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Improvement of Thyroid Function by Walnut Pulp Extract in Antidepressant-Treated Rats

Tiba Rami Abd[1]*, Jiheel, M.J.[2]

[1]Department of Life Sciences, College of Science, University of Wasit, Wasit, Iraq
[2] Department of Pathological Analysis, College of Science, University of Wasit, Wasit, Iraq

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ABSTRACT
The current study was carried out to discover the role of alcoholic extract of walnut pulp in reducing the side effects of citalopram as antidepressant drugs. Crude extract of walnut pulp was extracted by using ethanol alcohol at a concentration of 70%. The experiment lasted for sixty days from seventh of April to sixth of June 2021. Twenty-eight adult male rats were divided into four equal groups, with age 2.5-3 months old, weighting 150-250g. All animals were drenched orally with gavage needle as the first group was given 0.5 ml of tap water daily and was considered a control group. The second and third groups were given respectively 10 and 20 mg/kg alcoholic extract of walnut pulp in addition to 6.0 mg/kg citalopram daily. While fourth group was treated with 6.0 mg/kg citalopram only oral doses were given. At zero time and after thirty and sixty days, blood samples were drawn by heart pincher after anesthesia with ketamine and xylazine and the thyroid functions were evaluated by measuring the concentration of serum thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). Changes have been observed, such as loss of appetite, phenotypic changes such as pale-yellow skin color and hair loss. The results showed a decrease in thyroid-stimulating hormone in the second and third group who were dosed with walnut pulp alcoholic extract in addition to citalopram, and a decrease in the concentration of triiodothyronine and thyroxine in the second groups who dosed alcoholic extract of walnut pulp in addition to citalopram, and increase in serum triiodothyronine and thyroxine in the third groups who dosed 20mg/kg extract of walnut pulp in addition to citalopram. On the contrary, the results of the fourth group that was dosed with citalopram only, was recorded an increase in thyroid stimulating hormone and a decrease in both triiodothyronine and thyroxine. In conclusion, that citalopram has undesirable side effects on thyroid function. Alcoholic extract of walnut pulp has a role in reducing the side effects of citalopram, and the higher dose (mg/kg 20) showed a better effect than the lower dose.

Keywords: thyroid, citalopram, TSH, T3, T4

INTRODUCTION

Depression is a state of low mood which is classified medically as a mental and behavioral disorder (NIMH, 2020; Sartorius et al., 2021). The experience of depression affects a person's thoughts, behavior, motivation, feelings, and sense of well-being (de Zwart et al., 2019). The core symptom of depression is said to be anhedonia, which refers to loss of interest or a loss of feeling of pleasure in certain activities that usually bring joy to people (Gilbert, 2007). Depression (affective disease) has many types. Contracting depression is becoming a world problem. Data gathered by the World Health Organisation (WHO) and World Bank clearly show it. It can be seen that depression is the fourth most dangerous disease (currently it is estimated that 10% of population is afflicted) leading to death or disablement, it is also estimated that up to year 2020 depression will be the second most dangerous disease – less dangerous only than cardiac ischemia (Adamek et al., 2012).

Major depression is a serious medical illness that affects more than 13% of adults in the U.S. during their lifetime (Hasin et al., 2005). The vast majority of depressed patients have normal peripheral thyroid indices (Sokolov et al., 1994). However, the prevalence of subclinical hypothyroidism is significantly higher among depressed patients compared with the general population and over 63% of patients with subclinical hypothyroidism report depressive symptoms (Demartini et al., 2010).

Current evidence shows that antidepressants are among the most prescribed drugs in the most modern countries such as Europa. The most recent drug utilization studies conducted in Europe suggest an increase in the use of antidepressants over time, particularly for selective reuptake inhibitors (SSRIs), the most commonly prescribed type of antidepressant class. For example, according to a report published by the English National Health in 2016, prescription items for antidepressants increased by 3.7 million items (6.0%), from 61.0 to 64.7 million, between 2015 and 2016. Similar data emerge outside Europe Indeed, in the US, from 1999 to 2012, antidepressants prescription increased by 6.8%–13% (Kantor, 2015).

Antidepressants are prescribed worldwide to treat one of the leading causes of disability, depression, and other conditions such as panic disorders, generalized anxiety disorder and neuropathic pain, which often require medical intervention. However, antidepressants therapeutic effects may take days to manifest, and are often accompanied by unwanted side-effects, which may lead to premature interruption. More importantly, not all patients respond to antidepressant treatment (McIntyre et al., 2014). Knowing the mechanisms of actions of current antidepressants is the key to choosing the more effective therapy for the individual patient, to avoid undesired effects, as well as to develop more specific and faster-acting treatment. The adverse effects of antidepressants include their pharmacological side-effects, toxic effects, interactions with other drugs, and symptoms occurring during their withdrawal (Baldwin et al., 1991).

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavors, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has a continuous history of long use. The development and recognition of medical and financial aids of these plants are on rise in both industrialized and developing nations (Dar et al., 2017).

A plant that possesses therapeutic properties or exerts useful pharmacological impact is generally designated as "medical plant", it has been known as flora that naturally synthesize, and accumulate several secondary metabolites such as alkaloids, glycosides, tannins, volatile oils, minerals, vitamins and have medical properties (Wadood e t al., 2013).

Medical flora is the considered as main exporter of new drugs, as they have few or no side impact (Tariq et al., 2015). Walnut with the scientific name of Juglans regia, which belongs to its, Juglandaceae family, is one of the medical flora that, like most herbal medicines in traditional drug, are considered less, despite having great therapeutic potentials (Delaviz et al., 2017).

Walnut (*Juglanse persica*) is an important crop intended to produce nutritious nuts, key components of healthy diets correlated with quality lifespan (Rusu et al., 2019). some walnut by-products, such as leaves or green husks, were characterized and demonstrated to constitute good sources of bioactive molecules including tocopherols and phenolic compounds (Santos et al., 2013). Using herbs or their parts: leaves, roots, rhizomes, flowers, seeds, thallus, as well as their extracts or natural material, isolated metabolites can act both as a prophylaxis or a cure to diseases and enhance general organs functioning especially immunologic system. Many herbs have a role in curing mood disorders (Adamek et al., 2012.)

As it is also clear that walnut fruits have health benefits because they contain nutritional components that have many health benefits, so this study was conducted to reveal the role of walnut pulp extract in reducing the side effects of citalopram then the current study were done.

Materials and methods

Preparation of extract

In this study, walnut pulp (*Juglans persica* L) Iranian type which available in the local market (Alkut city) was used .

The pulp was ground and then soaked in alcohol (ethanol 70%) for 48 hours at room temperature, using 5 liters of ethanol per kilogram of walnut pulp. After 48 hours, the mixture was filtered with a filter cloth to obtain a mixture free of walnut husks. Then the mixture was placed in a 5-liter Pyrex and placed in an oven at a temperature of 45 °C for 24 hours, where the alcohol was taken by evaporation and an extract of dark brown color with a soft dough-like consistency was obtained. The extract was weighed by a sensitive scale and the result was 75 g of extract per kilograms of walnut pulp, the extract was preserved in a tightly closed glass container and placed in the freezer until use (Al-Shaher, 2020).

Design of the experiment:

The experiment ran for (60) days, starting on July 4, 2021, and ending on July 6, 2021. The animals were sorted into four groups, with 7rats in each group. The first group returned to the control group after receiving the necessary food and water. The second, third, and fourth groups were subjected to a two-month, dosage orally by gavage needle, the second group received 0.6 mg/kg citalopram as well as an alcoholic extract of walnut pulp in a dose of 10mg/kg body weight. The third group received walnut pulp extract 20 mg/kg in addition to 0.6mg/kg citalopram, and the fourth group received just 0.6mg/kg citalopram. Blood was collected from animals at a rate of (3) ml per animal in the beginning and after 30 days, at 10 o'clock, after the animals had been sedated with a combination of ketamine at a dosage of 90 mg/Kg B.W. Furthermore, xylazine, at a dosage of 40 mg/Kg B.W., an intramuscular route injectable that is safe (jiheel ,2015). Following completion of laboratory testing, they were compared with animals in the control group, and after 60 days, the same processes were performed. Blood samples placed in a centrifuge at a power of 3000 cycles/ After the centrifugation process, the serum layer was isolated from the rest of the blood components. The serum was withdrawn by a fine pipette and then placed in abendrov tubes for the purpose of conducting functional tests. After that, the serum samples were kept at a temperature of -20°C to preserve them from damage and until use.

Statistical analysis

The chi-square test in GraphPad Prism software was used to assess significant differences between study values at $P<0.05$ (Gharban, 2024).

RESULTS

Serum TSH

Table (1) demonstrated that there were no significant differences between treated groups (GII and GIII) after 30 and after 60 days compared with control group so as with zero time of experimental period. In the GIV group the TSH concentrations showed a significant elevation compared with control and other groups at the same time. The lowest result was record in GIII group in between other two groups, GII and GIV were recorded.

Serum Triiodothyronine (T3)

Table (2) showed a significant elevation in serum T3 was recorded after 30 and 60 days in animals of GIII group which treated by 20mg/kg walnut pulp extract with 0.6mg/kg citalopram compared with serum T3 concentration of control group. A significant elevation in serum T3 concentration was record between GIII on one side and GII, GIV in other side.

Serum Thyroxine (T4)

The results show in table (3-4) After thirty days of treatment with 0.6mg/kg B.W The alcoholic extract of walnut pulp and citalopram, mean value of serum T4 concentration of G II after 30 days showed a significant elevation compared with control group at same time. but a significant reduction was recorded after 60 days compared with the result of 30 days so as with control group. A significant elevation will be recorded after 30 and 60 days in mean values of T4 concentrations of GIII group compared with mean value of control group. A significant reduction in serum T4 concentrations were recorded in animals of GIV group which treated exclusively with citalopram after 30 days of treatment. and after 60 days compared with the control group.

Table (1) The effects of ethanolic extract of walnut pulp on serum thyroxine (TSH) of mature male rats treated by antidepressant drug (citalopram)

Study groups	TSH (ng\ml)		
	Zero time	After 30 days	After 60 days
GI	2.78±0.24	3.07± 0.38	2.98 ±0.33
GII	2.95±0.33	2.57± 0.38	1.31 ±0.2
GIII	3.07±0.36	1.44±0.36	1.89 ±0.6
GIV	3.24±0.36	3.89± 0.57	4.69 ±1.2

N=7

Table (2) The effects of ethanolic extract of walnut pulp on serum thyroxine(T3) of mature male rats treated by antidepressant drug (citalopram)

Study groups	T3 (ng\ml)		
	Zero time	After 30 days	After 60 days
GI	1.59 ± 0.22	1.60±0.19	1.63±0.21
GII	1.60±0.25	1.67±0.44	1.30±0.12
GIII	1.68±0.23	2.92±0.62	2.7±0.18
GI	1.86±0.16	1.61±0.38	0.79±0.11

N=7

Table (3) The effects of ethanolic extract of walnut pulp on serum thyroxine(T4) of mature male rats treated by antidepressant drug (citalopram)

Study groups	T4(nmol\L)		
	Zero time of study	30 days of study	60 days of study
GI	37.12 ±1.2	36.3±1.16	36.04±1.19
GII	35.3±1.02	38.72±3.12	29.83±4.39
GIII	36.15±1.3	80.78±4.25	44.17±2.70
GIV	34.9±0.99	25.84±1.4	10.23±0.51

N=7

Discussion

For a huge majority of the world's population, medicinal plants continue to play an essential role in healthcare, particularly in poorer countries where herbal therapy has a long history of use and has been demonstrated to treat

lifestyle-related illnesses. Thyroid function alterations have been connected to mental diseases, and clinical hypothyroidism has been linked to depression and a diminished response to antidepressants. In the total cohort, lower TSH levels were connected to less severe depression upon ingestion (Pae et al., 2009).

As show in these findings significant decrease in TSH hormone which it is secreted from pituitary gland as stimulating hormone in response to thyroid gland, our results suggest due to walnut positive effect on thyroid gland and increase their hormonal levels on other hand the TSH levels decreased, these our result in agreement with study explained The walnuts (Juglans persica) increased thyroxine secretion, which, by depleting body stocks of thyroid hormone, causes a rise in TSH secretion in order for the thyroid gland to maintain enough thyroxine production to compensate for the thyroxine loss (LINAZASORO et al., 1970). The walnut contains major components in its chemical composition, including Linoleic acid, which has positive effects on T3 and T4 thyroid hormones.

When we used 20 mg/kg walnut extract in group GIII, T3 and T4 levels were higher than normal, suggesting that linolenic acid is stimulating the thyroid gland and these result in agree with (McCoy et al., 2011). Another study looked at the effect of walnut extract on thyroid hormones and discovered that extracts made from J. persica fruits increased thyroid hormone levels (Öztürk et al., 1994). Another study, similar to ours, was undertaken in 2009 by Rath and Pradhan to examine the impact of J. persica fruit on depression, and the findings of the above study revealed that the hexane extract of fruits had antidepressant efficacy at 100 mg/kg or 150 mg/kg dosages owing to omega-3 fatty acid (Rath and Pradhan, 2009).

After sixty days, the effect of antidepressants on TSH is clearly visible. Thyroid function has a significant impact on mood and the development of mood disorders. Thyroid dysfunction can reduce the depression threshold, whereas an overabundance of thyroid hormones can lead to tense dysphoria (Pae et al., 2009). Increased TSH levels were linked to a longer time to recovery, whereas an increased incidence of subclinical hypothyroidism was described as one of the most common results in depressed individuals (Eker et al., 2008). The main in this study, the researchers discovered that citalopram antidepressants, which act through different mechanisms, have different effects on thyroid hormone levels, with T3 and T4 concentrations dropping to statistically significant levels after sixty days of treatment, indicating that citalopram, which has strong serotonergic activity, lowers thyroid hormone levels. Researchers (König et al., 2000) and (Gitlin et al., 2004) showed substantial declines in T4 and T3 readings with SSRI medication, which is in agreement with our results.

Our findings back up Joffe and Singer's (1990) findings that citalopram, chlorimipramine, or maprotilene therapy was linked to substantial decreases in thyroxine (T4) levels in individuals with serious depression. Furthermore, they discovered that treatment responders had considerably higher decreases in T4 and free T4 than non-responders. In individuals with established coronary Omega-3 fatty acids have been shown to reduce the incidence of sudden mortality caused by cardiac arrhythmias.

In people with heart disease. and all-cause mortality. Walnuts are also high in omega-3 fatty acids, which are important fatty acids; 100 g walnuts contain 9 g omega-3 fatty acids (Haider et al., 2011). Thyroid hormones are important for the growth, development, and operation of key organs including the central nervous system (CNS). When compared to the usual levels of these hormones, the omega-3 treated healthy volunteers exhibited a small change in T3 level, T4 level, and bodyweight, but a considerable rise in TSH level (Qazzaz et al., 2020).

We present the unique notion that diseases in thyroid function are linked with treatment response in primary depressive disorder based on our discovery that most antidepressant medications cause declines in thyroid function testing. Few studies have looked at the link between antidepressant medication and alterations in thyroid indices (Shelton et al., 1993). There have been no substantial changes. in TSH or total T4 levels after fluoxetine therapy, although there was a link between the fall in T3 levels and fluoxetine response. TSH variations following antidepressant therapy were less consistent than T3 and T4 changes, as seen in earlier research.

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