# ERECTOGENIC EFFECT OF THYME (*Thymus vulgaris)* EXTRACT IN NORMAL AND 5-FLUOROURACIL INDUCED OXIDATIVE STRESSED RATS


## ABSTRACT

This study determined the effect of oral administration of aqueous extract from Thyme (Thymus vulgaris) extract (TVE) on the antioxidant status and activity of some penile function enzymes (acetylcholinesterase (AChE), phosphodiesterase-5 (PDE-5), adenosine diaminase (ADA), and arginase) activity in normal and 5- Fluorouracil- induced oxidative stressed rats. Sixty adult Wister rats (210-225 g) were divided into ten (10) groups (n=6): Group 1: received oral administration of normal saline (NC), Group 2: received 100 mg/kg of thyme extract orally (TE 100 mg/kg), group 3 rats received 200 mg/kg of thyme extract orally (TE 200 mg/kg), rats in group four (4) were treated with 400 mg/kg of thyme extract orally (TE 400 mg/kg), Those in group 5: received 25 mg/kg of Vitamin C orally, while group 6 to 10 were induced with 150 mg/kg of 5-Fluorouracil solution (5-FLU, i.p), but group 7-10 were treated 100 mg/kg, 200 mg/kg, 400 mg/kg and Vitamin C (25 mg/kg) respectively. After fourteen (14) days of treatment, the rats were sacrificed and the penile tissue was carefully isolated and prepared into homogenate, which was used for antioxidant and enzymes biochemical analysis. The result revealed that i.p induction of 5-FLU caused a significant increase in malondialdehyde level, as well as AChE, ADA, PDE-5 and arginase activities wth concomitant decrease in thiol level when compared to control rats. However, the administration of TVE was found to reverse the effect of 5-FLU. The TVE was also found the reduced MDA level and all the enzyme activities, but boosted the thiol level in the normal rats when compared to control rats. Interestingly the effect of the TVE was found dose-dependently, and 400 mg/kg TVE was found to be more potent among all the doses used in both normal and 5-FLU-induced oxidative stress rats.



Keywords: Erectile dysfunction; thymus vulgaris; Fluorouracil; Arginase; Oxidative Stress


## 1.0 INTRODUCTION

5-Fluorouracil (5-FLU) has been regarded as one of the most frequently prescribed chemotherapeutic drugs for the adjuvant and palliative treatment of patients with cancers of the gastrointestinal tract, breast, head and neck [1] Comparable to other chemotherapeutic agents, 5-FLU generates excess reactive oxygen species (ROS) and overpowers the antioxidant defense mechanism [2]. ROS interaction with cellular biomolecules including lipids, proteins and DNA, and alters the essential cellular functions. Thus, ROS caused mutations, result into malignant transformation and the development of cancer [3]. The use of cancer drug is allied with cognitive impairment and possibly called chemobrain. Chemobrain results in impaired learning, memory and altered processing speed. Thus, could be as a result of decline in proliferating new brain cells and decreased myelination of axon. Biomolecules structural changes due to medications by oxidants could lead to loss or dysfunction of activities of these biomolecules. This oxidative modification effects could be combated through mechanisms such as protein refolding or degradation, lipid turnover and DNA base excision and repair. Brain neuronal homeostasis is distressed when this mechanism is bridged, oxidative stress therefore ensues [4]. Oxidative stress is a major risk factor implicated in several human diseases such as cardiovascular disease, brain and learning dysfunction, kidney and heart failure, erectile dysfunction among others [1].

Erectile penile dysfunction (ED) is the inability of man to have strong penile erection, enough for sexual activity [5,6]. Studies have described ED as a common condition that becomes more prevalent as men age [5]. ED accounts for 45% of male sexual dysfunction in Nigeria and a larger population of men worldwide have also been predicted to be affected with ED in the future [7,8]. Penile erection involves neurovascular events modulated by psychological and hormonal factors [9]. Proper erection occurs when upon sexual stimulation, there is a coordinated interplay between the penile vasculature, neural impulses, hormone level and cognitive behavior [10]. An important regulator of penile erection is nitric oxide (NO) and is released from both the endothelial cells as well as the neural tissue that supplies the corpora cavernosa [11]. NO is a gaso-transmitter which is involved in smooth muscle relaxation, promoting dopamine release and activating luteinizing hormone-releasing hormone thus controlling sexual behavior [12,5].

ED can arise from disturbances from vascular, neurologic, psychological or hormonal factors [13]. Medications and substances (such as tobacco, antidepressants) can also exacerbate ED [14]. As such, ED is a strong predictor for cardiovascular diseases (CVD), diabetes, testosterone deficiency, anxiety, Parkinson's disease, spinal cord disorder, multiple sclerosis, hyperlipidemia, and hypertension among others [15]. These mostly interrelated to lifestyle and endothelial dysfunction is prevalent in most instances [16]. Most of the antidepressant medications which includes the class of selective serotonin reuptake inhibitors (SSRI) such as paroxetine (Paxil), serotonin norepinephrine reuptake inhibitor such as venlafaxine have been associated to be common causes of ED [17,18].

The oral administration of phosphodiesterase-5 (PDE-5) inhibitors (for example sildenafil) is one of the first line therapies in the management of ED and is also part of the clinical approaches to mitigate sexual dysfunction associated with SSRIs [19]. PDE-5 inhibitors maintain erection by promoting the vasodilatory effects of NO [14]. The common adverse effects of PDE-5 inhibitors include headache, flushing, dyspepsia, hypotension, back pain among others [11, 5]. Sildenafil (viagra) is a potent PDE-5 inhibitor that is generically available and the first oral drug to be approved for the treatment of ED [14]. Studies have shown that it is effective within one hour of dosing and its usage can maintain sufficient erection for satisfactory sexual performance in both human and animal models [20]. Viagra when administered before sexual activity produces reliable efficacy, good tolerability and rapid absorption that yields prompt onset of action [21]. In clinical trials, viagra has been shown to increase the duration and rigidity of penile erection in response to visual sexual stimuli thereby enhancing the ability to achieve erections, leading to successful completion of intercourse [22]. Arginase is also a key regulator of the production of NO [23]. L-Arginine is a substrate for both arginase and NO synthase, as such, the inhibition of arginase leads to an increased bioavailability of NO thus reducing oxidative stress and enhancing normal vascular function while an increase in the activity of arginase brings about reduced NO production because L-arginine is available for NO synthase [12]. Acetylcholinesterase (AChE) is an enzyme that acts on the neurotransmitter, acetylcholine. Acetylcholine carries signal from the nerve cells to the muscle cells while acetylcholinesterase is found in the synapse between the nerve cells to the muscle cells [24].

One of the most difficult hurdles for many men is admitting that they have a problem [13]. The stigma associated with ED prevents men from seeking help and an important component of overall wellbeing in sexual health is pleasurable sexual activity [13,14]. ED has been reported to lead to lower levels of physical and emotional intimacy which could further lead to reduced satisfaction in a relationship. Many men with ED have low self-esteem and feel isolated because they are unable to discuss this sensitive issue with their

physician for fear of embarrassment [13]. As such, the likely worldwide increase in the prevalence of ED and the social stigma attached to the condition present a serious challenge for health care policy makers to develop and implement measures to manage ED [9].

Evidence have shown that ED has a significant negative impact on quality of life measures and that the successful treatment of ED can as such be associated with significant improvements of physical, psychological and emotional wellbeing [13,25]. Nevertheless, recent researches focus on cheap and natural sources having minimal or no side-effects with the aim of discovering a cure for ED and many spices/herbs have shown promising potential [26]. Dietary factors have been demonstrated over the years to play crucial role in the development of various human diseases [27]. Medicinal plant continues to provide valuable therapeutic agents in both modern medicine and traditional system [28]. In Nigeria and many other African countries, several plants have been used for many years to improve sexual stimulation and performance [13].

Approximately 150 species of *Thymus* are abundantly found, mainly in Asia, Africa, and North America. Recently, its range has been widely been extended to the Iberian Peninsula, with most of the species being endemic [29]. *Thymus vulgaris L.* (Lamiaceae) is a medicinal plant belonging to the Lamiaceae family. In folk medicine, some *Thymus* spp. are used for their anthelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidative, antivirotic, carminative, sedative, and diaphoretic effects. They are usually administered by infusion or are used externally in baths to cure rheumatic and skin diseases [30]. Thyme contains high concentrations of phenols, including thymol (12-61%), carvacrol (0.4-20.6%),1,8-cineole (0.2-14.2%), q-cymene (9.1-22.2%), linalool (2.2-4.8%), borneol (0.6-7.5%), a-pinene (0.9-6.6%), and camphor (07- 3%). Carvacrol and thymol are the main phenolic components that are primarily responsible for its antioxidative activity [31].In addition, thyme oil is widely used in phytotherapy, most notably to treat and offer protection from acne, hypertension, infections, and cancers [30]. The oil contains bioactive monoterpenes such as thymol, carvacrol, and linalool (Alireza *et al*., 2016).

### 1.1 JUSTIFICATON

The effective control of ED has been of major interests, and the use of several conventional drugs is yet to have any distinctive stance. As such, there have been a current shift to the use of plant-based bioactive compounds, Report have described *T. vulgaris* to be useful in the management of several human ailments However, there is limited information on the effect of erectogenic properties *T. vugaris*. Hence, this study is focused on the effects of *T. vugaris* on antioxidant and enzymes activities linked with erectile function in 5-FLU-induced oxidative stressed male rats.

## 2.0 MATERIALS AND METHODS

### 2.1 Chemicals and Reagent

Chemicals and reagents used: Acetylcholine Iodide, Gallic Acid and Folin-Ciocalteau reagent were obtained from Sigma-Aldrich (St Louis, MO, USA). Thiobarbituric acid (TBA), trichloroacetic acid (TCA), quercertin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-

deoxyribose were procured from Sigma-Aldrich Chemie (Steinheim, Germany). Sodium carbonate, ferric chloride, aluminium chloride, potassium acetate, potassium ferricyanide, tris salt, ferric sulphate as well as other reagents used were of analytical grade; glass distilled water was also used.

## 2.2 Plant Materials

Thyme (*Thymus vulgaris L., Lamiaceae*) was gotten from Erekesan market, Oja Oba, Akure, Ondo State, Nigeria, and was authenticated at the department of science laboratory technology, Rufus Giwa Polytechnic Owo.

## 2.3 Animals handlings

The handling and use of animals were in accordance with the NIH guide for the care and use of laboratory animals. The use of animal in this study was duly approved by the Animal Ethics Committee of our institution. In this experiment, sixty adults male Wistar rats weighing 210-225 g were purchased from the breeding colony of the Department of Biochemistry, Federal University of Technology Akure, Nigeria. Rats were maintained at 25 °C, on a 12-h light/12-h dark cycle, with free access to food and water. They were acclimatized under these conditions for 2 weeks before the commencement of the experiment.

## 2.4 Experimental design

Sixty adults male Wistar rats weighing 210-225 g were randomly assigned to either normal (control) or 5-FLU groups (n = 6). The animals were further divided into different groups and different doses of thyme extract, and saline solution, were administered through oral gavage while 5-FLU was administered intraperitoneally (i.p) as follows

- Group I: Normal control receiving saline (0.9% NaCl)
- Group II: rats were administered 100 mg/kg of thyme extract orally
- Group III: rats were administered 200 mg/kg of thyme extract orally
- Group IV: rats administered with 400 mg/kg of thyme extract orally
- Group V: rats were administered 25 mg/kg of Vitamin C orally
- Group VI: received 150 mg/kg of 5-FLU (i.p)
- Group VII: received 150 mg/kg of 5-FLU (i.p) and 100 mg/kg of thyme extract orally.
- Group VIII: received 150 mg/kg of 5-FLU (i.p) and 200 mg/kg of thyme extract orally.
- Group IX: received 150 mg/kg of 5-FLU (i.p) and 400 mg/kg of thyme extract orally.
- Group X: received 150 mg/kg of 5-FLU (i.p) and 25 mg/kg of Vitamin C orally.

The treatment lasted for 14 days; after which the rats were sacrificed penile tissue was collected and homogenized with cold sodium phosphate buffer (pH 6.9). The homogenate was centrifuge using refrigerated centrifuge. The supernatant obtained was used for biochemical analysis.

## 2.5 Biochemical assays

### 2.5.1 Biochemical assays

Lipid peroxidation assay quantification of thiobarbituric acid reactive species (TBARS) followed the method of [32]. Total thiol (T-SH) and non- protein contents were determined according to the method previously described by [33]. Protein was measured by the Coomassie blue method according to [34] using bovine serum as standard. Phosphodieterase-5 (PDE-5) activity was carried out using the previously described protocol of [35] with minor modifications. Arginase activity in the penile was assessed by the measurement of urea produced by the reaction of Ehrlich's reagent according to the method described by [36]. Acetylcholinesterase activity was assessed by a modified colorimetric method previously described by [37].

## 2.6 Data Analysis

The result of triplicate experiments was pooled and expressed as mean ± standard deviation and the mean was compared using the one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Statistical Package for Social Science (SPSS 17.0) for windows was used for the analysis. $P<0.05$ was considered to represent a significant difference in both analyses used and $IC_{50}$ was calculated using linear regression.

# 3.0 RESULTS AND DISCUSSION

## 3.1 RESULT

Figure 1 below illustrates the effect of studied plant extract on the level of thiobabituric reactive species (TBARS) produced in the experimental rats. From the result, it was observed that the normal rat treated with 400 mg/kg TVE had reduced level of TBARS when compared to control. In the pre-treated group and induced, the entire induced group pre-treated with TVE extract had reduced TBARS level when compared to untreated 5FLU induced group. Similar result was also observed in both total thiols (Fig. 2) and non-protein thiol level (Fig. 3)

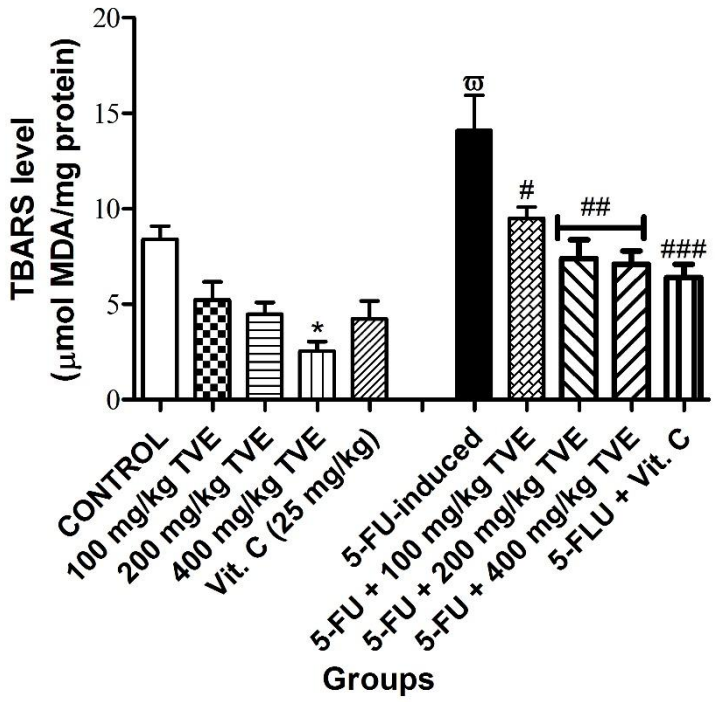


**Figure 1:** Effect of *T. vulgaris* extract (TVE) on the thiobabituric reactive acid species (TBARS) level in the penile of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

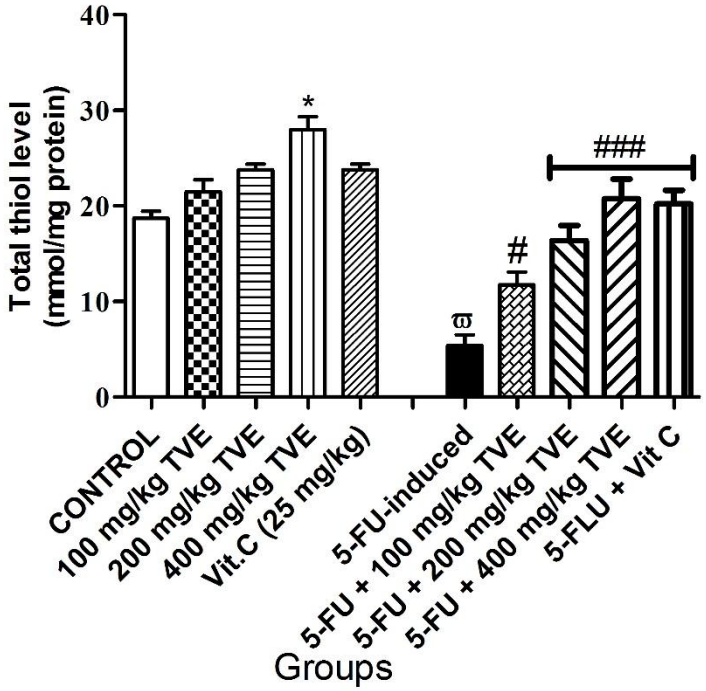


**Figure 2:** Effect of *T. vulgaris* extract (TVE) on the total thiol level in the penile of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

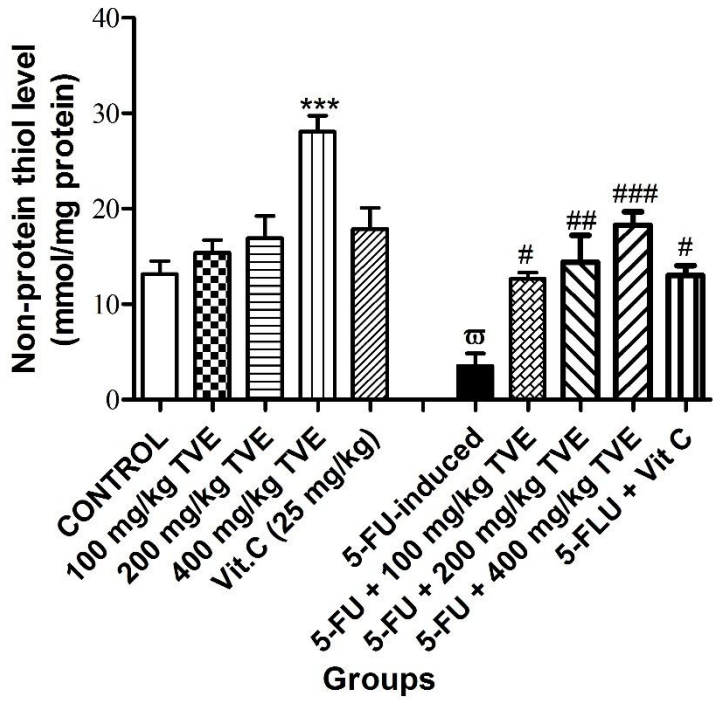


**Figure 3:** Effect of *T. vulgaris* extract (TVE) on the non-protein thiol level in the penile of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

Figure 4, illustrates the effect of *thymus* extracts on the phosphodiesterase-5 activity in the penile tissue of 5-FLU-induced oxidative stress. The result depicts that normal rats treated with the extract and Vitamin C alone reduced PDE-5 compared to the control. Interestingly rats treated with 400 mg/kg extract of *Thymus* had the highest effect. It was also observed that the levels of activities of PDE-5 in the groups of rats induced with 5-FLU had the highest PDE-5 activity, but those that were pre-treated the extract and Vit.C had reduced activity of PDE-5.

Figure 5 described the effect of TVE on arginase activity in the penile tissue of normal and 5-FLU-induced oxidative stress rats. The results depicted that the administration of TVE caused reduced arginase activity in normal rats but not ($p > 0.05$) significantly different from control, however there was significant increase in arginase activities in the rats induced with single dose of 5-FLU when compared to control rats. The result also showed that TVE pre-treated rats induced with 5-FLU had reduced arginase. Interestingly, 100 and 200 mg/kg TVE administered rats had reduced arginase activity ($p < 0.01$), while 400 mg/kg TVE and Vit C (25 mg/kg) had higher significant ($p< 0.001$) decrease arginase activity compared to 5-FLU induced rats.

AChE activity in the penile tissue of normal rats treated with TVE was also reduced but was not significant ($p > 0.05$) different except 400 mg/kg treated rat ($p < 0.05$) when compared to that of control rats. The result also revealed that single dose of 5-FLU caused significant ($p < 0.001$) increase in AChE activity compare to control rats, however, all the 5-FLU-induced rats pre-treated with TVE and Vit. C had reduced AChE activity ($p < 0.001$) when compared to the induced rats.

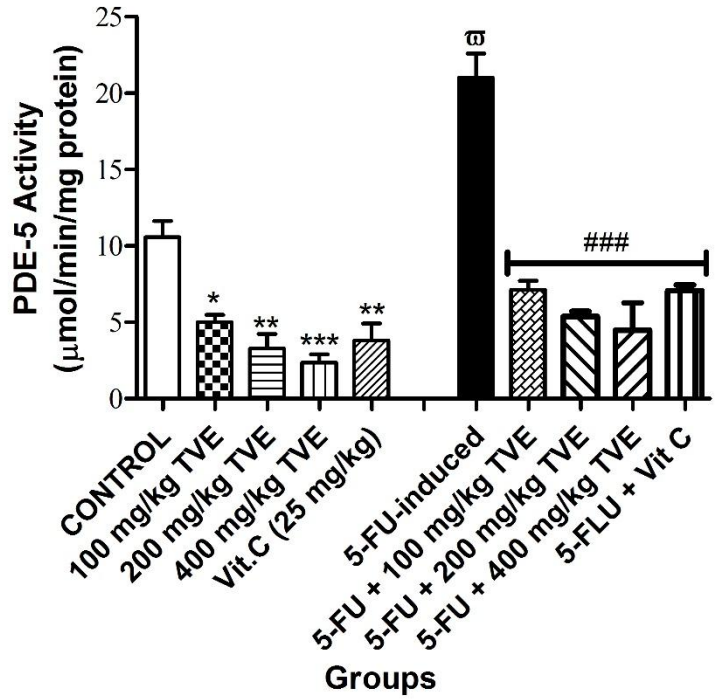


**Figure 4:** Effect of *T. vulgaris* extract (TVE) on the penile phosphodiesterase-5 activity of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

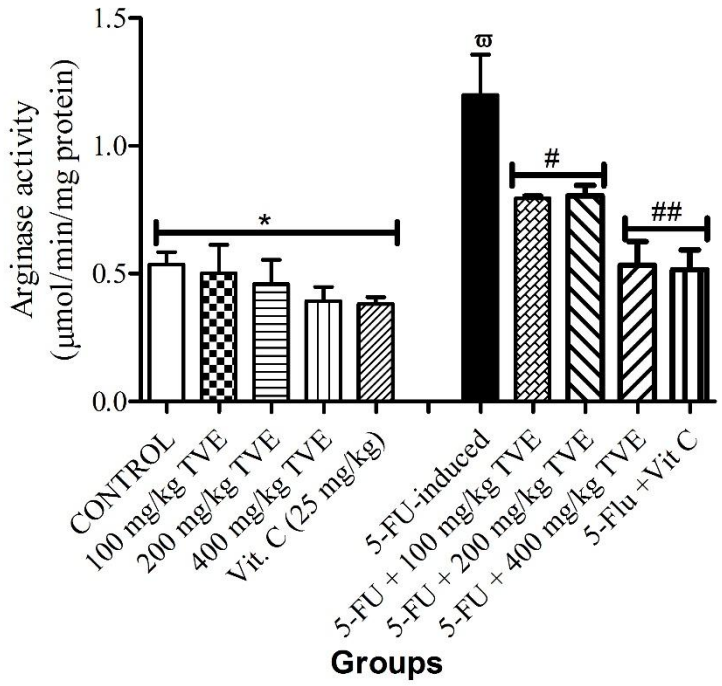


**Figure 5:** Effect of *T. vulgaris* extract (TVE) on the penile arginase activity of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

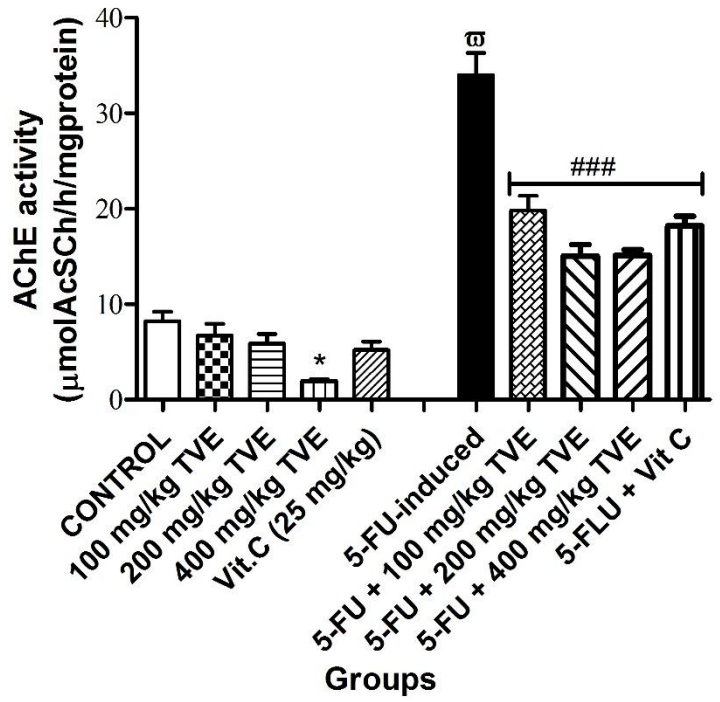


**Figure 6:** Effect of *T. vulgaris* extract (TVE) on the penile acetylcholinesterase (AChE) activity of normal and 5-fluorouracil (5-FLU)-induced oxidative stressed male rats

## 3.2 DISCUSSION

As popularly-known, the use of plant material as bankable sources for bioactive compounds for the medical treatment of various human diseases is from the time immemorial [27]). Thymus vulgaris (T. vulgaris) is among the most useful ancient medicinal plant, in the treatment of convulsions, respiratory diseases, among others, widely grown endemically in most part of the world, having the abilities to scavenge radicals [30]. The imbalance between pro-oxidants and the ability of antioxidants to scavenge free radicals bring about the occurrence of oxidative stress [38]. Free radicals catalyze the conversion of NO to peroxynitrite (ONOO*) thus limiting smooth muscle relaxation of the penile muscle [39]. Superoxide anion, a free radical, and peroxynitrite also impair endothelial function thereby causing ED [40]. The disruption of antioxidant inbalance in the body system can lead oxidative stress-induced ED via the initiation of lipid peroxidation and promoting the reduction of NO bioavailability. Hence, augmenting the body's antioxidant status could be a practical approach by which oxidative stress induced erectile dysfunction can be managed [41]. Results obtained from Figure 1 to 3, shows that T. vugaris has great potential in the management of oxidative stress. This could be as a result of some potential bioactive compounds such as phenolic compounds, essential oils such as thymol, carvacrol, 8-terpinene, p-cymene and a-pinene. Carvacrol and thymol, which have been reported as the major constituents T. vulgaris [42], which are capable to mitigate oxidative stress and enhancing its capability in the management/prevention of erectile dysfunction [43]. An important enzyme required for restoring basal smooth muscle tone, flaccidity and vaso-relaxation is phosphodiesterase-5 (PDE-5). PDE-5 regulates the activity of the second messenger in cells by cleaving to the phosphodiester bond of either cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) or both. As such, the

inhibition/reduced PDE-5 activity induces vasodilation, reduces cystolic Ca2+ and stimulates erection by enhancing the accumulation of cAMP/cGMP. From Figure 4, a result obtained explains that PDE-5 activity was inhibited by administration of the Thymus extract in both normal and pre-treated rats. This finding is in line with [44] reports that bioactive compounds such as polyphenols of the extract as they have been reported to inhibit PDE-5 as such potentiating the activity of cGMP- a potent vasodilator [45]. Furthermore, literatures have reviewed that polyphenols can cause endothelium-induced relaxation in vitro, as a result of inhibitory effects on phosphodiesterase, protein kinase as well as the influx of calcium ion [46]. A major predisposing factor of ED is decreased bioavailability of the vasoprotective endothelial NO which arises as a result of endothelial dysfunction or nerve injury [47]. Results obtained from Figure 5 explain that the studied extract reduced arginase activity in both normal and induced rats. Arginase is an important regulator of NO bioavailability and studies have explained that patients with ED commonly have increased arginase level due to decrease in the activity of the enzyme, nitric oxide synthase (NOS) thereby causing impairment in nitric oxide (NO) biosynthesis [48]. The utilization of L-arginine by arginase activity in vascular endothelia and smooth muscle cells of penile tissue can reduce NOS activity and consequently decrease concentration of NO: a major factor in erectile function [49]. Hence, the inhibition of arginase activity can be additional therapeutic targets for the management of ED as this could increase the bioavailability of L-arginine, contribute to the production of NO via reaction catalyzed by NOS, where overall result could facilitate penile erection [50]. Inhibition of arginase activity by the studied extracts is in line with some reports of plant extracts, which are rich in phenolics including epicatechin, quercetin, quercitrin and isoquercitrin and their arginase inhibitory potential [41,47]. The administration of TVE obtained shows that the concentration-dependent reduction of arginase activity in the penile tissue of the experimental rats is of importance as blood flow could be increased in the genitals during sexual arousal thus enhancing ED management. Interestingly, the inhibitory properties of the TVE could be associated to its phenolic components. This is because literatures by [51], have reported phenolics to have inhibitory effects on the activity of arginase. In addition, [52], explained flavonoids like quercetin and its derivatives (quercitrin and isoquercitrin) to be strong inhibitors of arginase activity and can be linked via the formation of hydrogen bond and hydrophobic interactions between these phenolic compounds and the hydrophobic active site of the enzyme. Acetylcholinesterase (AChE) is an enzyme that rapidly degrades the neurotransmitter, acetylcholine which enhances erection by acting on the vascular endothelium to release NO via the enzyme nitric oxide synthase thus increasing the cGMP level of the corpus cavernosum [53]. Hence, the cholinergic system plays crucial role in the production of penile erection via the inhibition of AChE to maintain Ach- induced NO production from nitric oxide synthase. Figure 6 demonstrates that the extracts inhibited acetylcholinesterase in a concentration-dependent manner. This may also be attributed to its phytochemical constituents as studies have reported the AChE inhibitory effects of some compounds [54,55]. Other medicinal plants which also contain phytochemicals like flavonoids have been reported in literatures to enhance sexual activities [56,8]. Furthermore, because flavonoids are therapeutically potentials and are widely distributed in flowering plants, they possess antioxidant and hemodynamic activities. The antioxidant potential of Allium cepa provides protection against cellular damage to erectile tissues that can bring about ED due to oxidative stress [8] or leydig cells damage causing decrease in testosterone level and loss of libido [57]. Several studies had reported that the penile tissue is rich in cholinergic nerves, and for sexual activity.

## 4.1 Conclusion

The current study has established that 5-FLU can also be used to induce erectile dysfunction in male rats. Although, several reports have shown the medicinal values of bioactive compounds responsible for the various medicinal properties of thymus. However, most of these compounds are heat sensitive and can be easily destroyed by cooking. As such, this study proves that the consumption of Thymus can be a safe and effective alternative remedy in sexual disorders by enhancing sexual activity.

Although, there exists a relationship between increased consumption of thymus extract and sexual function, However, it is apparent that more research is still needed to ascertain the medicinal benefits of sexual function of the extract in enhances sexual function.

DISCLAIMER

Products used for the research are commonly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products, hence the research is used for the advancement of knowledge. Also, the research work was funded by personal efforts of the authors.

ETHICAL APPROVAL

All procedures of this study described were reviewed and approved by the Institutional Animal Ethical Committee.



## REFERENCE


1) Longley DB, Harkin DP, Johnston PG (2003). 5-Fluorouracil: Mechanisms of Action Clinical Strategies. *Nature review*. 3:330-338.
2) Gelen V, Şengül E, Gedikli S, Atila G, Uslu H, Makav M (2017). The protective effect of rutin and quercetin on 5-FU-induced hepatotoxicity in rats. *Asian Pacific Journal of Tropical Biomedicine*, doi: 10.1016/j.apjtb.2017.06.013.
3) Conklin Kenneth A. (2004). Chemotherapy- Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. Integrative cancer therapies Volume: 3 issue: 4, page(s): 294-300 https://doi.org/10.1177%2F1534735404270335
4) Gaman AM, Uzoni A, Popa-Wagner A, Andrei A, Petcu EP (2016). The Role of Oxidative Stress in Etiopathogenesis of Chemotherapy Induced Cognitive Impairment (CICI)- "Chemobrain". Aging and disease, 7(3): 302-312.
5) Olabiyi A.A., Oboh G. and Adefegha S.A. (2016). Effect of Dietary Supplementation of Tiger Nut (*Cyperus esculentus I.*) and Walnut (*Tetracarpidium conophorum mull. Arg.*) on Sexual Behaviour, Hormonal Level and Antioxidant Status in Male Rats. *Journal of Food Biochemistry.* Page 1-9.
6) Oboh G., Ogunruku O.O., Oyeleye S.I., Olasehinde T.A., Ademosun A.O. and Boligon A.A. (2017). Phenolic Extracts from *Clerodendrum volubile* Leaves Inhibit Cholinergic and Monoaminergic Enzymes Relevant to the Management of Some Neurodegenerative Diseases. *Journal of Dietary Supplements.* Volume 14, Page 358–371.
7) Bacon C.G., Mittleman M.A., Kawachi I., Giovannucci E., Glasser D.B. and Rimm E.B. (2003). Sexual Function in Men Older Than 50 Years of Age: Results from the

Health Professionals Follow-up Study. *Annals of Internal Medicine.* Volume 139, Number 3, Page 161–168.
8) Dare A., Salami S.A., Kunle-Alabi O.T., Akindele O.O. and Yinusa R. (2015). Comparative Evaluation of the Aphrodisiac Efficacy of Sildenafil and *Carpolobia lutea* Root in Male Rabbits. *Journal of Intercultural Ethnopharmacology.* Volume 4, Number 4, Page 302-307
9) Lue T.F. (2000). Erectile Dysfunction. *New England Journal of Medicine.* Volume 342, Page 1802-1813.
10) Pocock, G. and Richards C. (2006). Human Physiology. The Basis of Medicine (3rd edition, Page 63). Oxford University Press.
11) McVary K.T. (2007). Clinical Practice. Erectile Dysfunction. *New England Journal of Medicine*. Volume 357, Number 24, Page 2472-2481.
12) Yang J., Wang J., Yang J. Rao K., Zhan Y., Chen R.B., Liu Z., LI M.C., Zhuan L., Zang G.H., Guo S.M., Xu H., Wang S.G., Liu J.H. and Ye Z.Q. (2013). S-Allyl Cysteine Restores Erectile Function Through the Inhibition of Reactive Oxygen Species Generation in Diabetic Rats. *Journal of Andrology*. Volume 1, Number 3, Page 487-494.
13) Solomon H., Man J.W. and Jackson G. (2003). Erectile Dysfunction and the Cardiovascular Patient: Endothelial Dysfunction is the Common Denominator. *Heart.* Volume 89, Page 251-254.
14) Rew K.T. and Heidelbaugh J.J. (2016). Erectile Dysfunction. *American Family Physician.* Volume 94, Number 10, Page 820-827A.
15) Hafez E.S.E. and Hafez S.D. (2009). Erectile Dysfunction: Anatomical Parameters, Etiology, Diagnosis and Therapy. *Archives of Andrology: Journal of Reproductive Systems.* Volume 51, Number 1, Page 15-31.
16) Esposito K., Marfella R., Ciotola M., Di Palo C., Giugliano F., Giugliano G., D'Armiento M., D'Andrea M. and Giugliano D. (2004). Effect pf a Mediterranean-Style Diet on Endothelial Dysfunction and Markers of Vascular Inflammation in the Metabolic Syndrome: A Randomised Trial. *Journal of the American Medical Association.* Volume 292, Number 12, Page 1440-1446.
17) La Torre A., Giupponi G., Duffy D. and Conca A. (2013a). Sexual Dysfunction Related to Psychotropic Drugs: A Critical Review- Part I: Antidepressants. *Pharmacopsychiatry*. Volume 46, Number 5, Page 191-199.
18) La Torre A., Conca A., Duffy D., Giupponi G., Pompili M. and Grözinger M. (2013b). Sexual Dysfunction Related to Psychotropic Drugs: A Critical Review—Part II: Antipsychotics. *Pharmacopsychiatry*. Volume 46, Number 6, Page 201-208.
19) Rizvi S.J. and Kennedy S.H. (2013). Management Strategies for SSRI-Induced Sexual Dysfunction. *Journal of Psychiatry and Neuroscience.* Volume 38, Number 5, Page E27-E28.
20) Brant W.O., Bella A.J. and Lue T.F. (2007). Treatment Options for Erectile Dysfunction. *Endocrinology and Metabolism Clinics*. Volume 36, Number 2, Page 465-479.
21) Chew K.K., Stuckey B.G. and Thompson P.L. (2000). Erectile Dysfunction, Sildenafil and Cardiovascular Risk. *Medical Journal of Australia.* Volume 172, Number 6, Page 279-283.
22) Heidelbaugh J.J. (2010). Management of Erectile Dysfunction. *American Family Physician*. Volume 81, Number 3, Page 305-312.
23) Numao N., Masuda H., Sakai Y., Okada Y., Kihara K. and Azuma H. (2007). Roles of Attenuated Neuronal Nitric-Oxide Synthase Protein Expression and Accelerated Arginase Activity in Impairing Neurogenic Relaxation of Corpus Cavernosum in

Aged Rabbits. *British Journal of Urology International.* Volume 99, Number 6, Page 1495-1499.
24) Ayajiki K., Hayashida H., Tawa M., Okamura T. and Toda N. (2009). Characterization of Nitrergic Function in Monkey Penile Erection *in vivo* and *in vitro. Hypertension Research.* Volume 32, Page 685-689.
25) Baldwin D.S., Palazzo M.C. and Masdrakis V.G. (2013). Reduced Treatment-Emergent Sexual Dysfunction as a Potential Target in the Development of New Antidepressants. *Depression Research and Treatment.* Volume 2013, Page 1-8.
26) Forouzan M., Hossein N., Allakbar T., Javad A.M. and Mahdi S. (2013). Effects of Herbal Medicine on Male Infertility. *Anatomical Sciences Journal.* Volume 10, Number 4, Page 3-16.
27) Banerjee S.K. and Maulik S.K. (2002). Effect of Garlic on Cardiovascular Disorders: A Review. *Nutritional Journal.* Volume 1, Number 4, Page 1-14.
28) El-Demerdash F.M., Yousef M.I. and Abou El-Naga N.I. (2005). Biochemical Study on the Hypoglycemic Effects of Onion and Garlic in Alloxan-Induced Diabetic Rats. *Food and Chemical Toxicology.* Volume 43, Number 1, Page 57-63.
29) Alireza Ansari-Moghaddam, Alireza Khorram, Mahmodreza Miri-Bonjar, Mahdi Mohammadi and Hossein Ansari (2016). The Prevalence and Risk Factors of Gallstone Among Adults in South-East of Iran: A population- Based Study. *Glob J Health Sci*. 8(4): 60- 67. doi: 10.5539/gihsv8n4p60
30) Hashim S and Gamil M (1988). Plants and herbs between the Iraqi folk medicine and scientific research. Baghdad, Dar revolution of Press and Publication.
31) Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B, Aruoma OI (1994). Antioxidant action of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology*, 32: 31-36.
32) Ohkawa, (1979). Determination of MDA activity, maker of Lipid Peroxidation in tissue homogenates. *Biochem Assay Protocols*
33) Ellman GL. (1959). *Arch Biochem Biophys.* 1959; 82:70–77.
34) Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.***72**, 248-254
35) Kelly, S. J.; Butler, L. G. (1977). Enzymic Hydrolysis of Phosphonate Esters. Reaction Mechanism of Intestinal 5′- Nucleotide Phosphodiesterase. Biochemistry., 16, 1102–1104. DOI: 10.1021/bi00625a011.
36) Kaysen, G.A. and H.J. Strecker, (1973). Purification and properties of arginase of rat kidney. *Biochem. J.*, 133: 779-788.
37) Perry N.S., Houghton P.J., Sampson J., Theobald A., Hart S., Lis-Balchin M., Hoult J.R.S., Evans P., Jenner P. Milligan S. and Perry E.K. (2003). *In vitro* Activity of *S. lavandulaefolia* (Spanish sage) Relevant to Treatment of Alzheimer's Disease. *Journal of Pharmacy and Pharmacology.* Volume 53, Number 10, Page 1347-1356.
38) Zhang, H., Davies, K. J. A., and Forman, H. J. (2015). Oxidative stress response and Nrf2 signaling in aging. *Free Radic. Biol. Med*. 88, 314–336. doi:10.1016/j.freeradbiomed.2015.05.036
39) Shukla K., Dilkshik P. and Jasvinder K. (2007). Ameliorative Effect of *Withania coaulans* on Dyslipidemia and Oxidative Stress in Nicotinamide Streptozotocin-Induced Diabetes Mellitus. *Food and Chemical Toxicology.* Volume 50, Number 10, Page 3595-3599.
40) Evgenov O.V., Pacher P., Schmidt P.M., Hasko G., Schmidt H.H. and Stasch J.P. (2006). NO- Independent Stimulators and Activators of Soluble Guanylate Cyclase:

Discovery and Therapeutic Potential. *National Review of Drug Discovery.* Volume 5, Page 755-768.

41) Oboh G., Ademiluyi A.O., Ademosun A.O., Olasehinde T.A., Oyeleye S.I., Boligon A.A. and Athayde M.L. (2015). Phenolic Extract from *Moringa oleifera* Leaves Inhibits Key Enzymes Linked to Erectile Dysfunction and Oxidative Stress in Rats Penile Tissues. *Biochemistry Research International.* Volume 2015, Page 1-8.
42) Ademiluyi A.O., Oyeleye S.I. and Oboh G. (2015). Biological Activities, Antioxidant Propertiesand Phytoconstituents of Essential Oil from Sweet Basil (*Ocimum basilicum* L.) Leaves. *Comparative Clinical Pathology.* Volume 25, Page 169–1768.
43) Sen G., Mukhopadhyay S., Ray M. and Biswas T. (2008). "Quercetin Interferes withIiron Metabolism in *Leishmania donovani* and Targets Ribonucleotide Reductase to Exert Leishmanicidal Activity," *Journal of Antimicrobial Chemotherapy*. Volume 61, Number 5, Page 1066–1075.
44) Siddig I.A., Abdelwahab H.M., Osama Y.M., Mahjoub O., Manal M.E.T., Syam M., Mohamed I.N., Mohd R.M. and Khalid M.A. (2012). Erectogenic Effects of *Clerodendron capitatum*: Involvement of Phosphodiesterase Type-5 Inhibition. *Evidence-Based Complementary and Alternative Medicine.* Volume 2012, Page 1-6.
45) Guohua, H., Yanhua, I., Rengang, M., Dongzhi, W., Zhengzhi, M., Hua, Z. (2009). Aphrodisiac Properties of *Allium tuberosum* Seeds Extract. *Journal of Ethnophormacology*. Volume 122, Page 579- 582.
46) Chan E.C., Pannangptech P. and Woodman O.L. (2000). Relaxation of Flavones and Flavonols in Rat Isolated Thoracic Aorta: Mechanism of Action and Structure-Activity Relationships. *Journal of Cardiovascular Pharmacology*. Volume 35, Number 2, Page 326-333.
47) Akomolafe S.F., Oboh G., Oyeleye S.I. and Boligon A.A (2016). Aqueous Extract from *Ficus capensis* Leaves Inhibits Key Enzymes Linked to Erectile Dysfunction and Prevent Oxidative Stress in Rats' Penile Tissue. *Nutrition and Food Science Journal.* Volume 4, Page 15-21.
48) Shodehinde S.A., Ademiluyi A.O., Oboh G. and Akindahunsi A.A. (2016). Contribution of *Musa paradisiaca* in the Inhibition of α-Amylase, α-Glucosidase and Angiotensin-I Converting Enzyme in Streptozotocin Induced Rats. *Life Sciences.* Volume 133, Page 8–14.
49) Mori M. and Gotoh T. (2000). Regulation of Nitric Oxide Production by Arginine Metabolic Enzymes. *Biochem Biophysic Res Commun.* Volume 275, Page 715–719.
50) Oboh Ganiyu, Adedayo Oluwaseun Ademiluyi, Sunday Idowu Oyeleye, Tosin Abiola Olasehinde and Aline Augusti Boligon (2017). Modulation of some markers of erectile and malonaldehyde levels in isolated rats penile tissue with unripe and ripe plantain peels: identification of the constituents of the plants using HPLC. *Pharm Biol*.55 (1): 1920-1926. doi: 10.1080/13880209.2017.1340966.
51) Akomolafe S.F., Oboh G., Akindahunsi A.A. and Afolayan A.J. (2015). Tetracarpidium conophorum (Mull.Arg) Hutch & Dalziel Inhibits $FeSO_4$-Induced Lipid Peroxidation in Rat's Genitals, *BMC Complementary and Alternative Medicine.* Volume 15, Page 57.
52) Dos Reis M.B.G., Manjolin L.C., Maquiaveli Cdo., Santos- Filho O.A and Da Silva E.R (2013). Inhibition of Leishmania (Leishmania) amazonensis and Rat Arginases by Green Tea EGCG, (+)-catechin and (-)- epicatechin: A Comparative structural Analysis of Enzyme- Inhibitor Interactions.'' *PLos ONE journal* Volume 8, Number 11 doi: 10.11371/journal.pone.0078387.

53) Andersson K.E. (2011). "Mechanisms of Penile Erection and Basis for Pharmacological Treatment of Erectile Dysfunction." *Pharmacological Reviews.* Volume 63, Number 4, Page 811–859.
54) Ramassamy C. (2006). Emerging Role of Polyphenolic Compounds in the Treatment of Neurodegenerative Diseases: A Review of their Intracellular Targets. *European Jounral of Pharmacology.* Volume 545, Page 51-64.
55) Oboh G., Akinyemi A.J. and Ademiluyi A.O. (2013). Inhibitory Effect of Phenolic Extract from Garlic on Angiotensin-I Converting Enzyme and Cisplatin Induced Lipid Peroxidation – *in vitro. International Journal of Biomedical Science.* Volume 9, Page 98–106.
56) Tabana R.J., Riserpey F.K., Yusuf T.Y. and Afolabi K.A. (2007). Paroxetine Plus Sildenafil in Patients with Premature Ejaculation. *Journal of Urology.* Volume 8, Page 2486-2490
57) Malviya N., Jain S., Gupta V.B. and Vyas S. (2011). Effect of Garlic Bulb on Paroxetine-Induced Sexual Dysfunction in Male Rats. *Asian Journal Pharmeutical and Biological Research.* Volume 1, Number 2, Page 281-221.