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Oestrogenic Effects of Onion and Garlic Extracts: Potential Alternatives to Synthetic Oestradiol?

¹RE Kadir, ¹A Ibrahim, ¹G Oderinde, ¹MS Gwadabe, ²LS Ojulari, ³SA Biliaminu

1.Department of Anatomy, University of Ilorin, Ilorin, Kwara State 2.Department of Physiology, University of Ilorin, Ilorin, Kwara State 3.Department of Chemical Pathology, University of Ilorin, Ilorin, Kwara State

Abstract

The aims of the study were to investigate the oestrogenic activities of onion and garlic and their effects on the uterus of adult female Wister rats. A total number of thirty (30) rats comprising of five (5) rats in six (6) groups were used. Group I received feeds and water only and served as the control. Group II (oestradiol group) was administered 10µg/kg of oestradiol orally. Groups III and IV were given 1.14gm/kg and 1.7gm/kg body weight of onion extract respectively while groups V and VI were given 1.14gm/kg and 1.7gm/kg body weight of garlic extract respectively for four weeks. The microscopic architectures of uterus of all the study animals were observed under light microscope following the preparation of the tissues (uterus) and subsequent staining with Hematoxylin and Eosin dyes; and serum oestrogen levels were also assayed and compared after four weeks of treatment. Animals in the control group have higher oestrogen levels than other treatment groups including oestradiol group. It was observed that the endometrium of animals in the low onion and oestradiol groups were thicker than the control and other treatment groups. Our study showed that low onion offered some protection to uterus in similar way synthetic oestradiol does. The use of onion or garlic as a potential alternative to synthetic oestradiol in the treatments of oestrogen depletion associated with menopause could not be established.

Key words: onion, garlic, oestrogen, oestradiol, Wister rat

Introduction

Menopause is a stage in women's lives characterised with oestrogen depletion which in turn is associated with disruptions or disturbances of normal reproductive physiology of women and other diseases. Oestrogen depletion is not limited to postmenopausal women only, pre-pubertal girls and women of all ages may develop low oestrogen¹.

Oestrogen is the primary female hormone; it promotes growth and wellness of the female reproductive organs and keeps the vagina moisturised, elastic (stretchy), and well vascularised². The natural decline in oestrogen levels during menopause can have significant effects in woman's health. Oestrogen associated reproductive system problems include vagina dryness (which results in pain during sexual intercourse), hot flushes, urogenital atrophy, etc. In addition, menopausal women are also at high risk of cardiovascular diseases, fluctuations in central nervous system functions which causes mood swings, migraine, depression, fatigue and trouble concentrating, osteoporosis, which are due to low levels of serum oestrogen³⁻⁷.

Synthetic oestradiol, an oestrogen therapy is often used to treat oestrogen related problems both in postmenopausal women and women of all ages with low serum oestrogen levels. Oestradiol is commercially available and can be prescribed alone or in combination with other hormones most especially progesterone depending on the diagnosis. Oestradiol therapy have been proven to be effective in the treatments of depressive disorders, osteoporosis, urogenital atrophy and sexual functions, which are associated with low serum oestrogen levels⁸⁻¹³. As efficacious as oestradiol therapy may be in the treatment of these associated problems, literatures have also reported various side effects which may pose serious health problems to patients such as breast pain, headache, vaginal itching and discharge, changes in menstrual periods, vaginal bleeding, thinning scalp hair, nausea, vomiting, bloating, stomach cramps etc. Therefore, naturally occurring substance such as quercetin which are oestrogen memetic can be a good replacement for treating hypoestrogenic states especially in women who cannot tolerate synthetic oestradiol side effects.

Quercetin, a natural pentahydroxyflavone is believed to exhibit oestrogenic and anticancer activity by acting as an effective radical-scavenger against oxidative cell damage. Various epidemiological studies have shown that quercetin and related isoflavonoids suppress cancerous tumour growth *in-vivo* and *in-vitro*¹⁴. Any structural and chemical similarities between the quercetin and synthetic oestrogen can also provide insights to their mode of action¹⁵. Many foods such as fruits and vegetables have been shown to be rich in quercetin. Examples of these foods include; onions, garlic, apple, apricot, black grapes, cocoa

Correspondence to:

RE Kadir

Department of Anatomy,
College of Health Sciences,
University of Ilorin.
e-mail: ennyshittabey@yahoo.com
Phone number: +2348023550366

powder, hot wax yellow peppers, red chili, Chinese chives, cashew shoots etc^{16,17}.

Onion and garlic are worldwide-consumed vegetables that are rich source of dietary quercetin known as strong anti-oxidant with ability to chelate metals and scavenge free radicals, which in turn inhibit lipid peroxidation. A great deal of research has focused on other biological activities of flavonoid, quercetin, which are found at particularly high levels in onions that may be beneficial to health. It can inhibit platelet aggregation and/or broad spectrum of enzymes and has been demonstrated to have anti-inflammatory properties, decrease cancer tumor initiation, promote healing of stomach ulcers, inhibit the proliferation of cultured ovarian, breast and colon cancer cells. Through these actions quercetin may contribute to protection from aging, vascular diseases and certain forms of cancer that suggests results of numerous epidemiology studies¹⁸. Garlic as well has been used for both culinary and medicinal purposes in similar ways to the onions¹⁹.

Onion and garlic which contain about 37.11mg/100g and 1.74mg/100g of quercetin on the average respectively were vegetables of choice for our study because of their easy accessibility and also they form part of our daily meals in this part of the world^{16,17}. Even though other flavonoids foods like Hot wax yellow pepper (50.65mg quercetin), Chinese chives (160.0mg quercetin), Cashew shoots (213.0mg quercetin), and Red chili (799.5 quercetin) have been shown to contain more quercetin than onions and garlic, onions and garlic were preferred to these as our study plants because these other flavonoids are either seasonal or not readily available and therefore many people in this part of the world are not quite familiar with them. The aim of this study is to determine the levels of oestrogenicity of onions and garlic and also compare them with that of synthetic oestradiol.

Materials and Methods

Extracts Preparation

The onion and garlic were purchased from a local market (Yoruba Road market) in Ilorin, Kwara state. The extracts were prepared according to a method described by Aletan, with some modifications²⁰. The plants were dried under a shady environment until all the moisture was dried off. The dry scaly parts of the onion bulbs were removed and the fresh bulbs were properly washed. A quantity of 100gm of the onions were weighed and blended in 100ml of deionized water using an electric blender. The homogenous mixture obtained after blending was filtered through a cheese cloth and the residue removed, dried and weighed. The solution left behind weighing 171.2gm was used as whole onion extract and from this stock; high and low doses were obtained for the experiment. Similarly, the dry scaly outer parts of the garlic cloves were removed

and the fresh cloves were properly washed. A quantity of 100gm of the garlic were weighed and macerated in 100ml of deionized water using an electric blender. The solution left behind was used as whole garlic extract of 161.7gm and from this, high and low doses were also obtained for the experiment.

Animal care

A total of 30 female wistar rats weighing about 140gm on the average were procured from Afolabi farm, Oko Olowo Ilorin, Kwara state. The rats were bred in the animal house of College of Health Sciences, University of Ilorin. They were housed in wooden wire-gauzed and mosquito netted cages and allowed to acclimatize for two (2) weeks under normal atmospheric conditions. The rats were fed with pelletized feed (growers mash) bought from Ogo-Oluwa feeds, Sango Ilorin. Their feeds were put in plates while water was given liberally. The animal care was as stipulated by the National Institutes of Health guide for the care and use of laboratory animals.

The weights of the animals were checked at the commencement of administration (week one), at the end of second week of administration (week four) and just prior of sacrifice (week six)

Animal grouping and treatment

The animals were randomly divided into six (6) groups of five (5) animals per group, group I served as the control group; group II was the standard group while groups III to VI served as the experimental groups. The control group I received food and water only while group II received Oestradiol orally (10ug/kg) in addition to the same food and water all the groups liberally had²¹. The experimental groups III to VI received the extracts as follows: Group III received Low Dose Onion (1.14gm/kg); group IV received High Dose Onion (1.70gm/kg); group V received Low Dose Garlic (1.14gm/kg); and group VI received High Dose Garlic (1.70gm/kg)²⁰. Animals in the experimental groups were force fed with the extracts using a ball tip needle via stomach gavage. The duration of treatment was 4 (four) weeks. Prior to the treatments, the estrous cycle of all the animals (including the control groups) was determined as contained in the Long and Evans protocol²².

Animal sacrifice and tissue processing

Sequels to completion of treatment, the rats were weighed and euthanized using 20mg/kg of ketamine (intraperitoneal), transcardially perfused by injecting 400ml paraformaldehyde. The animals were sacrificed at the proestrus stage (the stage characterized with high level of oestrogen) of the estrous cycle and blood samples were collected via cardiac puncture for determination of oestrogen level. One horn of each uterus was immediately fixed in 10% buffered formalin

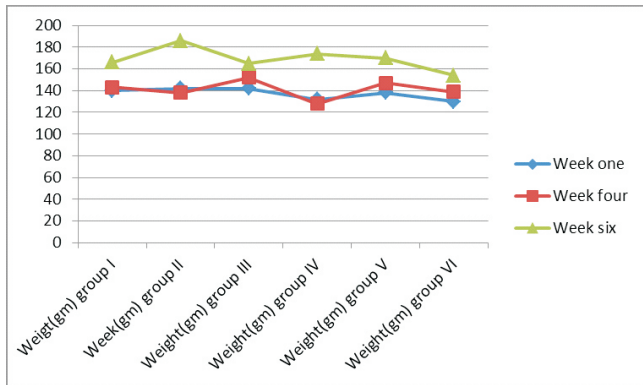


Figure 1: Average weights of study animals in grams during the course of the experiment (over the three assessment periods)

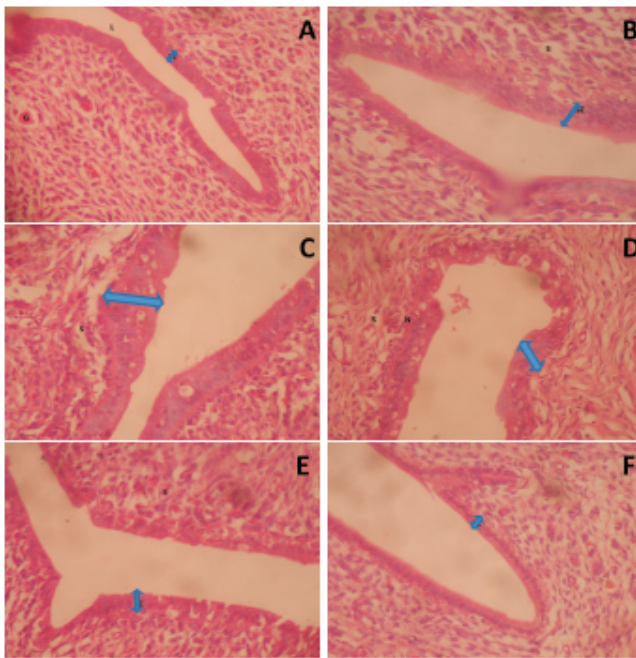


Figure 2: Photomicrograph showing the uterine horn of the study animals in all groups. A-Control; B-estradiol; C-low onion; D-high onion; E-low garlic; F-high garlic. The value thickness of the surface epithelium of the tissues (endometrium lining) indicated by the blue arrows are as follows: control group (9mm to 10mm); oestradiol group (about 22mm); low onion group (26mm to 34mm), high onion group (16mm to 18mm), low garlic group (14mm to 18mm); and high garlic group (7mm to 11mm). (H&E x100).

for histology and morphology of the luminal epithelial thickness. Sullivan method was employed to process tissue to obtain paraffin wax embedded blocks for staining to demonstrate the thickness of luminal epithelia. The histological sections were done in paraffin wax embedded sections in line with Gray method and stained with Haematoxylin and Eosin as described in Fischers protocol^{23,24}.

Procedure for determination of oestrogen levels

Immunoenzymic determination of 17 β -Oestradiol serum level was carried out in all the study rats' serum samples. The assay kit was procured from Eagle Biosciences, Inc. USA. The 17 β -Oestradiol was assayed according to manufacturer's enclosed protocol

Table 1: Mean difference of Serum Oestradiol level using Turkey HSD post hoc test

Extract ^B	Extract ^A Mean Difference in Oestrogen level (A B)	p value
High onion		
Low onion	50.000	0.812
High garlic	- 145.000	0.089
Low garlic	-81.667	0.454
Oestradiol	- 85.000	0.336
Control		
Low onion		
High onion	- 50.000	0.812
High garlic	-195.000	0.014*
Low garlic	-131.667	0.076
Oestradiol	-135.000	0.042*
Control	-260.000	< 0.001*
High garlic		
High onion	145.000	0.089
Low onion	195.00	0.014*
Low garlic	63.333	0.823
Oestradiol	60.000	0.824
Control	-65.000	0.774
Low garlic		
High onion	81.667	0.454
Low onion	131.667	0.076
High garlic	-63.333	0.823
Oestradiol	-3.333	1.000
Control	-128.333	0.087
Oestradiol		
High onion	85.000	0.336
Low onion	135.000	0.042*
High garlic	-60.000	0.824
Low garlic	3.333	1.000
Control	-125.000	0.066
Control		
High onion	210.000	0.001*
Low onion	260.000	< 0.001*
High garlic	65.000	0.774
Low garlic	128.333	0.087
Oestradiol	125.000	0.666

*: p value less than 0.05 (i.e. statistically significant)

Light microscopy

The uterus sections on glass slides were captured using Olympus binocular research microscope connected to a 5.0 MP Amscope Camera (Amscope Inc, USA.).

Data analysis

The quantitative outcomes were analysed using Turkey HSD post hoc test, which was used to compare the mean differences and this was displayed in tables at $p < 0.05$ level of significant.

Results

Figure 1 shows average weights (grams) of animals studied during the course of the experiment

(over the three assessment periods)

There was a significant difference between the mean serum oestrogen levels in the study ($F: 10.297$; p value < 0.001); Post hoc test done (Turkey HSD) showed a significant difference between the high onion group and control ($p = 0.001$), low onion and high garlic ($p = 0.014$), low onion and oestradiol ($p = 0.042$) and low onion and control ($p < 0.002$) groups. Detail results are presented in table 1.

Histological analysis was done on the horn of the uterus of the animals for all groups. The uterine tissue of all groups shows the myometrium, consisting of multiple interwoven layers of well-vascularized smooth muscle. The endometrium of the uterus contains the basal ends of the uterine glands which are round to oval, some elongated and tortuous and many small arteries embedded in a distinctive connective tissue stroma. The surface epithelium (blue arrows) lining the endometrium is columnar epithelium. The micrographs are presented in figure 2.

Discussion

This study aims to determine the oestrogenic properties of onion and garlic and also to compare these properties with that of synthetic oestradiol which currently serve as the gold standard therapy for treating oestrogen related disorders. Though oestrogen has been over the years used as the drug or therapy of choice by the gynaecologists to treat women with low level serum oestrogen, oestrogen is not completely safe as substantial number of patients complain of side effects associated with this drug. Oestradiol is an essential steroid hormone responsible for the wellness of female reproductive system. The interaction of oestradiol and progesterone primarily controls female reproductive tract function. In the uterus, oestradiol initiates a series of responses in uterine cells in preparation for the possibility of pregnancy, including cell hypertrophy and hyperplasia. Oestradiol is also known to play a significant role in the regulation of myometrial contractility²⁵.

Natural oestrogens are secreted principally by the ovarian follicles and also by the adrenals, corpus luteum, and placenta and in males by the testes. Exogenous oestrogens (natural or synthetic) elicit, to varying degrees, all the pharmacological responses usually produced by endogenous oestrogens. Due to side effects associated with the synthetic oestrogen, keeping natural oestradiol at optimum levels is crucial for normal physiological function. Unfortunately keeping oestrogen at a balance is not possible in postmenopausal women because low levels oestrogen is a normal physiological process in this cohort. Therefore, natural oestrogen mimetic products could serve as the alternative to synthetic oestradiol. Flavonoids such as onion and garlic have substantial amount of quercetin, an important constituent of oestrogen. Therefore, these natural foods may exhibit some oestrogenic activities.

This study reveals that onion given orally to

rats at low dose has some agonistic oestrogenic effects on the uterus of the rats as evidenced by increased tallness of the endometrium which is similar to what is observed in the oestradiol group. The oestrogenic effects of low dose onion observed in this study agreed with what was reported by Zeinab and colleagues, though they administered onion extract to their study rats through intravenous injections at a concentration of 30mg/kg body weight²⁶. However, this may suggest that onion extracts at low dose (below 1.5mg/kg body weight) is best used orally in rats, though the mechanism on how low onion increase the thickness of the endometrium in our study was not demonstrated, which is one of our limitations in this study. Garlic extracts whether low or high did not show appreciable effects on the endometrium of the rats as compared to that of low onion and oestradiol though the uterus of rats in these groups were better protected than the control as demonstrated in the photomicrographs. This is also in conformity with findings from Zeinab and colleagues' study²⁶.

The mean difference of serum oestrogen levels in all treatment groups and oestradiol group was low compared to control group (group I). This may be explained by exogenously administered oestrogen suppressing the natural endogenous production, as occurs with hormones.

In conclusion, low dose of onion extracts increases endometrial thickness of the uterus in a way synthetic oestradiol does. However, the use of onion or garlic extracts as a potential oestrogen booster cannot be established in this study. A study on understanding mechanism of how low onion extract offers this protection is next to be explored, as well as simulating this study in ovariectomized female animals.

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