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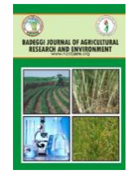

Research Article

Neuro Protective Effect of Lycopene and *Tetracarpidium Conophorum* Extracts on Heat-Stressed Cockerels

Ojo, O. A[1]., Akintunde, A. O[2] Jimoh O. A[3] and Baraje M. S[1].

[1]Department of Animal Production, Kwara State University, Malete.
[2]Department of Agriculture and Industrial Technology Babcocke University, Ogun State
[3]Animal Production Technology, Federal Polytechnic Ado Ekiti, Ado Ekiti, Nigeria
Correspondence e-mail: ykmelodya1@yahoo.com

Abstract

The study was conducted to determine the neuro protective potential of lycopene and *Tetracarpidium conophorum* leave Extract (TCLE) on acetyl cholinesterase activity and oxidative status of heat-stress cockerels. A total of 54 cockerels of 25- week old were used for this experiment. There were nine treatments: 0 ml of extract/ 250 ml of water (control)(T1), 30 ml of lycopene/250 ml of water (T2), 60 ml of lycopene/250 ml of water (T3), 30 ml of TCLE/250 ml of water (T4), 60 ml of TCLE /250 ml of water (T5), 15 ml of lycopene + 15 ml of TCLE/250 ml of water (T6), 30 ml of lycopene + 30 ml of TCLE/ 250 ml of water (T7), Vitamin C 0.1g/250 ml of water (T8), Cooler temperature (25±2^{o}C) + Cold water 4^{o}C (T9). Total Protein (TP), brain antioxdant; superoxide peroxidase (SOD), catalase (CAT), Glutathione peroxidase (GPx), Glutathiones-S transferase (GST), Glutathione (GSH), Malondialdehyde (MDA) Acetylcholinesterase (AChe) and phytochemical quantitative/ qualitative analysis of TCLE were evaluated.Results showed that value obtained for crude protein (7.24%), moisture (4.66%), ash and crude fibre content (9.75% and 2.49%) of TCL were depressed, while carbohydrate value (65.30%) was elevated. Also TCLE contain essential compounds as saponin, tannin, phenolics, flavonoids, alkaloids, steroids, glycosides, coumarins, triterpenes, terpenoids and fixed oils, which are present in low quantity(+). There was non-significant effect (P>0.05) of lycopene and *Tetracarpidium conophorum* on TP, MDA, SOD and catalase of cockerel brain, however, significant elevation was observed in the brain GST was significantly elevated treatment 4 (1490.45 U/mg protein), GPx (1009.04 U/mg protein), GSH (71.03 U/mg protein) and Acetylcholinesterase activities were significantly (P<0.05) influenced by the treatments. It was observed that the experimental inclusions did not significantly impose any negative effect on the cockerels.

Keywords: *Acetylcholin esterase, Brain antioxidant, Heat-stress, Lycopene, Tetracarpidium conophorum.*

Introduction

Human brains share a remarkable similarity with those of chickens, rats and mice, among other mammals (Beechamp et al., 2022). This has necessitated the use of these species in research, aimed at understanding human brains, particularly at early developmental stages, steamed from the easier access to the embryo in the egg of chickens. According to Squire (2012) and Lushchak et al. (2021), although brain comprises of as low as 2% of the body weight, yet, it employs close to 25% of the body's total glucose engaged for use and 20% of oxygen used by the body. The brain is an organ with a high metabolic requirement, due to its intense heat production (Howarth *et al.*, 2012). Acetylcholine is a germane neurochemical, having an expanded variability and functionality in relation to the brain and other parts of the body. This is because it relates as a released chemico-neural impulse, indulging an interface within and

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among several other specialized cells, such as myocytes and glandular tissues (Sam and Bordoni, 2023). Stress has been shown to unfavorably affect the normal physiological performance of the central nervous system, causing structural changes in different parts of the brain (Lupien *et al.*, 2009 ; Vona et al., 2021), including acetylcholine. According to Chu et al. (2023), when exposed to favourable stress-free environment, the brain is in relatively phychological homeostatic condition. However, an incessant stress challenge resulting from either intrinsic, extrinsic, tangible or even intangible are described as stressors. Novel findings clearly depict that overly production of free radicals have negative effects on the antioxidant fortification, resulting into oxidative stress. Oxidative stress occurs due to redox inequality, at either cellular or whole organism level (Pizzino *et al.,* 2017). Stress is expressed as a condition of conflict (cacostasis), impeded by a complicated symphony of physiologic and behavioral responses, which targets rectification mechanism of endangered homeostasis, known as adaptive stress response. This type of behavioural response is conciliated by certain complex and integrated stress mechanism inclusive of the neuroendocrine, cellular and molecular architecture, domiciled in the central and peripheral nervous systems (Tsigos et al., 2000). Although the mechanism by which a single act of stressful event moderates the neurophysiological extension of neuronal plasticity and acetylcholinesterase inhibition is not fully understood (Colavitta and Barrantes 2023). However, acute stress evokes a momentary elevation in the expressed quantity of neurotransmitter acetylcholine, thereby improving excitations at the neural junctions. Kaufer et al. (1998) has also delineated that there is a close synergistic transformation of genes, which modulate acetylcholine obtainability, following stress and obstruction of acetylcholinesterase. Recent finding revealed alignment of cholinergic neurotransmission with a series of important psychological processes including, but not limited to memory, learning process, waking and sleeping cycles and stress response (Stanciu et al., 2020). Novel findings clearly depict that overly production of free radicals have negative effects on the antioxidant fortification, resulting into oxidative stress. Oxidative stress occurs due to redox inequality, at either cellular or whole organism level (Liu *et al.*, 2018). Bremner (2012) attested that chronic stress causes brain mass atrophy, associated with detruded weight, which negatively influences acetylcholinesterase (AChe) functions. An enzyme known as AChe breaks down the neurotransmitter, referred to as acetycetylcholine and heightens transmittance of the next nerve impulse transversely within the gap at the synaptic cleft. Report showed that AChe flag the hippocampus in knowledge attainment, while reminiscencing and controlling the amygdala in arbitrating fear reaction, which are noted to be critical nodes in mechanism of animal systems adaptation and subsequent reaction to stress (Belujon and Grace, 2011 ; da Costa et al., 2023).

As a way of exploring ameliorative solution, there has been a frequent use of phyto-derived natural antioxidants such as lycopene produced from fresh tomatoes and Tetracarpidium conophorum leaves (TLC), having similar activities with synthetic antioxidants (Yashin *et al.,* 2013). Studies on TC showed that leaves contain phenolic compounds such as gallic acid, catechin, chlorogenic acid, caffeic acid, coumarin, rutin, quercitrin, quercetin, kaempferol, and luteolin, enhancing it as a potential antioxidant herbal supplement. The neuroprotective action of lycopene in cell culture and animal models has been extensively studied along with incidence of oxidative damage to DNA in vitro (Mirzaee *et al.*, 2021). A critical study of the cockerel brain is paramount as it controls physical gesturing, maintenance of the balance of the animal or subject, regulation of muscle tension and the function. Therefore, any damage to the brain is a damage to the whole animal, there is therefore an urgent need to proffer solution to this impending problem. Chijoke *et al.* (2017) stated that the leaf extract of *Tetracarpidium conophorum leaves* (TCL) has been noted as significantly effective as an antioxidant on the reproductive pathway of the rats in a dose-dependent manner. Although lycopene has been documented to have neuro-protective substance, but the combined effect of Lycopene and TCL in protecting brain cell damage in cockerels exposed to heat stress is scanty. This study is geared towards evaluating the neuro-protective potential of lycopene and TLC extracts on the acetylcholinesterase activity and oxidative status of heat stressed cockerel

Materials and Methods

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Kwara State, Nigeria. The University is located within latitude 8.7100˚N and longitude 4.4666˚E and lies in the North Central part of Nigeria. It has an average temperature of 26.5˚C and relative humidity of 70% with rainfall of 1217mm.The experiment was carried out at the early dry season (October - December) with an average temperature and relative humidity of 28.5^{0}C and 70 %

A total of 54 cockerels of 25 week old were used for the experiment. Birds were procured from a reputable farm in Ilorin metropolis. The birds were individually wing-tagged for identification purpose and birds under each replicate were housed accordingly. Birds were given clean water and fed *ad-libitum* with commercial breeder mash containing 17.5% crude protein and 2700 kcal metabolizable energy. Medications and vaccinations were done as required. The cockerels were subjected to nine (9) treatments, each replicated thrice, with 2 birds per replicate in a completely randomized design. The treatments were:

T1: Treatment 1: control group (0ml) of extract/litre of water
T2: Treatment 2: 30ml of lycopene / litre of water
T3: Treatment 3: 60ml of lycopene /litre of water
T4: Treatment 4: 30ml Tertracarpidium conophorum leaf extract (TCLE) /litre of water
T5: Treatment 5: 60ml of Tertracarpidium conophorum leaf extract /litre of water
T6: Treatment 6: 15ml of lycopene + 15ml of TCLE /litre of water
T7: Treatment 7: 30ml of lycopene + 30ml of TCLE /litre of water
T8: Treatment 8: 0.1g Vitamin C per /litre of water
T9: Treatment 9: cold environment (25±2^{O}C) of Cold temperature + Cold water (5-7^{o}C)

Locally available bright red tomatoes (*Solanum lycoperisicum*. L) were procured from local market. The tomatoes were washed under running stream of water to remove all dirt, dust and foreign materials attached to their surface. De-heading and trimming of the tomatoes were carried out manually using a sharp knife. The fresh bright red fully mature tomatoes were grinded extensively and the resulting tomato pulp were properly homogenized. It was cooked for 3 hours at 99 ± 2^{o}C and then reduced to a thick, red concentrate. Likewise, fresh sample of Tertracarpidium conophorum leaves (TCL) were obtained from the Ore, in Ondo State. The leaves were cleaned, air dried, macerated and blended into powdery form and then stored for subsequent usage.

Extraction of Lycopene: This involves the simple application of organic solvents for lycopene extraction as adopted by Roldan-Gutierrez *et al.* (2007). The tomato paste was properly homogenized to break the cell structure of these natural food materials for efficient extraction of the lycopene. About 20 g of the sample (Lycopene) and was taken into 250 ml of the conical flask. Samples were extracted overnight in the orbital shaker, with the solvent mixture of 200 mL of hexane and acetone in the ratio of 75:25 respectively at room temperature. The extract from each flask was filtered with Whatman No. 1 filter paper. The solvent from extract was separated at 50°C in a rotary vacuum evaporator (EYELA, N-N series, Japan) leaving behind crude extract only, stored at 4°C until use.

Preparation of Extract from *Tetracarpidium conophorum* leaves (TCL): TCL were obtained from a Town called Ore, in Ondo State, Nigeria. The freshly collected leaves were separated, air dried, and milled into powder using an electric blender. The total weight of the powdered leaves obtained for this study was Tertracarpidium conophorum leaf of ethanol and kept in conical flask. The resulting weight of the powdered leaves was 542.6g, it was extracted using ethanol at ratio 1:5 using a reciprocating shaker for 24 hours to achieve continuous agitation at 150 revolution/minute. This enhanced thorough mixing and complete elucidation of active materials to dissolve in the respective solvent. Then extract was filtered by using muslin cloth, followed by filtration using Whatman filter paper. Solvent from the extract was removed, using rotary evaporator at 75^{O}C and transferred to water bath with temperature $50^{O}C$ for further removal of sample moisture. Residue was finally collected for the experiments.

After the completion of the experiments, 3 cockerels were randomly selected per treatment (1 per replicate) were slaughtered and whole intact brain was carefully removed from each replicated and incubated on ice and homogenized prior to analysis (Schmidt, 2005).

AChE activity was determined using the colorimetric. The brain sample were tested and 40µl were added to test the sample and was gently mixed. HRP-conjugate 100µl were added and incubated using closure plate membrane and incubated for 60 mins at $37^{0}C$, the liquid was configured by diluting it with distilled water and dried by swing, washing buffer were added and still for 30 seconds, then drained and repeated for five times. Chromogen solution A and Chromogen solution B of 50µl were added and were evaded for 15 min at $37^{0}c$, then the reaction were stopped by adding stop solution of 50µl and the blue color changed to yellow colour. The AChE activity is expressed as AChEng/mL of tissue protein.

Collected data were subjected to Two-way analysis of variance using GLM module of Statistical Package for Social science (SPSS) version 23 (IBM, 2015). Significant difference in mean were separated using Duncan Multiple Range.

Results and Discussion

Table 1 **s**hows the proximate composition of TCL. The results showed that it contains crude protein content of 7.24%, crude fibre (2.49%), crude lipids (10.56%), carbohydrate (65.30%), moisture content (4.66%), Ash (9.75%) and calorific value (1609.69KJ/100g). TCLE contained low amount of protein, similar to the value (8.76) as reported by Ojobor*et al.* (2017). The ash content (9.75%) of TCL observed in this study is also low. This is an indication of low amount of inorganic nutrients. A very low moisture content (4.66%) of TCL observed in this study shows a high storage capacity, to prevent microbial attack is desirable. This is in line with the findings of Chikezie (2018), who also reported low moisture content of 5.46. Protein is an important part of animal structure and metabolism, as it forms a major component of muscle connective tissue and cell membrane (Ojo, 2017). The carbohydrate content is elevated (65.30%) reflecting TCL as a good source of energy. The value for carbohydrate in this study is higher than 24.36 reported by Ojobor *et al.* (2017). The crude fibre content obtained in this study is depressed; lower value of 1.24 has also been reported by Chikezie (2018). The crude lipid and energy value observed in this study are higher compared with the value of 3.77% (crude lipid) and 234.57kj/100g (energy value) reported by Ojobor *et al.* (2015). Evaluation of nutrients in herbal plants through assessment of the proximate composition, minerals and vitamin content of vegetables play important roles in understanding the nutritional values and medicinal worth of such plant species(Akomolafe et al., 2017), as well as its ability to enhance the scavenging properties of the plants when used in ameliorating heat stress effects in heat-stressed animals. The proximate composition result recorded is an expression of the

The quantitative analysis of *Tetracarpidium conophorum* leaves is as shown in Table 2**.** The saponin content in TCL is 23.80, the steroids (289.27), flavonoids (182.49), phenolic (163.40), the terpenoids content (141.83), coumarins (29.35), the glycosides (2.42), the alkaloids (183.56), while the value for triterpenes and tanin are 120.93 and 6.67 g/kg, respectively. It can be observed that alkaloid, flavonoids and steroids content of *Tetracarpidium conophorum*is very high. Generally, alkaloids have medicinal and physiological function, pure isolated alkaloids from plants and their derivatives possess analgesic, anti-spasmodic and anti- bacterial effects (Okwu and Emelike, 2006). Alkaloids are the effective plant substances used therapeutically as analgesic, anti-microbial and antibacterial properties. Steroids have been reported to have antibacterial potential (Raquel, 2007), and they are very important compounds, especially due to their relationship with compounds, such as sex hormones (Okwu, 2001). Ajaiyeoba and Fadare (2006), reported that TCL has been reported to cure male-related infertility. Some plant steroids are also useful for their effects when consumed by human beings, because their presence decreases the amount of cholesterol in the blood stream.

(Wisegeek, 2013). The leave has a high amount of flavonoids 182.49 mg/100g. Flavonoids have been reported to have anti-oxidative property, free radical scavenging capacity, coronary heart disease preventions and anti-cancer activity (Salah *et al.,* 1995; Del-Rio *et al.,* 1997). The leaves also contain a high amount of phenols. Phenols and phenolic compounds have anti-microbial and anti-oxidant protective effects and are anti-tumor agents (Omoboyowa*et al.,* 2012). There were appreciable quantity of saponin and tannin. The presence of tannins suggests the ability of this plant to play a role as antidiarrhoeic and antihaemorrhagic agent. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Tannins bind to proline – rich protein and interfere with protein synthesis. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003).

Generally, phytochemicals are natural antioxidants, which represent one of the prominent ingredients needed in animal diets to improve the potentials of antioxidant enzymes in scavenging activities in heat-stressed animals, with positive feedbacks reflected by improved apparent performance, productivity and internal physiological changes (Lee et al., 2017). Result in this studies showed that the phytochemicals in the herbal supplements enhanced the performance of the heat-Stressed cockerels.

Table 2 shows the Phytochemical screening of *Tetracapidium conophorum* leaves. The results showed that *Tetracapidium conophorum* contains essential compounds such as saponin (+), tannin (+), phenolics (+), flavonoids (+), alkaloids (+), steroids (+) and glycosides (+). Others compound such as coumarins (+), triterpenes (+), terpenoids (+) and fixed oils are equally present, while compounds such as anthocyanin, phlobatanin and amino acid were not present in *Tetracapidium conophorum* leaves. Phytochemicals are biologically active compounds, found in trace amounts, which are not established nutrients, but which nevertheless contribute significantly to protection against degenerative diseases (Dreosti, 2002). The presence of trace of flavonoids and tannins is consistent with the report of Ojobor*et al.* (2015) who also observed trace amount of these compounds in *Tetracapidium conophorum* leave. Ayoola *et al.* (2011) also reported the presence of moderate amount of tannins, saponin, phenolics, and alkaloids in *Tetracapidium conophorum*root. The findings in this study are similar with what was obtained by Akomolafe and Ojobor (2017b).

Acetylcholinesterase activities of the brain of heat-stressed cockerels given lycopene and TCLE is shown in Figure 1. It was observed that the experimental inclusions significantly imposed a depressive effect on the brain acetylcholinesterase activities of experimental cockerels under treatment 2 (T2), however, T3 and T9 showed higher values compared with the control.ACh is a neurotransmitter at the sweat glands, and at the piloerector muscle of the sympathetic autonomic nervous system (ANS). The predominant function of AChE is to abolish neuronal transmission and signaling betwixt synapses via hydrolysing the neurotransmitter acetylcholine into acetic acid and choline at brain cholinergic synapses and neuromuscular connection to avert ACh dissemination and vivification of nearby receptors (Seth, 2009; Amy and Paras, 2021). The catalyzing activity of AchE stem into anatomization of ACh, thus terminating its action on ACh receptors (Silman, 2010). According to Nordberg et al. (2013), cholinesterase inhibitors depresses the breaking down of synaptic acetylcholine, improves brain acetylcholine levels in a dose-dependent manner and thereby boosting cholinergic transmission in patients with Alzheimer's and other similar cases. This reduction in the AchE activity affected the behaviour, survivality of individuals and performance of the animals in question. Extensive research has established the involvement of cholinergic system in regulating stress, as evident from past studies. Perhaps, the observed elevation in the AchE values in T3 and T9 (Figure 1) might have resulted from the thermal assault, which the birds were exposed to. Stress can be rated as either acute or chronic type, depending on the duration of exposure. According to Marana et al. (2010), both initiate neuroendocrine pathways that alter the physiological proceedings in order to stablilize homeostasis and to ascertain the safe existence of the animal This is in congruent with the report of Mark *et al.*(1996) who stated that acute inescapable stress enhanced release of ACh and induced expression of genes that regulate ACh availability in the hippocampus and prefrontal cortex. An increased AchE value noted in T2 is in tandem with the findings of Świergosz-Kowalewska *et al*. (2014), who also observed depressed values of AchE after studying the interraction between temperature effects and two chemical stressors on the AchE of Bank voles, which caused depressed overall performance. Contrary to this, Nitika *et al*. (2020), reported an escalation in the AchE value when he tested the effect of Citrus limon fruit juice on the AchE of rat brain after been challenged with heavy metals and pesticides. **T**he influence of lycopene and *Tetracapidium conophorum* leaf extract on the brain oxidative biomarkers and total protein are as presented in Table 5. Observation showed that the total protein, SOD U/mg and MDA values were not significantly influenced by the experimental phytogenics. However, the Glutathione S-transferase (GST) U/mg, Glutathione (GSH) U/mg, Glutathione peroxidase (Gpx) and Catalase (CAT) U/mg level of the brain were significantly affected. The GST value was noted to be significantly elevated in T6 (322.48) with statistical similarity to T3 (154.58), while the least value was observed in T7 (56.62). T1 has significant increase in the values of GSH, CAT, but lower in the Gpx value. However, the recorded GPx value was raised significantly in T3, T4 and T6. The total protein U/mg value across the treatment ranged within 12.43 to 15.20 U/mg with birds of T6 having the numerically highest value (15.20). Glutathione S-transferases are purifying enzymes that catalyze the concormittance of glutathione with harmful electrophilic molecules, either autogenously or extracellularly produced, thereby safeguarding cells from carcinogenic factors. Enzymatic antioxidants, such as plasma GSH-Px and SOD donates to the intracellular antioxidant oxidative sheltering of animal tissues via catalysing the reduction of hydrogen and lipid peroxides (Halliwell& Chirico, 1993) and is also considered an indicator of oxidative stress (Tüzün et al., 2002). Studies in grazing sheep have revealed that glutathione-peroxidase (GSH-Px) activity is impacted by season (Andrés et al., 1999).Studies in dairy goats have also shown that blood GSH-Px activity is diminished during the postpartum period, suggesting some degree of oxidative stress and lipid peroxidation, influence its serum concentration (Celi et al., 2008). Superoxide dismutase has been noted to dismutates superoxide into hydrogen peroxide, which is in turn degraded by catalase or by glutathione peroxidase in line with the report of Ankita et al. (2019). Higher plasma concentration of these antioxidant enzymes would be highly beneficial to the animal as this would enhance the enzymatic "mopping up" mechanism of the generated Reactive Oxygen Species (ROS).. Cellular damage would be reduced to the lowest level to achieve maximum performance in the animals system. The observed elevation in the GST and GPx values of birds under T6 could have occured as a result of the numerically increased total protein value in the same treatment. In line with the findings of Feoli et al. (2006), protein malnutrition increased oxidative damage to lipids and proteins after he studied rat brain areas exposed to malnutrition and resulting into improvement in antioxidant enzymes in protein repletion. This might be an indication of the importance of observable changes in brain development traceable to malnutrition and or stress. Proteins have long been considered as germane to antioxidants as a result of their abundance in biological systems, backed up by an increasing evidence about the significant antioxidant activity in proteins (Medina-Navarro et al., 2010). New concepts have considered albumin component of total protein as the major antioxidant in plasma constantly exposed to oxidative stress. So an increase in the plasma concentration of total protein could have improved the "mopping up" potential of GST and GPX observed in T6. The numerical elevated values in Superoxide dismutase (SOD), notable in T4 and T7 respectively is proportional to the values recorded for catalase (CAT) under similar treatments. According to Frei (1994) and Kehrer (1994), SOD activity increases H_2O_2 production, whereas, protective mechanism from reactive oxygen could only be attainable by a simultaneous increase in catalase. This might have reflected in the numerically lowest malondialdehyde (MDA) values recorded especially under T7(0.32±0.02). Catalase is a very important enzyme in protecting the cell from oxidative damage through reactive oxygen species (ROS), by converting hydrogen peroxide molecules to water and oxygen per second. (Ankita et al., 2019;Vona et al., 2021).

Conclusion

TCL contains elevated carbohydrate value, while essential compounds as saponin, tannin, phenolics, flavonoids, alkaloids, steroids, glycosides, coumarins, triterpenes, terpenoids and fixed oils are present. The combination of lycopene and *Tetracarpidium conophorum* had no influence on TP, MDA and catalase of cockerel brain, however, brain GST, GPx, GSH, SOD and Acetylcholinesterase activities were influenced by the treatments. It was observed that the experimental inclusions did not significantly impose any negative effect on the cockerels.Supplemental inclusion of lycopene and *Tetracarpidium conophorum* leave extract could enhance brain antioxidants potential which is better expressed when used in the ratio 15 ml of lycopene + 15 ml of African walnut leaf extract /litre of water. Further research is recommended to investigate the effect of the duo phytogenics on other vitagenes to ascertain its efficacy in alleviating adverse effect of heat stress in chicken.

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Ethics Statement

This study was duly approved by the Ethical Committee of the Kwara State University. The manuscript is be in line with the recommendations for the Conduct, Reporting, Editing and publication of scholarly work in animal study.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' Contributions

Ojo O. A designed analyzed the raw data and wrote the manuscript . Akintunde A .O ensured the implementation of the lab analysis Jimoh O.A editted the manuscript to standard and Mustapha S. managed the experimental animals and collected data .All authors approved the final article.

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Table 1:Proximate Composition of *Tetracarpidium conophorum* Leave Extract (TCLE)

Proximate composition	Values
Moisture%	4.66
Ash%	9.75
CHO%	65.30
Calorific Value Kj/100g	1609.69
Crude Lipids%	10.56
Crude Fibre%	2.49
Crude Protein %	7.24

CHO- Carbohydrate

Table 2: Quantitative Analysis of *Tetracarpidium conophorum* Leave (TCL)

Phytoconstituents	Quantitative abundance (mg/g)
Saponin	23.80
Steroids	289.27
Flavonoids	182.49
Phenolos	163.40
Terpenoids	141.83
Coumarins	29.35
Glycosides	2.42
Alkaloids	183.56
Triterpenes	120.93
Tanin	6.67

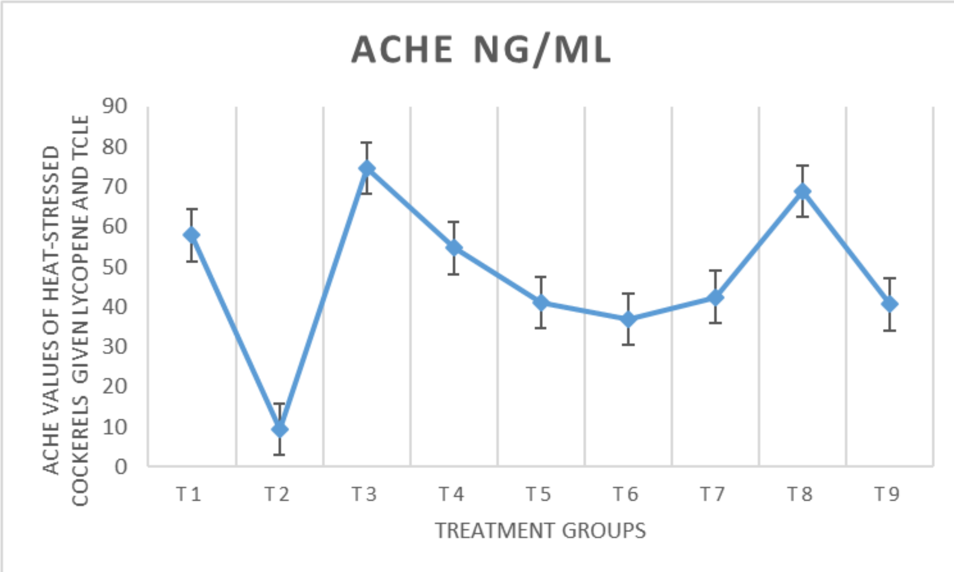

Figure 1: The acetylcholinesterase activities of the brain of heat-stressed cockerels given lycopene and AWLE