata, 2000).

Conversely, S-methylcysteine sulfoxide isolated from garlic had been shown to reduce both the severity of atherosclerosis and blood cholesterol levels thereby acting as a preventive chemical against cardiovascular diseases (Sainani et al., 1979; Warshafsky et al., 1993). Collectively, garlic is also reportedly known to have protective effects against coronary thrombosis and stroke (Ali et al., 1990; Block et al., 1986). In addition, it is reported to manage, atherosclerosis (Fudler, 1989., Turner, 1994), platelet aggregation (Makheja and Bailey, 1990; Srivastava and Tayagi, 1993; Thomson et al., 2000), vascular disorders and infections (Block et al., 1986).

Theologically, the Jewish slaves in captive in Egypt were fed with garlic in order to make them strong and boost their productivity (Rivlin, 2001). In other areas around the world especially Greece, garlic was utilized in the treatment of ailments such as intestinal and lung problems (Farbman et al., 1993). In ancient India, Hippocrates and physicians were reported to have used garlic externally for cancer treatment (Hartwell, 1968; Moyers, 1996). Postulates from China indicate that an increase in garlic
consumption led to a reduction of nitrites in the stomach via inhibition of nitrate reduction by bacteria (Mei et al., 1982).

Natural antioxidant compounds have been favored more than the synthetic ones according to recent trends in controlling and treating diseases. *Allium sativum* which is commonly used as food additive worldwide has been regarded highly because of its medicinal properties (Craig and Beck, 1999). Garlic’s beneficial properties are attributed to the organosulfur compounds, especially to sulfur-bearing compounds such as allicin, S-allyl-cysteines, diallyl-di-sulfide and diallyl-sulfide (Koch and Lawson, 1996).

Aluminium, which has been widely distributed in the environment, is an extensively used metal in daily life and thus is greatly exposed to human beings (Kumar and Gill, 2009). *Allium sativum* has been used in the amelioration of aluminium induced toxicity. Moreover, it has been studied to prevent abnormalities caused by aluminium poisoning due to its role in stabilizing the cell membrane and also protecting the tissue from free radical mediated toxicity (Belaid-Nouira et al., 2012; Wen et al., 2012).

Garlic has also been extensively used in the treatment and control of leishmaniasis. It has also anti-diabetic properties, which has been attributed to the allicin-type of compounds found in the plant (Mathew and Augusti, 1973; Chang and Johnson, 1980). Its hypoglycemic potency has been attributed to the sulphur compounds [di (2-propenyl) disulphide and 2-propenyl propyl disulphide, respectively]. Garlic is also known to improve lipid profile including the reduction of serum cholesterol levels (Knipschild and Ter-Riet, 1989). Another mechanism of the anti-cholesterol property of
A. sativum L. is through inhibition of some enzymes such as the hydroxyl methyl glutaryl CoA reductase involved in cholesterol synthesis (Gebhardt, 1991; Gebhardt and Beck, 1996).

Most conventional ways of treating and managing fungal infections, microbial infections, cancer, hypercholesterolemia, oxidations, metal toxicities and viral infections are costly and may also possess undesired side effects, unbearable withdrawal symptoms to the patients in terms of pain. These emerging limitations involved in the usage of conventional methods to manage the above ailments require the need for an alternative safer and effective remedy devoid of the named shortcomings in order to contain and manage these disorders (Gebhardt, 1991; Gebhardt and Beck, 1996).

Traditionally, A. sativum L. has been used as an anti-cancer, antifungal, antimicrobial, antiviral, hypolipidemic and also an antioxidant, but its safety in terms of administration has been scientifically studied or even validated. Therefore, it was against this background that the present study was undertaken to scientifically test the in vivo safety of DCM-MeOH extract of A. sativum L. in normal mice. This will avail additional information regarding its safety through the study of various parameters in normal mice models.

1.2 Problem statement and justification

Allium sativum L. as an alternative medicine for a wide range of ailments such as bacterial, cancer, viral and viral infections is economically viable as compared to the
conventional drugs. However, the indiscriminate use of *A. sativum* L. in the treatment and management these diseases pose a great risk to patients. One of the major risks of the indiscriminate use of this plant is toxicity caused by the organosulfur compounds and also its metabolites. It has been researched that *A. sativum* L. when consumed by animals such as dogs and cats, leads to oxidative hemolysis which can potentially lead to anaemia, impaired oxygen transportation and also methemoglobinemia. It is important to understand and scientifically test how much in terms of concentration of the herb is safe and what is unsafe. There has been no scientific data and safety margin on the *in vivo* safety of DCM-MeOH extract of *A. sativum* L. Changes in body weight of the animals, hematological and biochemical parameters were the indicators of either safety or toxicity of the extract in the assay. This information will prove to be important in the both scientific and societal realms.

### 1.3 Hypotheses

i. Dichloromethane-Methanolic extract of *A. sativum* L. has no effect on hematological parameters in Swiss albino mice.

ii. Dichloromethane-Methanolic extract of *A. sativum* L. has no effect on liver and kidney functions in Swiss albino mice.
1.4 Objectives

1.4.1 General objective

To determine the *in vivo* safety of DCM-MeOH extract of *A. sativum* L. in Swiss albino mice.

1.4.2 Specific objectives

i. To determine hematological effects of DCM-MeOH extract of *A. sativum* L. in Swiss albino mice.

ii. To determine the effects of DCM-MeOH extract of *A. sativum* L. on liver and kidney functions of Swiss albino mice.

iii. To determine qualitative phytochemical composition of DCM-MeOH extract of *A. sativum* L.
CHAPTER TWO
LITERATURE REVIEW

2.1 Allium sativum L.

2.1.1 Plant description

Allium sativum L. commonly known as garlic is found in the onion genus Allium. There has been a controversy for a long time in the taxonomic position of Allium and other related genera (Fritsch and Friesen, 2002). Allium sativum’s hierarchy is as follows: Class-Liliopsida, Subclass-

Liliidae, Superorder- Liliinae, Order- Amaryllidales, Family- Alliaceae, Subfamily- Allioideae,

Tribe-Allieae and Genus- Allium (Takhtajan, 1997).

The garlic plant is composed of a bulb which is rounded and has about 15 smaller bulblets (catherine). These bulbs have been assayed to contain S-allylcysteine sulfoxide (alliin) which is liberated when the plant is cut and there is destruction of the parenchyma (Block et al., 1986). Garlic also possesses 4-12 long, sword shaped leaves attached to an underground stem. Its flowers are borne in a dense spherical cluster on a flower stalk which is approximately 25cm long. The garlic plant has been known to produce leafless flower stem (a scape), sterile flowers that produce bulbils (small cloves) rather than seeds; moreover the species is propagated clonally from the cloves and bulbils (Block, 2010).
2.1.2 Geographical distribution

Garlic’s origin is believed to be from Central Asia specifically in Kazakhstan, Uzbekistan and Western China (Ensminger, 1994). It has been documented that China produces ¾ of the total yield according to garlic production and in 2008 it produced 22 million metric tons harvested from approximately 1.4 million hectares (FAOSTAT, 2011). This was after confirmation by phylogenetic analysis based on molecular and biochemical markers and the indication of a secondary diversity centre in the Caucasus. This plant
was already grown in Egypt in 1600 BC and is an ancient crop in India and China (Simonetti, 1990).

In Kenya, *A. sativum* L. is best and conversely suited for medium to high altitudes of 500-2000m. In addition, for optimal bulb development high temperatures of about 30ºC are necessary. The precursor for continued plant establishment is usually cooler temperatures in early stages. Clay soils are also avoided since they may result in malformed bulbs and therefore well drained fertile soils are highly recommended (Njeru *et al*., 2013). This plant grows well in soils of pH ranging between 5.5-6.8 units and should be planted on raised seed beds, ridges at a spacing of 30cm between rows and 15-20cm between plants (Biamah, 2005).

### 2.1.3 Classification

There are three main types of garlic varieties, each blended with their own unique properties and they include the softneck, hardneck and the elephant garlic (Block, 2010).

#### 2.1.3.1 Softneck garlic

This is usually the one mostly found in almost all the grocery stores and is the most common of all the garlic. It is also the easiest to grow in a backyard vegetable garden and comprises of a white, paper-like skin and its head comprises of several layers of garlic cloves surrounding the core. The Softneck garlic has a fairly strong taste and
sweet pungent smell. A few varieties of Softneck garlic include California Early, California Late and Creole (Block, 2010).

2.1.3.2 Hardneck garlic

It has characteristic features of tan or purple marking and is a less common garlic type. This type of garlic usually feature a fairly strong taste and sweet, pungent odor and usually produce larger, fewer cloves on each head. They do not store for long periods of time as the softneck ones and are generally thin skinned hence easy to skin. Hardneck garlic varieties include the German Extra Hearty and Roja (Block, 2010).

2.1.3.3 Elephant garlic

Just like the softneck garlic, elephant garlic is also commonly found in grocery stores. One of its characteristic feature is a large head which is comprised of very large cloves. It is also been proven to have a very subtle flavor and mild odor and its taste is more than often equated to leeks or shallots than with other types of garlic by purists (Block, 2010).

2.1.4 Nutritional composition

*Allium sativum* L. has been known to contain 0.1-0.36% of a volatile oil, with which these volatile compounds are the ones responsible for pharmacological garlic properties. Garlic is ultimately composed of approximately 33 sulfur compounds including alliin, allicin, ajoene, allylpropl, dialyl, trisulfide, sallylcysteine, vinylthiines, S-allylmercaptocystein and other sulfur compounds, namely peptides, steroids, terpenoids,
flavonoids and phenols which have been recognized as possible active ingredients. Garlic is an essential functional food, and highly nutritionally complete (Borek, 2001).

This botanical plant’s medical effects have been reported to emanate from the sulfur containing compounds, high trace mineral content and also enzymes. The two abundantly found sulfur compounds in garlic cloves are namely S-alkylcysteine sulfoxides and γ-glutamyl-S-alkylcysteines. Alliin (S-allylcysteine sulfoxide) which is the most abundant sulfur compound in garlic can be contained in almost 10mg/g of fresh garlic or 30mg/g dry garlic (Lawson, 1998).

Garlic possesses a variety of mineral substances and trace elements and they include calcium, copper, bromine, magnesium, phosphor, potassium, selenium, sulphur, zinc, iron, selenium among others. It also has a variety of vitamins namely provitamins A, B1, B2, B3 or PP, B5, B6, C, E and carbohydrates. There are approximately 20 amino acids and enzymes within the garlic plant (Borek, 2001).

2.1.5 Medicinal uses

*Allium sativum* L. has been traditionally used in the treatment of pulmonary problems, coughs, and also tuberculosis. As garlic is metabolized, it produces various sulfur compounds that, together with their breakdown products, yield a characteristic pungent taste and odor that may persist on the breath and body for up to 30 hours (Block, 2010). One of the derivatives of garlic, allicin, has been shown to combat fungal infections and parasites; it also lowers blood cholesterol, treats arteriosclerosis, and promotes circulatory function (Reuter *et al.*, 1996; Block, 2010).
Garlic has highly been associated with medicinal benefits and has also been used in the prevention of a variety of diseases for instance common flu and gastrointestinal disorders. In countries like China, there has been anecdotal evidence suggesting that successful treatment of gastrointestinal diseases via consumption of fried garlic and eggs maybe related to its function of improving the human gastrointestinal environment (Block, 2010). This has led to increased interests in the analysis of garlic compounds and its constituents such as the essential oil, selenium, amino acids and their contributions to the claimed benefits have grown in recent years (Irkin and Korukluoglu, 2009; Kim and Kim, 2011; Leong et al., 2011). A variety of research has suggested that garlic contain fructans 12.5% to 23.5% on a wet weight basis and also more than 75% on a dry weight basis. Ultimately, fructan is responsible in the protection against gastrointestinal diseases by improving the microbial gastrointestinal environment (Losso and Nakai, 1997; Baumgartner et al., 2000).

Garlic preparations have been greatly used in the treatment of insect stings and improvement of scar healing (Block, 2010). Dietary garlic consumption is known to lower the risk of various cancers (Reuter et al., 1996). Inhibition of the growth of cancer is ultimately one of the most prominent functions of garlic as a plant. The main known mode of action of garlic towards cancer alleviation is via the stimulation of immune effectors cells including T-cell and natural killer cells (Reuter et al., 1996). This plant is highly attributed to prevention against digestive tract cancers such as esophageal, stomach and colon cancer. Another prominent mechanism of garlic in cancer treatment is via the ability to modulate the activity of several metabolizing enzymes that activate
cytochrome P450s or detoxify glutathione S-transferases carcinogens and inhibit formation of DNA adducts in several target tissues (Hassan, 2004). Garlic’s inhibitory activity against cancer cells is highly influenced by the presence of promoters including tumor promoter 12-0-tetradecanoylphorbol-13-acetate (Hikino et al., 1986). It is also reported that garlic reduces the risk of patients with prostate cancer (Hsing et al., 2002).

Allicin, which is responsible in reducing the formation of carcinogenic compounds on gastrointestinal tract, is the main factor in enhancing the antioxidant effect. Studies and research also proves that, A. sativum L. has protective effects against hepatotoxins cyclophosphamide, Adriamycin methylcholanthrene, gentamicin, 4-nitroquinoline 1-oxide and bromobenzene (Galeone et al., 2006).

This plant has also been used in combating diabetes and blood purification. It has been established that garlic extracts significantly decrease serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate aminotransferase and alanine amino transferase levels. Even when compared with other antidiabetic drugs like glibenclamide, garlic was found to be having more antidiabetic effect (Eidi et al., 2006).

The most used form of dosage for garlic in order to achieve its protective, anti-viral, anti-bacterial and anti-parasitic (Candida albicans) efficacy is 2 to 4 cloves per day (Eidi et al., 2006).

It has been established that a combination of garlic with Callistemon citrinus and Moringa stenopetala is highly effective in the fight against cutaneous leishmaniasis. In more than 70 countries worldwide, cutaneous leishmaniasis is the most common
widespread disease (Reithinger et al., 2007). Due to garlic’s anti-microbial, anti-viral and its antiparasitic activities, it has been proven that the combinational therapy is better as than monotherapeutic regimen. This technique of combinational therapy of antilesmanial drugs improves efficacy and reduces resistance tolerance and toxicity levels of drugs (Nyakundi et al., 1994; Melaku et al., 2007; Sunder et al., 2008).

As an antibacterial agent, garlic has been known to inhibit the growth of Gram positive and Gram negative bacteria such as *Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia* and *Helicobacter pylori* (Tsao and Yin, 2001). Allicin is the main antibacterial agent produced by garlic and is highly potent in crushed garlic extracts (Hahn, 1996).

Ajoene is a topical antifungal agent which is an active compound found in garlic and is known to inhibit growth of fungal diseases just as equal as the drug ketoconazole when tested on the following fungi; *Malassezia furfur, Candida albicans, Aspergillus, Cryptococcus* and other *Candida* species (Shams-Ghahfarokhi et al., 2006). The antifungal nature of garlic has been uniquely attributed to its ability to stimulate phagocytic activity within a host immune system. Moreover, garlic oil has also been reported to treat ringworms, skin parasites and also warts when applied externally (Sabitha et al., 2005).

Garlic can also be found commercially in various forms of preparations including the garlic oil, garlic powder, and pills which are the widely used forms for therapeutic purposes, improving lipid profile and lowering blood pressure (Elkayam et al., 2003). This can be easily understood as garlic antilipemic activity which is chiefly cholesterol
lowering in the body of individuals through inhibition of 3-hydroxy-3-methylglutaryl-CoA. This plant via research has been known to prevent blood clotting and increase the rate at which blood clots are broken down (Auer et al., 1990). The plant is also highly known for its antihypertensive activity through a variety of study and analysis (Schulz et al., 1997). Studies also documented that there is also a response to garlic showing a significant reduction in systolic blood pressure (SBP) and in the diastolic blood pressure (DBP) (Tsai et al., 1985; Silagy and Neil, 1994).

Garlic has also potential diuretic properties, whereby reports indicated that it helps to get rid of body liquids via urine. This is quite an important factor in correlation to dealing with diseases such as rheumatism, gout, arthritis, hidropesia and edemas (Ali, 1995). As an antimicrobial agent, garlic was first put to test by Louis Pasteur who’s uniquely displayed its antibacterial properties. It was also nicknamed as “Russian penicillin” due to its vast use as a topical and systemic antimicrobial agent (Borek, 1998).

Aged Garlic Extract (AGE) has been assayed and reported to effectively reduce hyperhomocysteinemia. This is a condition whereby there are high blood levels of homocysteine which is a well-established risk factor for arteriovascular disease and also result to folate deficiency (Yeh et al., 1999). Aged Garlic Extract is also responsible in prevention of sickle cell anaemia whereby; it inhibits dense cell formation by 50% along with other effective nutrients like black, green tea extracts, pycnogenol, α-lipoic acid, vitamin E, coenzyme Q10 and β-carotene (Amagase et al., 2000).
Antioxidative and radio-protective properties of garlic have been reported via AGE and its various constituents. The unique constituents of AGE have been known to protect the white blood cells against radiation damage, liver cells from lipid peroxidation and also vascular endothelial cells from oxidant injury. Moreover, they have been assayed to scavenge hydrogen peroxide, to inhibit the formation of thiobarbituric acid reactive substances to enhance heart protection from cardiotoxicosis, anticancer drug doxorubicin, and also to protect the kidneys from the antibiotic gentamicin (Oshiba et al., 1990).

Garlic has also been highly used as a natural immune booster especially with the emergence of frightening diseases such as HIV/AIDS. Its abundance in sulfur containing amino acids and other compounds that initiate increased activity in the immune system, makes it possible for a patient’s body to fight against these kinds of diseases (Lau, 1991). Garlic uniquely acts as an impressive conductor of the body immune system that leads to the stimulation of the immune functions by making macrophages or killer cells more active. One of the major minerals in garlic known as germanium is one of the indispensable mineral that offers excellent immune stimulation (Lau, 1991).

The neuroprotective abilities of aged garlic in vitro have been studied for multiple benefits including the treatment of the classic Alzheimer beta-amyloid plaque (Peng et al., 2002). Garlic has also been known to possess cumulative benefits and thereby enhance neuroprotection by the virtue of being “natural statin”, “natural NSAID”,
“natural anti-oxidant”, “natural anti-apoptotic agent” and “memory enhancer (Peng et al., 2002).

Garlic thereby seems a prudent recommendation for prevention and treatment of Alzheimer’s given its multiple-mechanistic possibilities and minimal risk associated with its use. Furthermore, it has been known to inhibit aluminium toxicity due to its antioxidative stress properties. Aluminium is a potent neurotoxin associated with Alzheimer’s disease causality for decades and garlic uniquely attenuates oxidative stress by scavenging various free radicals (Fukao et al., 2007).

*Allium sativum* L. is also a major antithrombotic agent whose properties are attributed to the allyl propyl disulfide, diallyl disulfide, and other sulfur compounds present in the essential oil (Fukao et al., 2007). In addition to this, it has been reported by researchers that a daily consumption of 1 clove of fresh garlic for 6 months resulted in an 80% decrease in serum thromboxane B2 in middle-aged men (Ali et al., 1995).

Osteoporosis is usually attributed to boneloss and garlic has been reported to counter this disease. Garlic oil extract highly promotes intestinal transference of calcium by modulating the activities of both intestinal alkaline phosphatase and Ca (2+) activated ATPase. This clearly demonstrates that garlic is responsible in enhanced calcium transference and a better preservative of bone mineral content (Mukherjee et al., 2006).
2.1.6 Potential toxicity

Research findings indicate that powders and extracts of *A. sativum* L. are toxic to *Callosobruchus maculatus* F. adults (Coleoptera: Bruchidea), which infests cowpeas (Osuji, 1985). The *A. sativum*’s toxicity to adults and to immature stages of this pest indicates the appropriate time of application. In another study, it was reported that *A. sativum* had cytotoxic activity against overwintering *Cacopsylla chinensis* (Hemiptera: Psyllidae) with an LC50 value of 1.42µg per adult (Tian *et al*., 2015). The two main compounds associated with acute toxicity against *C. chinensis* include diallyl trisulfide and diallyl disulfide (Xin *et al*., 2014).

The pharmacologically garlic’s active agents such as allicin and ajoene are potent cardiac and smooth muscle relaxants, vasodilators and hyptensive agents (Malik and Siddigui 1981; Mayeux *et al*., 1988; Martin *et al*., 1992). These compounds have been shown to cause hemolytic anaemia in wether dogs (Lee *et al*., 2000). *Allium sativum* L. active agents lead to Heinz body formation, increase in erythrocyte –reduced glutathione concentration and eccentricytosis major diagnostic feature of garlic-induced hemolysis in dogs (Lee *et al*., 2000; Yamato *et al*., 2005).

Garlic is also potentially known to cause halitosis and also causes body odor which is a result of the pungent-like “garlicky” smell in an individual’s sweat (BBC, 2010). These effects of toxicology are caused by a particular compound in garlic known as allyl methyl sulfide. This compound has been reported to be readily absorbent in blood thereby, making it possible to be transported to the lungs and finally find its way outside via exhaling (Block, 2010).
Another form of garlic toxicity includes allergies that a number of people usually suffer from. These particular allergies are preceded by symptoms such as irritable bowel, diarrhea, mouth and throat ulcerations, nausea, breathing difficulties and at times show causes of anaphylaxis. The main active ingredients present in garlic that have shown to result to allergic reactions include, diallyl disulfide, allylpropyldisulfide, allylmercaptan and allicin (Block, 2010).

Moreover, garlic burns have been reported resulting from topical application of garlic for various purposes such as naturopathic and acne treatments. Young children are not allowed to be treated with garlic topically as opposed to other category of people. There are number of side effects that have been keenly researched and identified such as gastrointestinal discomfort, sweating, dizziness, allergic reactions, bleeding and menstrual irregularities (Garty, 1993).

Garlic toxicity has also been investigated to cause low milk production in lactating mothers (Hogg, 2002 and WM, 2010). Apart from the low milk production during breastfeeding, it was proved that the garlic odor was noted to come from the milk. Further research has proven that high dosages of garlic when taken in combination with an anticoagulant medication leads to even higher risks of bleeding (Brown et al., 2015).

2.1.7 Effects of toxicants on hematological parameters

Blood is a specialized and vital circulatory tissue whose main function is maintaining homeostasis (Isaac et al., 2013). There are a variety of hematological components that
are found in the blood and they comprise of red blood cells (RBCs), white blood cells (WBCs), mean corpuscular volume and mean corpuscular hemoglobin. These hematological parameters are used in monitoring toxicity patterns and progression (Oyawoye and Ogunkunle, 2004). RBCs are chiefly involved in oxygen transport and therefore, a reduction in RBC count synergistically leads to a reduction in the oxygen capacity within body tissues. As the level of oxygen is depleted within the tissues, the level of carbon dioxide returned to the lungs will also be reduced (Ugwuene, 2011; Isaac et al., 2013; Soetan et al., 2013).

WBCs and its resultant differentials are involved in body defense by the act of phagocytosis of foreign organisms. They are also established and researched to produce, transport and distribute antibodies required in an immune response. It is therefore very risky for individuals with low WBCs count since they will be exposed to high risk of disease infection. However, those at very high and abnormal counts are capable of generating antibodies during phagocytosis which prove to have a high degree of resistance against disease causing virus (Soetan et al., 2013).

During tissue injury the blood platelets are involved in the process of blood clotting. The process of clot-formation is thus highly dependent on the amount of platelet count. This is to mean that, if there is low platelet count then there prolonged clot-formation and this will lead to excessive blood loss. Packed Cell Volume (PCV) which refers to the percentage of red blood cells in blood is a unique vehicle in the transport of oxygen and absorbed nutrients. Hemoglobin is also involved in the transport of oxygen in the body.
tissues and helps in the removal of carbon dioxide by transporting it to the lungs where it is finally exhaled (Purves et al., 2003).

### 2.1.8 Biomarkers of liver injury

An ideal biomarker has a variety of characteristics such as accessibility, non-invasive, sensitive, specific, inexpensive, translational, accurate and predictive of the injury extent. Biomarkers are essential in a body system since they indicate the normal biological process, pathogenic process or sometimes pharmacological responses towards a therapeutic interaction (Schomaker et al., 2013).

Hepatotoxicity can be easily diagnosed by certain biochemical markers which include Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and also bilirubin. Liver toxicity is usually established when there is an elevation in the serum enzyme levels. On the other hand, elevations or increases in both total bilirubin and conjugated bilirubin are relevant measures in the overall function of the liver (Reuben, 2004).

There are two main groups of characterizing hepatotoxicity each having a different mechanism of injury: that is hepatocellular and cholestatic. Hepatocellular injury is coupled by the modest increases in alkaline phosphate levels, predominant initial elevations in serum aminotransferase levels and also preceding increases in total bilirubin levels. Cholestatic injury is as a result of an elevation in the levels of the predominantly initial alkaline phosphatase which is usually more prominent than serum
aminotransferase elevation levels (Navarro and Senior, 2006; Chang and Schaino, 2007).

### 2.1.9 Biomarkers of kidney injury

Serum creatinine is a specific biomarker in renal function testing. Creatinine is formed through the metabolism of creatine in the skeletal muscles and is nitrogenous in nature. An increase in the level of serum creatinine is an indicator of poor creatinine clearance and therefore a resultant state of kidney injury. A low level of serum creatinine on the other hand, is an indication that there is decreased muscle mass (Akanda et al., 2013).

Blood urea nitrogen (BUN) is also a biochemical marker of the renal function although; it gives an indirect and rough measurement of the urea nitrogen in serum (Robert et al., 2010). Diet, specifically proteins, directly affects the amount of urea in the blood. An increase in the amounts of urea is known as uremia and is ultimately caused by impairment of the kidney function (Lesley et al., 2006).

Positively and negatively charged ions known as electrolytes found within cells and extracellular fluids are also used in kidney function testing. The measurement of sodium, potassium and chloride ions is important in the diagnosis of various physiological processes such as homeostasis (Owiredu et al., 2012). Electrolytes have extensive roles especially sodium and potassium ions which are largely free in body fluids. Furthermore, cellular tonicity and fluid balance between varieties of cellular components are maintained by electrolytes (Yousafzai et al., 2011).
The main extracellular cation which is a major factor in homeostatic water control and extracellular fluid volume is sodium. An increase in the plasma sodium is associated with low amount of water and to compensate on this the body prompts for oral water intake and secretion of anti-diuretic hormone from the pituitary gland. The excess amount of sodium is excreted in the urine via the kidneys and finer control done by the tubular reabsorption (Cheesbrough, 2009).

However, low levels of sodium concentrations are attributed to too much water in the body. The glomerular filtrate in the kidney is responsible in the removal of the excess water. Renal injury may therefore occur as a result of hyponatremia whereby, there is an abnormal intake of water. On the other hand, renal injury can also be caused by hypernatremia that leads to renal water loss (Richard, 2010).

As an intracellular cation, potassium is a useful electrolyte which depends on ATP within cells in the sodium pump mechanism. Potassium is indispensable in the acid/base status in cellular transport system. The most convincing electrolyte marker of renal failure has been researched to be serum potassium (Yousafzai et al., 2011). A high level of serum potassium (hyperkalaemia) is highly regarded as life-threatening and most significant in correlation to renal failure. Moreover, hypokalaemia which means low serum potassium concentration usually occurs as result of various factors including intake of K\(^+\) lowering drugs, vomiting, acid-base disorders and also excessive urination (Richard, 2010).
2.1.10 Phytochemicals and constituents

There are a number of researches done on this plant and it has been documented that it possesses a lot of phytochemicals. These phytochemicals have been associated with both the plants’ nutritional and medicinal uses. Phytochemical analysis profiles have proved that *A. sativum* L. has presence of flavonoids, alkaloids, saponins, tannins, cardiac glycosides, terpenoids, anthraquinones and phenolics (Borek, 2001).

Other constituents of garlic include sulphur containing compounds such as allicin, which is derived from alliin through an enzymatic reaction of the allinase enzyme as shown below:

![Allicin biosynthesis](https://example.com/figure2.2)

**Figure 2.2: Allicin biosynthesis (Retrieved from Borek, 2001)**
CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection and preparation of plant material

Garlic (*A. sativum*) was collected from Kiambu market. A voucher specimen was deposited in the Kenyatta University herbarium. The plant material was washed thoroughly in tap water and put in liquid nitrogen, and then grounded into small particles using a pestle and mortar. The powdered samples were labeled, weighed and recorded (Evans, 2009; Gautam, 2014).

3.2 Extraction

A 200g measure of the powdered extract of the plant material was put in a beaker and DCM-MeOH mixture in 1:1 ratio added in order for the dry powder to be submerged in the solvent. The mixture was kept for 24 hours at room temperature. The mixture was then decanted, filtered using muslin cloth and then with Whatman filter paper No.1. The whole process was repeated three times and supernatant collected and pooled together. The extract was concentrated by rotary evaporator at 40°C to obtain the dry extract as described by Evans (2009).

3.3 Laboratory animals and experimental design

Thirty healthy mice aged between 8 and 12 weeks were obtained from the Department of Biochemistry and Biotechnology animal house. They were then be kept under standard environmental conditions 25°C, 12 hours light and 12 hours dark cycle and
were supplied with standard pellet diet and water *ad libitum*. The mice were allowed to acclimatize for a period of 7 days. The control group received DMSO while the experimental groups received extracts orally dissolved in 1% DMSO, using a drenching syringe, in doses of 100, 500, 1000 and 2000mg/ kg body weights of the extract for 28 days. The mice were observed daily for any abnormal clinical signs and in case of mortality the mean lethal dose (LD50) was to be established. Their body weights were taken at the end of every week.

Following overnight fasting on the 29th day, all animals were euthanized in a jar containing diethyl ether. Then they were sacrificed and 6 ml blood was collected by cardiac puncture and tail bleeding using sterile needle syringe from each mouse. Three millilitre portion of the blood was put in bottles containing EDTA to prevent coagulation. The EDTA blood samples were immediately used to determine hematological parameters (full haemogram) using automated analyzer (Model: Changchun Dirui Industrial Co., LTD). The remaining 2ml blood sample for biochemical tests was collected in bottles without EDTA and kept at 4°C for 4 hours to let it clot and centrifuged at 1500 rpm for 15 minutes to obtain serum. The serum was refrigerated at -22°C and used for biochemical assays.

**3.4 Determination of hematological parameters**

A full haemogram was conducted using a chemistry analyzer (Model: Changchun Dirui Industrial Co., LTD). It was analyzed following the protocol described by Hayes (2005). Erythrocytic parameters that were analyzed included the hemoglobin count
(HB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV) and red cell distribution with (RDW). The leukocytic parameters compromised of the percentage neutrophils (%NE), percentage lymphocyte (% LYM), percentage eosinophils (% EO), percentage basophils (%BA) and percentage monocyte (% MON). While platelets parameters were the platelets count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW).

3.5 Determination of biochemical parameters

Liver and kidney function tests were investigated by an automated chemistry analyzer (Model: Changchun Dirui Industrial Co., LTD) as outlined by Hayes (2005) and Stine (2006). The liver function markers of importance included alanine aminotransferase (ALT), total bilirubin (TBILI), aspartate aminotransferase (AST) and direct bilirubin (DBILI). Kidney function markers included Blood urea nitrogen (BUN), creatinine and.

3.6 Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by Harbone (1998) and Kotake (2000). The secondary metabolites tested included flavonoids, terpenoids, cardiac glycosides, alkaloids, steroids, tannins, saponins and phenols.
3.6.1 Saponins (Froth test)

To test for saponins, 2ml of the plant extract was mixed with a few drops of sodium bicarbonate solution and shaken vigorously. The extract was then allowed to stand for 15-20 minutes and was classified for saponins content as follows:

Negative - No froth
Weakly positive - Froth less than 1 cm Positive - Froth 1.2 cm high
Strongly positive - Froth greater than 2 cm high

3.6.2 Alkaloids

The extracts were tested for alkaloids by first acidifying 5 ml of each extract with 1M HCl. This acidic medium was heated and then treated with Dragendroff’s reagent. The formation of an orange or reddish brown precipitate was regarded as positive for the presence of alkaloids.

3.6.3 Terpenoids (Salkowski test)

To 0.5 g of each of the extract was added 1 ml of ethyl acetate/petroleum ether and then mixed into 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H₂SO₄) was carefully added alongside to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.
3.6.4 Flavonoids (Sodium hydroxide test)

Extracts were tested for flavonoids by mixing 2 ml of each extract with 2 ml of diluted sodium hydroxide (NaOH). An intense/golden yellow precipitate indicated positive results.

3.6.5 Cardiac glycosides (Keller-Kilian test)

To test for cardiac glycosides, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drop of 10% ferric chloride (FeCl₃) solution. This was under-layered with 1 ml of concentrated sulphuric acid. A brown, violet or greenish ring at the interphase indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

3.6.6 Steroids

To test for steroids, 0.5 g of each of the extract was dissolved in 2 ml of chloroform. 3ml Concentrated H₂SO₄ was carefully added by the sides of the test tube to form a layer. A reddish brown colour at the interface indicates the presence of steroidal ring.

3.6.7 Phenols

The extracts were screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of each extract. Formation of blue to green colour indicated the presence of phenolics.
3.6.8 Tannins

Ten milliliter of distilled water was added to the crude extract followed by two drops of 5% iron chloride. Blue-black coloration indicated presence of tannins.

3.7 Data management and statistical analysis

Experimental data on effect of treatment with A. sativum extract over time was compared between the control group and the treated groups, recorded and tabulated on a broad sheet using Ms Excel program. Analysis of the data was done using Minitab statistical software. The results were expressed as mean ± standard error of mean (SEM) for analysis. Statistical significance of difference among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for pairwise separation and comparison of means. P ≤ 0.05 was considered significant.
CHAPTER FOUR

RESULTS

4.1 Effect of DCM-MeOH extract of *A. sativum* on body weight in normal mice

The DCM-MeOH extract of *A. sativum* induced body weight changes in normal mice at different dosages over the 28 days of administration. It was observed that an increase in the concentration of DCM-MeOH extract of *A. sativum* led to a significant change in body weight of the experimental group in comparison to the control group (P<0.05; Table 4.1).

In the first week of the extract administration, there was an insignificant change in body weight of mice in control group compared with mice in all the experimental groups (100, 500, 1000 and 2000mg/kgbw) (P>0.05; Table 4.1).

In the second week of extract administration at the four doses, there was significant increase in body weight of mice in the control group compared with the mice in groups administered with the extract at doses of 500, 1000 and 2000mg/kgbw in a dose-dependent manner (P < 0.05; Table 4.1). However, the change in body weight of mice in the group administered with 100mg/kgbw dose of the extract was insignificant compared with the control group (P > 0.05; Table 4.1). In addition, there was no significant difference in the body weight of mice administered with the extract at doses of 100, 500 and 1000mg/kgbw (P > 0.05; Table 4.1). Similarly, there was an insignificant change in body weight of mice administered with the extract at doses of 1000 and 2000mg/kgbw (P > 0.05; Table 4.1).
In the third and fourth week of extract administration at various doses, there was a significant change in body weight of mice in the control group compared with mice in the four experimental groups ($P < 0.05$; Table 4.1). However, the weight levels were not reduced in a dose-dependent manner. Moreover, the change in body weight among the mice in the experimental group was not significantly different ($P > 0.05$; Table 4.1).

There was significant increase in the average weight change per week of mice in the control group compared with mice in the experimental groups ($P < 0.05$; Table 4.1). However, the average weight change per week among mice in the experimental groups was not significantly different ($P > 0.05$; Table 4.1).
Table 4.1: Effect of oral administration of DCM-MeOH extract of *A. sativum* on body weight of normal mice

<table>
<thead>
<tr>
<th>Treatment (mg/kgbw)</th>
<th>Weekly weight of mice (g)</th>
<th>Weight/Week (g/Week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>19.40±0.51(^a)</td>
<td>0.68±0.32(^a)</td>
</tr>
<tr>
<td>100</td>
<td>19.40±0.60(^a)</td>
<td>-1.30±0.95(^a)</td>
</tr>
<tr>
<td>500</td>
<td>18.60±0.40(^a)</td>
<td>-0.96±0.86(^a)</td>
</tr>
<tr>
<td>1000</td>
<td>19.40±0.51(^a)</td>
<td>-1.2±0.81(^a)</td>
</tr>
<tr>
<td>2000</td>
<td>19.20±0.25(^a)</td>
<td>-1.34±0.22(^a)</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05. (Analysed by ANOVA followed by Tukey’s post hoc test). Key - \(^a\)represents change in


4.2 Effect of DCM-MeOH extract of *A. sativum* on Erythrocytic parameters in normal mice

The DCM-MeOH extract of *Allium sativum* induced changes on erythrocytic parameter profiles in normal mice (Table 4.2). There was significant increase in RBC count in mice administered with the extract at a dose of 2000mg/kgbw compared with mice in the control group (*P* < 0.05; Table 4.2). However, there was insignificant change in RBC count in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000mg/kgbw (*P* > 0.05; Table 4.2). Moreover, there was no significant change in RBC count in mice administered with the extract at doses of 500, 1000 and 2000mg/kgbw (*P* > 0.05; Table 4.2). The increase in RBC count was dose-dependent among the experimental groups.

Similarly, there was a significant increase in HB levels in mice in the control group compared with mice administered with the extract at a dose of 100, whereas, there was a significant decrease in HB levels in mice in the control group compared to mice administered with the extract at a dose of 2000mg/kgbw (*P* < 0.05; Table 4.2). It was also observed that there was no significant change in HB levels among mice administered with the extract at three concentrations (500, 1000 and 2000mg/kgbw) (*P* > 0.05; Table 4.2). In addition, there was insignificant change in HB levels in mice in the control group compared with the mice administered with the extract at doses of 500 and 1000mg/kgbw (*P* > 0.05; Table 4.2). However, the HB levels were not increased in a dose-dependent manner.
Moreover, there was a significant decrease in HCT count in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000mg/kgbw ($P < 0.05$; Table 4.2). There was also an insignificant change in HCT count in mice in the control group compared with mice administered with the extract at doses of 100 and 500mg/kgbw ($P > 0.05$; Table 4.2). Moreover, there was no significant change in HCT count in mice administered with the extract at doses of 100, 500, 1000 and 2000mg/kgbw of the extract ($P > 0.05$; Table 4.2). Increase in the HCT count was in a dose-dependent manner.

However, the DCM-MeOH extract of *A. sativum* did not have any significant change in MCV, MCH, MCHC and RDW profiles in mice in the control group compared with the mice in all the experimental groups after the 28-days ($P > 0.05$; Table 4.2). In addition, the MCH and MCHC levels were not reduced in a dose-dependent manner. However, the MCV and RDW levels were reduced in a dose-dependent manner.
Table 4.2: Effect of DCM-MeOH extract of *A. sativum* on Erythrocytic parameters in normal mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Erythrocytic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC</td>
</tr>
<tr>
<td>(mg/kg bw)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.31±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>7.30±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>7.64±0.53&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1000</td>
<td>7.78±0.74&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>7.88±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05. (Analyzed by ANOVA followed by Tukey’s post hoc test). **Key:** RBC-red blood cell count, HB-hemoglobin, HCT-hematocrit, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, RDW-red cell distribution width
4.3 Effect of DCM-MeOH extract of *A. sativum* on Leukocytic parameters in normal mice

The DCM-MeOH extract of *A. sativum* caused changes in leukocytic parameters (total WBC and differential WBC count) in normal mice. There was a significant decrease in WBC count in mice in the control group compared with mice administered with extract at a dose of 2000mg/kgbw ($P < 0.05$; Table 4.3). However, there was no significant change in WBC count in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000mg/kgbw ($P > 0.05$; Table 4.3). Similarly, there was an insignificant change in WBC count among mice administered with the extract at doses of 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.3). The increase in WBC count was dose-dependent.

Moreover, there was a significant decrease in neutrophil count in mice in the control group compared with mice administered with the extract at a dose of 2000mg/kgbw ($P < 0.05$; Table 4.3). It was also observed that there was no significant change in neutrophil count in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000mg/kgbw ($P > 0.05$; Table 4.3). There was also insignificant change in neutrophil count among mice in all the experimental groups ($P>0.05$; Table 4.3). There was a dose-dependent manner in the increase of neutrophil count.
There was a significant increase in lymphocyte count in mice administered with the extract at doses of 100, 500, 1000 and 2000mg/kgbw compared with the mice in the control group \((P < 0.05; \text{Table 4.3})\). However, there was no significant change in lymphocyte count among mice in all the experimental groups \((P > 0.05; \text{Table 4.3})\). Lymphocyte count increase was dose-dependent.

The DCM-MeOH extract of *A. sativum* did not show a significant change in monocyte and eosinophil count in mice in the control group compared with mice in the experimental groups (100, 500, 1000 and 2000mg/kgbw) \((P > 0.05; \text{Table 4.3})\). However, the monocyte and eosinophil count were not increased in a dose-dependent manner.

Conversely, there was a significant increase in basophil count in mice in the control group compared with mice administered with the extract at a dose of 2000mg/kgbw \((P < 0.05; \text{Table 4.3})\). There was also a significant increase in basophil count in mice in the control group compared with the mice administered with the extract at a dose of 100mg/kgbw \((P < 0.05; \text{Table 4.3})\). However, there was an insignificant change in basophil count in mice in the experimental groups \((P > 0.05; \text{Table 4.3})\). The basophil count did not reduce in a dose-dependent manner.
Table 4.3: Effect DCM-MeOH extract of *A. sativum* on Leukocytic counts in normal mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukocytic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC</td>
</tr>
<tr>
<td>(mg/kgbw)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.26±1.30a</td>
</tr>
<tr>
<td>100</td>
<td>8.56±2.28a</td>
</tr>
<tr>
<td>500</td>
<td>8.02±0.35a</td>
</tr>
<tr>
<td>1000</td>
<td>9.05±1.69ab</td>
</tr>
<tr>
<td>2000</td>
<td>11.26±0.94b</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05. (Analyzed by ANOVA followed by Tukey’s post hoc test). **Key**: WBC-white blood cell count, NE-neutrophil count, LY-lymphocyte count, MO-monocytes, EO-eosinophils, BA-basophils
4.4 Effect of DCM-MeOH extract of *A. sativum* on Platelets and their related parameters in normal mice

The DCM-MeOH extract of *A. sativum* induced changes in platelets and their related parameters in normal mice. There was no significant change in PLT, PCT, MPV and PDW levels in mice in the control group compared with mice in all the experimental groups (*P > 0.05*; Table 4.4). In addition, the PLT, PCT and MPV levels were not increased in a dose-dependent manner. However, the PDW levels were increased in a dose-dependent manner.

**Table 4.4: Effect of DCM-MeOH extract of *A. sativum* on Platelets and their related parameters in normal mice**

<table>
<thead>
<tr>
<th>Treatment (mg/kg bw)</th>
<th>Thrombocytic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLT</td>
</tr>
<tr>
<td>Control</td>
<td>947.00±131.00(^a)</td>
</tr>
<tr>
<td>100</td>
<td>865.00±66.30(^a)</td>
</tr>
<tr>
<td>500</td>
<td>1014.0±108.00(^a)</td>
</tr>
<tr>
<td>1000</td>
<td>1131.0±166.00(^a)</td>
</tr>
<tr>
<td>2000</td>
<td>986.60±80.50(^a)</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at *p ≤ 0.05*. (Analyzed by ANOVA followed by Tukey’s post hoc test). **Key:** PLT-platelet count, PCT-platelet crit, MPV-mean platelet volume, PDW-platelet distribution width.
4.5 Effect of DCM-MeOH extract of *A. sativum* on liver functions in normal mice

The DCM-MeOH extract of *A. sativum* induced changes in liver functions in normal mice. There was a significant increase in ALT levels in mice in the control group compared with mice administered with the extract at a dose of 2000mg/kgbw (*P* < 0.05; Table 4.5). However, there was no significant change in ALT levels in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000mg/kgbw (*P* > 0.05; Table 4.5). The ALT levels were not increased in a dose-independent manner.

Moreover, there was a significant increase in AST levels in mice in the control group compared with mice administered with the extract at doses of 100, 1000 and 2000mg/kgbw (*P* < 0.05; Table 4.5). There was also a significant increase in AST levels in mice administered with the extract at a dose of 2000mg/kgbw as compared with mice administered with the extract at doses of 100, 500 and 1000mg/kgbw (*P* < 0.05; Table 4.5). In addition, there was no significant change in AST levels in mice in the control group compared with mice administered with the extract at a dose of 500mg/kgbw (*P* > 0.05; Table 4.5). Similarly, there was an insignificant change in AST levels in mice administered with the extract at doses of 500 and 1000mg/kgbw (*P* > 0.05; Table 4.5). Moreover, the AST levels were not increased in a dose-dependent manner.
There was a significant decrease in TBILI levels in mice in the control group compared with the mice administered with the extract at a dose of 2000mg/kgbw ($P < 0.05$; Table 4.5). However, there was an insignificant change in TBILI levels in mice in the control group compared with the mice administered with the extract at doses of 100, 500 and 1000mg/kgbw ($P > 0.05$; Table 4.5). There was also an insignificant change in TBILI levels in mice among the groups administered with the extract at doses of 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.5). The increase in the TBILI levels was in a dose-dependent manner.

It was also observed that there was a significant decrease in DBILI levels in mice in the control group compared with mice administered with the extract at a dose of 2000mg/kgbw ($P < 0.05$; Table 4.5). In addition, there was insignificant change in DBILI levels in mice in the control group compared with the mice administered with the extract at doses of 100, 500 and 1000mg/kgbw ($P > 0.05$; Table 4.5). There was also no significant change in DBILI in mice administered with the extract at doses of 500, 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.5). In addition, the DBILI levels were increased in a dose-dependent manner.
Table 4.5 Effect of DCM- MeOH extract of *A. sativum* on liver functions in normal mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg bw)</th>
<th>ALT</th>
<th>AST</th>
<th>TBILI</th>
<th>DBILI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.48±1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.00±3.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>52.50±3.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>213.00±22.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>38.80±4.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.20±16.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.12±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1000</td>
<td>55.00±18.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>247.10±59.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.08±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>62.00±19.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>288.00±65.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.52±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05. (Analyzed by ANOVA followed by Tukey’s post hoc test). **Key**- ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, TBILI-Total bilirubin, DBILI-Direct bilirubin.
4.6 Effect of DCM-MeOH extract of A. sativum on kidney functions in normal mice

The DCM-MeOH extract of A. sativum induced alterations in kidney functions in normal mice. There was a significant decrease in BUN levels in mice in the control group compared with the mice administered with the extract a dose of 2000mg/kgbw ($P < 0.05$; Table 4.6). However, there was an insignificant change in BUN levels in mice in the control group compared with the mice administered with the extract at doses of 100, 500, and 1000mg/kgbw; the increase in BUN levels was dose-dependent ($P > 0.05$; Table 4.6).

There was significant increase in creatinine levels in mice administered with the extract at a dose of 100mg/kgbw compared with the mice in the control group ($P < 0.05$; Table 4.6). In addition, the change in creatinine levels was insignificant among mice in the control group and mice administered with the extract at doses of 500, 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.6). There was also an insignificant change in creatinine among mice in the experimental groups administered with the extract at doses of 100, 500 and 1000mg/kgbw ($P > 0.05$; Table 4.6). Moreover, the Cr levels increase was in a dose-dependent manner.

For Cl$^-$ ions there was a significant decrease in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000mg/kgbw ($P < 0.05$; Table 4.6). However, the change in Cl$^-$ ions among mice in the control group and mice administered with the extract at doses of 100 and 500mg/kgbw was not significantly different ($P > 0.05$; Table 4.6). In addition, there was an insignificant change in Cl$^-$ ions
in mice administered with the extract at doses of 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.6). Similarly, there was no significant change in Cl$^-$ ions in mice administered with the extract at a dose of 100mg/kgbw compared with mice administered with the extract at a dose of 1000mg/kgbw ($P > 0.05$; Table 4.6). However, the Cl$^-$ ions increase was not in a dose-dependent manner.

There was a significant increase in K$^+$ ions in mice in all the experimental groups compared with mice in the control group ($P < 0.05$; Table 4.6). However, there was an insignificant change in K$^+$ ions among mice administered with the extract at doses of 100, 500 and 2000mg/kgbw ($P > 0.05$; Table 4.6). There was also no significant change in Na$^+$ ions in mice administered with the extract at doses of 500, 1000 and 2000mg/kgbw ($P > 0.05$; Table 4.6). In addition, the K$^+$ ions increase was not in a dose-dependent manner.

On the other hand, there was a significant decrease in Na$^+$ ions in mice in the control group compared with the mice administered with the extract at doses of 100 and 500mg/kgbw ($P < 0.05$; Table 4.6). However, there was no significant change in Na$^+$ ions in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000mg/kgbw. Similarly, there was an insignificant change in Na$^+$ ions in mice in the experimental groups administered with the extract at doses of 100, 500, 1000 and 2000mg/kgbw; however increase in Na$^+$ was not in a dose-dependent manner ($P > 0.05$; Table 4.6).
Table 4.6: Effect of DCM-MeOH extract of *Allium sativum* on kidney functions in normal mice

<table>
<thead>
<tr>
<th>Treatment (mg/kgbw)</th>
<th>BUN</th>
<th>Cr</th>
<th>Cl⁻</th>
<th>K⁺</th>
<th>Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.13±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.80±2.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.38±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>210.04±4.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>3.75±0.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.53±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.46±1.77&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>12.06±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>234.28±5.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>3.85±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.68±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>98.78±1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.77±0.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>227.78±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1000</td>
<td>4.32±0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.72±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>106.78±1.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.47±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>222.74±3.95&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>5.06±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.86±2.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.80±1.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>220.20±4.51&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as Mean SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05. (Analyzed by ANOVA followed by Tukey’s post hoc test). **Key:** BUN-blood Urea Nitrogen, Cr-creatinine, Cl⁻-chloride ions, K⁺-potassium ions, Na⁺-sodium ions.
4.7 Phytochemical screening

Qualitative phytochemical screening of the DCM-MeOH extract of *A. sativum* revealed the presence of alkaloids, flavonoids, phenolics, steroids, saponins and cardiac glycosides. However, terpenoids were not detected in the extract (Table 4.7).

**Table 4.7: Qualitative phytochemical screening of DCM-MeOH extract of *Allium sativum***

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Presence/Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign while + (trace) denote slightly present phytochemicals.
CHAPTER FIVE

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

Discussion

Generally, search for alternative medication continues due to the existing association of synthetic drugs with several limitations including cost and side effects. *Allium sativum* has been highly researched and proven to be a broad-spectrum natural therapeutic herb. However, the plant’s safety in regard to its dosage is also important to understand in order to prevent over-dose.

The current study was designed to evaluate the *in vivo* safety of DCM-MeOH extract of *A. sativum* in normal mice models. The first aspect of *A. sativum* safety was how the body weight of the albino mice were affected by various administered dosages. The control group was administered with DMSO for 28 days and every interval of 7 days body weight taken.

In this study, there was a reported decrease in the body weight of the test albino mice after being administered with *A. sativum*. This was in agreement with Banerjee and Maulik (2002), who observed that prolonged feeding of rats with garlic 40 and ginger 40 mg/kg may cause weight loss, anaemia and stunted growth in rat models. However, this was in contrast with the findings of Al-Sarraf (1982) who observed an increase in body weight of male chicks injected subcutaneously with ethanol extracted garlic at two doses of (100 and 200mg/kgbw).
The observed weight loss may have been as a result of minimal food and water intake, a phenomenon that is due to the feeling of fullness and appetite loss after the extract administration (Joseph et al., 1989). It may also be attributed to the triglyceride-lowering activity of A. sativum (Kyo et al., 1999). The anti-lipidemic effect of the extract can also be the cause the weight loss of the albino mice (Arora and Arora, 1981). At 2000mg/kgbw dose of the extract, the average weight change per week was the highest in terms of weight loss as compared to other groups. This was likely due to increased toxicity which probably led to decreased food and water intake as reported by Joseph et al. (1989), who was working with aqueous extract of A. sativum bulbs on mice and rats.

Deleterious effect of foreign compounds, toxins, chemicals and plant extract on blood constituents of humans and animals can easily be detected through assessment of hematological parameters. In humans and most animals, these blood indices are usually used in the diagnosis of anemic episodes. Anemia is usually a condition where the body has too few red blood cells or can be understood as the body having little amount of hemoglobin to transport oxygen to tissues. There was observed decreases in red cell indices (MCV, MCH and MCHC) in this study. The observed decreases are in agreement with findings of Corzo-Martinez et al. (2007) that indicated a significant decrease of MCH and MCV in fish fed with highest A. sativum doses of (0.45 and 0.6g/kg).
The counts of RBC, HB and HCT increased significantly as the concentration of administered *A. sativum* extract increased. This is in agreement with Iranloye (2002), who reported increases in RBC and HB concentrations in rats following 30-days of garlic consumption at a dosage of 200mg/kg. Increase in the RBC count is associated with the blood promoting action of the garlic extract as established by Griffiths *et al.* (2002); who observed that garlic is a well known folk cure, which is rich in flavonoids such as quercetin and sulfur compounds in allyl propyl disulphide which are beneficial to humans.

Increase in RBC, HB and HCT counts may also be attributed to the stimulatory effect garlic on particular pathways especially involving hematopoietic growth factors (cytokines). These growth factors are involved with interaction of specific receptors on the surface of hematopoietic cells thereby, regulating the proliferation and differentiation of progenitor cells and functioning of mature cells. Phytochemicals such as flavonoids and alkaloids from garlic act as oxygen scavenger competing with hemoglobin in RBCs thereby leading to hypoxia which in turn stimulates the kidney to secrete erythropoietin hormone (Ohlsson and Aher, 2009). The end product of garlic metabolism may also step up Hb synthesis and RBC production through their indirect effect on erythropoietin. In addition, erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce RBCs (Ohlsson and Aher, 2009). Erythropoietin highly affects the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues (Polenakovic and Sikole, 1996; Oyedeji and Bolarinwa, 2013).
Although erythropoiesis is restricted to the bone marrow, Akgul et al. (2010) reported that garlic can enhance erythropoiesis in the spleen which is termed as garlic-induced extramedullary haemopoiesis. In this study, it is assumed that increase RBC, HB and HCT counts was a result of stimulation of splenic erythropoiesis in mice by garlic components that was mediated by hypoxia which led to production of erythropoietin (Akgul et al., 2010).

Moreover, it is assumed that an increase or decrease in the blood indices is as a result of a defense reaction against the administered garlic extract which takes place via erythropoiesis stimulation. The stimulation process occurs through competition between phytochemical components present in garlic, which act as active oxygen scavengers, with hemoglobin in the RBC for oxygen which later on leads to hypoxia. This, therefore, leads to the stimulation of the kidney causing direct synthesis and secretion of erythropoietin (Corzo-Martinez et al., 2007).

The extract of *A. sativum* significantly increased the levels of WBC, neutrophils, lymphocytes, monocytes and PLT counts. This is in agreement with the findings of Iranloye (2002), who, after 30 days of garlic administration a dosage of 200mg/kg in rats, reported increases in total leucocyte count, neutrophils, lymphocytes, monocytes, RBC and HB concentration. A significant increase in the level of leukocytic count was also reported by Salah et al. (2008). However, this study does not agree with that of Michael et al. (2009), who reported a significant decrease in the level of total WBC. Ugwu and Omale (2011) also reported a non-significant decrease in the WBC.
Moreover, the findings of Tatfeng and Enitan (2012) which reported significant increases in the level of total leucocyte count, absolute lymphocytes count, neutrophils and monocytes, eosinophils and basophils summation during their work on effects of onion and garlic (750mg/kg/d) extracts on immunologic cells strongly confirms the findings of this study. This is in line with the findings reported by Sumiyoshi (1997) and Bjarnsholt et al. (2005) that A. sativum is a stimulator of the immune functions in rats.
Garlic’s role in leucopoiesis may be attributed to its ability to stimulate the production of a number of colony stimulatory factors (CSF) such as the Granulocyte-CSF, monocyte-CSF and Granulocyte-Macrophage-CSF. In addition, leucopoiesis may be as a result of the effect of end product of garlic’s metabolism on cytokine production regulation, normal systemic defense maintenance (against pathogens) and bio-regulation. Cytokines activate intracellular signaling pathways which can result to myeloid and lymphoid cells proliferation and differentiation into white blood cells (Albert et al., 2002).

Increase in the levels of WBC count after oral administration of DCM-MeOH extract of A. sativum maybe attributed to the various phytochemical substances that possess antibiotic effects which cause the proliferation of circulating white blood cells that protect the body against teratogens as reported by Augusti (1996) after confirming that there was a significant increase in total white blood count in garlic-treated animal models. Increase in monocytes might have resulted from the anti-microbial and the antioxidant effects of garlic (Banerjee and Maulik, 2002). Monocytes can directly be activated by microbial products and this leads to production of pro-inflammatory and with some delay of anti-inflammatory cytokines. A variety of cytokines produced by monocytes include the tumor necrosis factor (TNF), interleukin-1 and interleukin-2 (Swirski et al., 2009).

Eosinophils are mainly involved in fight against multicellular parasites and various infections in vertebrates. They are also involved in the control of various mechanisms that are associated with episodes of allergy and asthma via the help of mast-cells
(Young et al., 2006). On the other hand, the decreases in the level of eosinophils maybe indicative of the antioxidant property of the garlic extract (Riet et al. 1995; Vazquez-Prieto et al., 2010).

Increase in PLT count may be associated with the effects of garlic extract on thrombopoietin production. Thrombopoietin is a glycoprotein mainly produced by the liver, kidney and bone marrow. The chemical components in garlic or the end products of its metabolism are believed to act on thrombopoietin which is responsible for the production, proliferation and maturation of megakaryocytes and differentiation of megakaryocytes into large numbers of platelets (thrombopoiesis) (Kandil et al., 1987).

The liver is primarily the target organ of acute toxicity when exposed to foreign substances. It is also the central site for biotransformation of xenobiotics. Principally, ALT enzyme is found in the liver where it catalyzes the amino group transfer (Cheesbrough, 1991). In any liver cell damage, the serum or plasma levels of the ALT and AST rise. The degree of liver damage is usually determined by a rise in plasma levels of these enzymes. Hepatotoxicity maybe caused by a variety of substances such as alcohol, drugs and herbs. These substances cause necrosis of the hepatocytes, increased permeability and later lead to leakage of cellular enzymes from liver cytosol into the bloodstream. In the current study, there was a significant increase in both ALT and AST enzymes upon administration of A. sativum at 2000mg/kgbw. This, therefore, indicates that the high concentration of the garlic extract may have had cytotoxic effect on the liver (hepatotoxicity) (Cheesbrough, 1991).
The garlic extract may have affected the permeability of the liver cell membrane thereby making the membrane leaky. The permeability of the cell membrane subsequently induces the release of these enzymes from the cell into the bloodstream thereby resulting in high serum or plasma concentration of these enzymes (Emerson et al., 1993). This did not agree with findings of Augusti et al. (2001) who reported that lipid parameters and enzyme activities including AST, ALT and ALP in rat serum decreased significantly when fed with a diet containing 5% garlic.

The ultimate breakdown product of hemoglobin is bilirubin and is a very useful tool in the diagnosis of liver and blood disorders (Johan, 2008). Bilirubin may either be direct or indirect. Bilirubin in the blood is collectively termed as total bilirubin. In this study, it was found that there was significant increase in the concentration of total and direct bilirubin. Therefore, this is indicative of hepatotoxicity, which may have been as a result of high concentration of the garlic extract. This observation is in agreement with the finding of Zbinden (1991) and Ballet (1997).

Creatinine is the by product of muscle metabolism and is derived from creatine and phosphocreatine. Serum creatinine is mostly used in indirect measure of glomerular filtration rate and is filtered and excreted by the kidney. Creatinine clearance can be calculated by measuring the levels of creatinine in blood and urine, which also reflects the glomerular filtration rate (GFR) (Delanghe et al., 1989). The creatinine levels were also seen to be increasing as the concentration of the garlic extract increased. Adelman et al. (1981) argues that an increase in the creatinine levels is usually due to nephrotoxicity. This shows that the high concentrations of the garlic extract caused
kidney malfunctions and, therefore, not safe. Blood urea nitrogen (BUN) is a waste product formed in the liver after protein breakdown, which is later filtered and excreted by the kidneys. The elevated levels of BUN may have been as a result of nephrotoxicity (Adelman et al., 1981).

Serum electrolytes were also analyzed and there was a reduced level of serum sodium as the concentration of the garlic extract increased. This maybe as a result of change in the glomerular filtration or a change in the renal blood flow (Zanchetti et al., 1985). Moreover, this may be caused by an interference with aldosterone secretion and/or aldosterone action on the distal tubules (Otsuka et al., 2000). Ultimately, the garlic extract may have been involved in the interference of the adrenergic sodium handling thereby causing its decrease. The serum potassium levels in the experimental groups showed a significant increase as compared to the control group. This maybe attributed to the alteration in the potassium transport system that may have been caused by the administration of the garlic extract (Zanchetti et al., 1985).

In addition, after the photochemical screening, various phytochemicals were observed which included alkaloids, flavonoids, steroids, saponins, cardiac glycosides and phenolics whose biological and physiological roles have been documented by Fenwick and Hanley (1985). These phytochemicals are believed to play a major role in gene expression, enzyme activity, stimulation of the immune system and organ related to blood cell formation (in the bone marrow) (Jeorg and Lee, 1998).
It may also be assumed that the increase in WBC counts, neutrophils, monocytes and lymphocytes was as a result of the phytochemicals stimulating the immune system. Flavonoids, cardiac glycosides and alkaloids may have been responsible for the increase in the RBC count due to their antioxidant properties. This probably could have been achieved by their good free radical scavenging properties that protect the RBCs against oxidative damage. In additions, saponins which have anti-nutritive effects may have resulted in RBC turnover in this study (Sparg et al., 2004).

However, an increase in the concentration of the extract, which may be assumed to cause an increase in the level of saponins, may have resulted to a decrease in red blood cell indices (MCV, MHC and MCHC). This may have exhausted the erythropoietic capacity of the mice models therefore, leading to anaemia (Sparg et al., 2004).

Terpenoids were seen to be absent in the phytochemical analysis of this study even though other researchers had found it present in their assays. Therefore, this can be attributed to divergence in soil profiles and also the environmental conditions such as climate and surrounding vegetation which can lead to either a lack of, a decrease or even an increase of a given phytochemical (Block, 2010).

5.2 Conclusion

From the study it can be concluded that;

i. The extract promoted erythropoiesis, leucopoiesis and thrombopoiesis as the concentration was increased.
ii. The extract was hepatotoxic and nephrotoxic at higher concentrations

iii. The extract had phytochemicals associated with the observed effects on experimental animals.

The present study, therefore, clearly shows that *A. sativum* is safe at particular doses as indicated by changes in hematological parameters. On the other hand, the study also shows that plant extract is not safe at high doses as indicated by change in the liver and kidney parameters which showed hepatotoxicity and nephrotoxicity respectively, in this study, the alternative hypothesis is hence accepted.

**Recommendations**

i. The dosages above 1000 mg/kg body weight of *A. sativum* L. can be considered to be unsafe and therefore should for used.

ii. Quantitative analysis of phytochemicals that could be toxic at a higher dose in the extract can be pursued.

iii. Histopathological preparations can be carried out to assay various toxicological effects of the extract on liver and kidney tissues.

**Suggestions for further studies**

iv. Need to carry out chronic *in vivo* safety study using the extracts of *A. sativum* and compare the outcomes.

v. Perform a similar study using alternative routes of extract administration other than the oral route used in the current study and compare the toxicity outcomes.
REFERENCES


**Borek C. (1998).** Recent advances on the nutritional benefits accompanying the use of garlic as a supplement. Newport Beach, CA.


APPENDICES

Appendix 1.1: Image of *A. sativum* L. (photograph taken in November 2015 at Kiambu Market)
Appendix 1.2: Descriptive image of *A. sativum* L. (Retrieved from Block, 2010)
### Appendix 1.3: Normal Hematology Reference Values (CDC, 2008)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>35.1 - 45.4</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.0 - 15.1</td>
</tr>
<tr>
<td>RBC (x 10^6/μL)</td>
<td>6.36 - 9.42</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>14.1 - 19.3</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.2 - 34.2</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>45.4 - 60.3</td>
</tr>
<tr>
<td>WBC (x 10^3/L)</td>
<td>1.8 - 10.7</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>6.6 - 38.9</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>55.8 - 91.6</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.0 - 7.5</td>
</tr>
</tbody>
</table>