The biochemical effect of bee stings on thyroid function in hyperthyroid women

By:

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Dedication

To the soul of my father.

To my lovely mother.

To my brothers.

Sisters and friends.

Raga
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<table>
<thead>
<tr>
<th>LIST OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication.................................................................</td>
</tr>
<tr>
<td>Acknowledgement..........................................................</td>
</tr>
<tr>
<td>List of contents...........................................................</td>
</tr>
<tr>
<td>List of tables.............................................................</td>
</tr>
<tr>
<td>List of figures............................................................</td>
</tr>
<tr>
<td>Abstract...............................................................................</td>
</tr>
<tr>
<td>Arabic abstract.............................................................</td>
</tr>
<tr>
<td>Introduction.........................................................................</td>
</tr>
<tr>
<td>Chapter one: Literature review..........................................</td>
</tr>
<tr>
<td>1.1. The thyroid gland....................................................</td>
</tr>
<tr>
<td>1.1.1. Structure and function............................................</td>
</tr>
<tr>
<td>1.1.2. Thyroid hormones...................................................</td>
</tr>
<tr>
<td>1.1.2.1. Thyroid hormones synthesis....................................</td>
</tr>
<tr>
<td>1.1.2.2. The transport of the thyroid hormones.....................</td>
</tr>
<tr>
<td>1.1.2.3. The biochemical and physiological functions of the thyroid hormones</td>
</tr>
<tr>
<td>1.1.2.4. Regulation of the thyroid hormones secretion............</td>
</tr>
<tr>
<td>1.1.3. Hyperthyroidism......................................................</td>
</tr>
<tr>
<td>1.1.3.1. Chemical therapy for hyperthyroidism.......................</td>
</tr>
<tr>
<td>1.4. Apitherapy...............................................................</td>
</tr>
<tr>
<td>1.4.1. Bee venom...............................................................</td>
</tr>
<tr>
<td>1.4.1.1. The chemical composition of the bee venom..............</td>
</tr>
<tr>
<td>1.4.1.2. Bee venom therapy.................................................</td>
</tr>
<tr>
<td>Section</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chapter two: Materials and methods</td>
</tr>
<tr>
<td>2.1. The experimental design</td>
</tr>
<tr>
<td>2.2. Subjects</td>
</tr>
<tr>
<td>2.3. The method of bee venom treatment</td>
</tr>
<tr>
<td>2.4. Blood collection</td>
</tr>
<tr>
<td>2.5. Hormonal determination</td>
</tr>
<tr>
<td>2.5.1. Determination of serum total thyroxine concentration</td>
</tr>
<tr>
<td>2.5.2. Determination of serum total triiodothyronine concentration</td>
</tr>
<tr>
<td>2.5.3. Determination of serum thyroid stimulating hormone concentration</td>
</tr>
<tr>
<td>2.6. Statistical analysis</td>
</tr>
<tr>
<td>Chapter three: Results and discussion</td>
</tr>
<tr>
<td>3.1. The effect of bee venom on serum T4 level</td>
</tr>
<tr>
<td>3.2. The effect of bee venom on serum T3 level</td>
</tr>
<tr>
<td>3.3. The effect of bee venom on serum TSH level</td>
</tr>
<tr>
<td>Conclusion</td>
</tr>
<tr>
<td>References</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table (1): The chemical composition of bee venom</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table (2): The effect of bee venom on serum thyroid hormones and thyroid stimulating hormone in hyperthyroid women</td>
<td>38</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. (1)</td>
<td>Relationships between the thyroid gland and other organs and tissues of the body</td>
<td>4</td>
</tr>
<tr>
<td>Fig. (2)</td>
<td>The structure of the thyroid hormones</td>
<td>6</td>
</tr>
<tr>
<td>Fig. (3)</td>
<td>The effect of bee venom on serum $T_4$</td>
<td>39</td>
</tr>
<tr>
<td>Fig. (4)</td>
<td>The effect of bee venom on serum $T_3$</td>
<td>42</td>
</tr>
<tr>
<td>Fig. (5)</td>
<td>The effect of bee venom on serum TSH</td>
<td>45</td>
</tr>
</tbody>
</table>
Abstract

This study was carried out in Elkhalia Investment Company in Khartoum during September to December 2003. The effect of bee venom on serum thyroid hormones and thyroid stimulating hormone in hyperthyroid women was studied.

Fifteen women suffering from hyperthyroidism were included in this study, their ages range between 30-60 years and all had the symptoms of the disease. Fifteen healthy women were also used as a control.

The bee venom treatment was started by two stings on the first day then the number was increased to four stings and repeated daily for two weeks.

The results showed that, bee venom treatment resulted in a significant reduction of the serum thyroid hormones levels and a significant increase of the serum thyroid stimulating hormone level.
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Introduction

Apitherapy is medicinal use of the bee products to promote health and healing. The term comes from the Latin (Apis) which means (bee) and (therapy) which means (treatment) (American Apitherapy Society, 2002).

The bee product includes royal jelly, propolis, pollen, bee venom and honey, which contain minerals and vitamins that are needed for health and nourishment (Michael, 1999).

For more than 60 years thousand of individuals use apitherapy to treat different disease conditions, for example the use of the bee venom in the treatment of auto-immune diseases particularly arthritis and multiple sclerosis (Ryan, 1954).

The royal jelly was found to have some cholesterol lowering proprieties (Vittek, 1995), while propolis was found to be effective against hepato-toxic agents (Amoros, et al. 1992) and also enhances wound healing (Filho and Carralho, 1990).

Hyperthyroidism is the condition in which there is too much thyroid hormones in the body. This most commonly results from a generalized over activity of the entire thyroid gland or it may result from the activity of one or more nodules in the thyroid gland, and also it may be caused by taking too much thyroid hormone in a tablet form (American Thyroid Society, 2002).
There is no one treatment for all patients with hyperthyroidism and this differ according to the age, sex, type of the disease, size of the gland and other medical conditions (Ernst, 1965). The best treatment includes antithyroid drugs, radioactive iodine and surgery. However, all these types of chemical therapy for hyperthyroidism have their disadvantages like the development of hypothyroidism in case of radioactive iodine treatment, (Nygaard, et al. 1991) rash and agranulocytosis in case of antithyroid drugs treatment and the transient hypocalcaemia and hypothyroidism in case of surgery (Edwards, et al. 1995).

As in the folkloric medicine bee venom is used to treat hyperthyroidism (personal communications, 2002). Therefore, the objective of this study is to investigate scientifically the effect of bee venom on the treatment of hyperthyroidism in women.

**The Parameters investigated were:**

1. Triiodothyronine (T<sub>3</sub>).
2. Tetraiodothyronine (T<sub>4</sub>).
3. Thyroid stimulating hormone (TSH).
1.1. The thyroid gland:

1.1.1. Structure and function:

The thyroid gland is defined as the tissue which is capable of accumulating iodide in great excess and combining it into an organic compound known as tetraiodothyronine (T₄) or thyroxine (Ernst, 1965).

In mammals, the thyroid gland consists of two lateral lobes, one on each side of the trachea close to its junction with the larynx, the lobes are connected by an isthmus crossing the ventral surface of the trachea (Sisson, 1969).

Microscopically the thyroid gland is made up of numerous closed follicles, these are lined with a single layer of low cuboidal epithelial cells and are filled with viscid colloid (Hawak and Oser, 1954).

The functions of the thyroid gland are to collect and transport iodine, synthesize thyroglobulin and secrete it into the colloid then remove the thyroid hormones from thyroglobulin and secrete them into the circulation (Ganong, 2001). There is a closely relationship between the thyroid gland and other organs and tissues of the body as illustrated in Fig. (1).

Fig. (1): Relationships between thyroid gland and other organs and tissues of the body:
1.1.2. Thyroid hormones:

The principle secretion of the thyroid gland contains the hormones thyroxine or tetraiodothyronine (T$_4$) which represents 93% of the secreted compounds and triiodothyronine (T$_3$) which represent 7% (Arthur and Hyton, 1994).

The structural formula of these hormones is shown in Fig. (2). The thyroid gland also secretes small amount of reverse T$_3$ (r T$_3$) and other iodinated compounds.

T$_3$ was found to possess biological potency greater than that of T$_4$, however, it is present in the plasma in a considerably small amount than T$_4$, whereas, r T$_3$ is inactive (Ganong, 2001). Approximately 85% of T$_3$ is produced by mono - deiodination of T$_4$ in other tissues such as the liver, muscle and the kidney, T$_4$ is probably not metabolically active until converted to T$_3$ and may be regarded as a prohormone (Edwards, et al. 1995).
Fig. (2): The structure of the thyroid hormones:

3, 5, 3, Triiodothyronine (T₃)

3, 5, 3, 5, Tetraiodothyronine (thyroxine, T₄)
1.1.2.1. Thyroid hormones synthesis:

The synthesis of the thyroid hormones depends solely on the presence of the trace element iodine which is very essential for both man and animal. Iodine occurs in traces in most foods and is present mainly as inorganic iodide the form in which it is absorbed from the digestive tract, then trapped in the thyroid gland (Berger and Quinn, 1982 and Guyton, 1991).

The thyroid gland has special affinity for iodine (Dukes, 1960). The average amount of iodine in the normal thyroid gland of mammals has been shown by Hawak (1954) to vary from 2.2 to 3.5 mg of dried thyroid gland.

Berger and Quinn (1982) and Guyton, (1991) described five distinct steps for thyroid hormones biosynthesis as follows:

1. Thyroidial trapping of serum iodide: The thyroidial follicle cells effectively trap the iodide through an active transport process.

2. Enzymatic oxidation of iodide to active iodine by a peroxidase and its accompanying hydrogen peroxiodase.

3. The iodination of tyrosyl residues which is a part of thyroglobulin amino acid sequence in a reaction that involves thyroperoxidase to produce mono and diiodotyrosine (MIT and DIT), this reaction, some times called organification, it occurs within seconds in luminal thyroglobulin.
4. The oxidation and condensation of MIT and DIT to form the thyroid hormones \((T_4 \text{ and } T_3)\).

5. Proteolytic cleavage of thyroglobulin by a thyroglobulin protease to free the MIT, DIT, \(T_3\) and \(T_4\), the MIT and DIT are deiodinated by iodotyrosine deiodinase and the iodide is recycled.

1.1.2.2. The transport of the thyroid hormones:

Thyroid hormones are transported by thyroid binding globulin (TBG), one-half to two-thirds of the thyroid hormones in the body is extrathyroidial, and most of this circulates in a bound form. There are two specific binding proteins including thyroxine binding globulin TBG and thyroxine binding prealbumin (TBPA). TBG which is a glycoprotein with a molecular mass of 50 KD, is quantitatively the most important. It binds \(T_4\) and \(T_3\) with 100 times the affinity of TBPA (Murray, et al. 1996). About 99.9% of the thyroid hormones in the plasma bound to the transport protein TBG, it is the only free fraction of unbound which diffuse into tissues and exerts its metabolic action. It is possible to measure the concentration of the total or free thyroid hormones but the advantage of the free hormones measurement is that they are not influenced by changes in concentration of binding protein, for example in pregnancy where, TBG levels increased and...
total T₃ and T₄ may be raised but free thyroid hormones levels are normal (Edwards, et al. 1995).

1.1.2.3. The biochemical and physiological functions of the thyroid hormones:

The thyroid hormones increase the metabolic activities of almost all the tissues of the body. About 90% of the thyroid hormones secreted by the gland are T₄ and 10% is T₃. However, most of the T₄ is eventually converted to T₃ in the tissues, so that both are important functionally (Guyton, 1991).

Biochemically thyroid hormones increase utilization of carbohydrates, proteins catabolism and greater oxidation of fats (Abebe and Eley, 1992).

The thyroid hormones stimulate carbohydrate metabolism including the rapid uptake of glucose by the cells, enhanced gluconeogenesis, increased rate of glucose absorption from the gastrointestinal tract, increased insulin secretion (Barker, 1964) and enhanced glycolysis in liver and muscles (Vanhardeveld and Clausen, 1984). There is experimental evidence that thyroid hormones have a diabetogenic action and that thyroidectomy inhibits the development of diabetes. In human, the fasting blood glucose level is elevated in hyperthyroid patients and is decreased in hypothyroid ones. However, hyperthyroid patients apparently utilize glucose at a normal
or increase rate, whereas, hypothyroid ones have a decrease ability to utilize glucose and less sensitive to insulin (Murray, et al. 1999).

Thyroid hormones lower circulating level of cholesterol. They stimulate cholesterol synthesis and the hepatic mechanisms that remove cholesterol from the circulation, the decline in plasma cholesterol level may occur because the rate of its removal from the circulation exceeds the rate of its synthesis (Warda, 1995).

In Nubian goats T₃ treatment was found to decrease serum, liver and heart triacylglyceroles, cholesterol and phospholipids concentrations, (Ibrahim, et al. 1984) this was found to be accompanied by an increase in the activity of lipoprotein lipase in the heart and skeletal muscles.

In addition to that thyroid hormones stimulate protein catabolism and anabolism. Brown and Millward, (1983) reported that T₄ administration in hypophysectomized animals stimulates both rates of protein synthesis and decreases protein breakdown. Barker (1964) showed that catabolism of protein is not a direct effect of thyroid hormones but is most likely produced indirectly through the mobilizing activity of pituitary growth hormone and adrenal glucocorticoids secreted in response to the demand for carbohydrates.
The thyroid hormones have a number of physiological effects, their effects on oxygen consumption, pulse rate, cardiac output, glucose absorption and body temperature are well known (Cole, 1980).

Also they have profound effects on the heart and peripheral vascular system (Klein and Ojamaa, 1992).

In addition to that thyroid hormones are essential for normal postnatal growth and skeletal maturation (Fisher, 1985). They Potentiate the action of insulin-like growth factor -1 (IGF-1) (Holder and Wallis, 1977), and Stimulate growth hormone synthesis by anterior pituitary gland (Solomon and Greep, 1959).

Changes in the circulating levels of the thyroid hormones may be particularly important during puberty when there is rapid growth and changes in energy expenditure (Danger, et al. 1990).

1.1.2.4. Regulation of thyroid hormones secretion:

The right amount of thyroid hormones must be secreted at all times to maintain normal levels of metabolic activities in the body.

The thyroid hormones secretion is regulated by two general mechanisms including:

1- Thyroid stimulating hormone (TSH), thyrotropin or thyrotropic hormone which is a glycoprotien secreted by the basophilic cells in the anterior lobe of the pituitary gland that accelerates most aspect of intermediary
metabolism in the thyroid gland and stimulates the synthesis and secretion of
the thyroid hormones (Wilson, 1992). The specific effect of TSH on the
thyroid gland are: increased proteolysis of the thyroglobulin stored in the
thyroid follicles with the resultant release of thyroid hormones, increased
activity of the iodine pump, which increases the rate of iodine trapping in the
glandular cells. Increase iodination of tyrosine and increase coupling to form
the thyroid hormones, increase size, secretory activity and number of the
thyroid cells (Guyton, 1991).

Pituitary secretion of TSH is in turn regulated by two ways:

Firstly by thyrotropin releasing hormone (TRH) which is a tripeptide
of hypothalamic origin stimulating secretion and synthesis of TSH secretory
mechanism (Warda, 1995).

Secondly by the circulating thyroid hormones which inhibits TSH
synthesis and release from the pituitary gland (Michalkiewicz, et al. 1987
and Brabant, et al. 1991). The pituitary secretion of TSH is more affected by
the circulating T4 this is because of the rapid deiodination of T4 to T3 by the
pituitary cells (Edwards, et al. 1995). Increased concentration of thyroid
hormones suppress the secretion of TSH, and a decreased concentration
augment secretion, of TSH, this regulatory arrangement has been designated
as a negative feed back system (Berger, 1982). The effect of thyroid
hormones on TSH secretion is exerted in part at the hypothalamic level, but
it is also due in large part to an action on the pitutary (Ganong, 2001).
Cascedo, *et al.* (1976) reported that the injection of T$_4$ resulted in a decline in the serum TSH level in rats, in line with that the level of TSH in patients with hypothyroidism was found to be raised above the normal range (Selwa, 1999).

2- The other way of thyroid hormones regulation is not TSH dependant and its most important role is to modify the response to TSH probably by modifying the generation of (cAMP) consequent to TSH stimulation (Wilson, 1992).

**1.1.3. Hyperthyroidism:**

Hyperthyroidism is the clinical syndrome which results from the exposure of the body tissues to excess circulating levels of thyroid hormones. It is a commonly disorder in women, male are affected 5 times less frequently. In over 90% of patients the hyperthyroidism is due to graves’ disease, toxic multinodular goitre or toxic adenoma (Edwards, *et al.* 1995).

Graves’ disease occurs as a result of the production of thyroid stimulating immunoglobulins (TSI) which activates the TSH receptor in the follicles. This cause diffuse enlargement of the thyroid and excessive production of thyroid hormones, since the production of TSI is not under feed back control (Alter and Moshange, 1991). This form of hyperthyroidism is distinguished from the other forms by the presence of diffuse thyroid enlargement. It can occurs at any age but is unusual before puberty and the most commonly affected age group is 30-50 years old (Edwards, *et al.* 1995).
The second common cause of hyperthyroidism is the over activity of one or more nodules in the thyroid gland and the condition is known as toxic nodular or multinodular goiter. Like Graves’ disease, this form is more common in women, with a mean age of 60 years. Thyroid hormone levels are usually slightly elevated, but as an older age is affected cardiovascular features such as cardiac failure predominates (Edwards, et al. 1995).

Finally, the person may become hyperthyroid if he takes too much thyroid hormone tablets, but till now no specific causative agents like viruses or bacteria have been identified (American Thyroid Society, 2002).

1.1.3.1. Chemical therapy of hyperthyroidism:

The selection of a particular form of treatment for hyperthyroidism is determined by the patient status and circumstances, this includes the age, sex, associated disorders and the size of the gland (Ernst, 1965).

The aim of the therapy in the presence of hyperfunctioning thyroid gland is to diminish the excessive secretion of the thyroid hormones. Most of the drugs that inhibit thyroid function act by interfering with the iodide trapping mechanism or by blocking the organic binding of iodine (Warda, 1995). In either case, TSH secretion is stimulated by the decline in circulating thyroid hormones and goiter is produced (Ganong, 2001).

The antithyroid drugs that interfere with the formation of thyroid hormones, resulting in the enlargement of the thyroid gland, are called
goiterogenic substances or goitrogens (Devlin, 1986). The goiterogens can be classified according to their mode of action as mentioned by Mc Donald (1975) into:

1. monovalent anions, example potassium perchlorate which inhibits iodine trapping and transport in the thyroid gland.
2. Organic compounds, example thiouracil which inhibits iodination of tyrosine.
3. Radioactive iodine, which destroys the thyroid tissue.
4. Miscellaneous anions and cations, example cobalt, calcium, but their mode of action is unknown.
The chemical therapy that used in the treatment of hyperthyroidism includes, radioactive iodine and antithyroid drugs.

Radioactive iodine (RAI) is used increasingly as a first line treatment of hyperthyroidism, it exerts its action through the destruction of the thyroid cells which is induced by the local radiation, though it takes several months to be fully effective. RAI is contraindicated in pregnancy because the incidence of thyroid injury in the fetus is high after the first trimester (Wilson, 1992 and Harbert, 1984), also it is contraindicated in lactating patients since iodine is secreted in the milk (Wilson, 1992).

Although RAI is the treatment of choice for hyperthyroidism but concerns remains regarding its safety with respect to risk of malignancy as well as infertility and congeial abnormality in off springs (Lee, et al. 1992). Carcinoma of the thyroid gland and leukemia were also reported theoretically as a side effects of RAI therapy (Schlumberger and Vathaine 1996). But still there is no practical study to confirm this evidence. Also Lee, et al. (1992) reported that many studies failed to reveal an increased risk of solid tumours in hyperthyroid patients treated with RAI.
As hypothyroidism is the most common long term complication of iodine therapy (Harbert, 1984), Nygaard, et al. (1998) stated that it will be impossible to modify radioactive iodine therapy in away to achieve both early control of hyperthyroidism and the incidence of hypothyroidism.

The second form of chemical therapy for hyperthyroidism is the antithyroid drugs, thiocyanate propylthiouracil and inorganic iodine are the best known antithyroid substances.

Thiocyanate which is one of the goitrogenic substances, exerts a direct effect on the thyroid gland to disrupt one of the several steps in the biosynthesis and secretion of the thyroid hormones through inhibition of the iodine trapping mechanism. Capin (1994) and Rao and Lakshmy, (1995) mentioned that the addition of thiocyanate to food deprived of KI brought down significantly the circulating levels of thyroxine in rats. In line with that the addition of sodium thiocyanate in camels resulted in a significant increase in serum thiocyanate concentration which was accompanied by a significant decrease in serum total thyroxine and total T₃ levels. This reduction in thyroid hormones level might be attributed to thiocyanate overload, as it competes with iodine at entry into the thyroid gland, increasing its urinary losses and producing a relative or absolute iodine

Guyton, (1991) stated that the same pump that pumps iodide into the thyroid cell can also pump the antithyroid substances and therefore, their administration in high enough concentration can cause competitive inhibition of iodine transport into the cells. The decrease availability of iodide into the glandular cell does not stop the formation of thyroglobulin, it merely prevents the thyroglobulin that is formed from becoming iodinated and therefore, from forming the thyroid hormones.

The antithyroid drugs also include thiourylenes which is a group of compounds related to thiourea inhibit the organic binding of iodide and block the coupling reactions, the two used clinically are propylthiouracil and methimazole, the iodination of tyrosine is inhibited because the drugs compete with tyrosine residues for iodine and become iodinated in addition propylthiouracil but not methimazole inhibits 5-deiodinase reducing the conversion of $T_4$ to $T_3$ in many extrathyroidial tissues, also both drugs may act by suppressing the immune system and thereby depressing the formation of TSH- receptor stimulating antibodies, they may also inhibit biosynthesis or alter the structure of thyroglobulins (Ganong, 2001).

Another substance that inhibits thyroid functions in hyperthyroidism is iodide itself, it was found to be effective in the treatment of hyperthyroidism for many centuries (Winkler, 1946). It acts by inhibiting the organic binding
mechanism, reduces the effect of TSH on the thyroid gland or inhibits proteolysis of thyroglobulin (Ganong, 2001).

The several side effects of antithyroid drugs therapy include, myalgia, arthralgia, fever and rash which referred to as the antithyroid arthritis syndrome. It has been found that patients treated with propylthiouracil or methimazole were considered to have these complications (Warda, 1995). Beside that, Edwards, et al. (1995) reported a rash, nausea, vomiting and agranulocytosis as side effects in case of antithyroid drugs treatment.

1.4. Apitherapy:

Apitherapy is the medicinal use of honey bee products which includes honey, pollen, propolis, royal jelly, bee wax and venom. It is an old practice, it was mentioned in Chinese texts that it is 2000 years old. It began as a part of folkloric medicine and even to day, most of the people using it are either doing it themselves or with the help of practioners (Michael, 1999)
1.4.1. Bee venom:

Bee venom is one of the most important product of the honey bee. It comes from the venom sac, located at the level of the last segment of the worker bee abdomen. Venom is what comes out of the stinging apparatus of the bee, its quantity depends on the age and the race of the bee and the composition depends on the pollen consumed and the age of the bee.

The stinger of the bee is about 2 mm long and has a sharply pointed end. The diameter of the end is approximately 0.1 mm and after the evaporation each one sting was found to contain 0.1 mg of pure dried venom (American Apitherapy Society, 2001).

The venom is synthesized mainly as a defensive mechanism against predators, in order to be of defensive value. The venom must induce pain, cause damage or have some other pharmacological activity in the potential predators (Schmidt and Buchmann, 1992).

1.4.1.1. The chemical composition of the bee venom:

The basic informations dealing with the bee venom constituents and their pharmacological effects were done since 1950 and 1960 (Krell, 1996).

Bee venom contains several biochemical or pharmacologically active substances, including at least the following melittin, apamin, mast cell
degranulating peptide (MCDP), minamine, histamine, dopamine, phospholipase, A₁ and A₂ and hyaluronidase (Habermann, 1972).

Munjal and Elliot (1971) reported that at least eight fractions of the honey bee venom constituents can be separated, of which phospholipase, melittin and apamin are the major ones.

Root (1975) reported that bee venom consisted of eight components of which histamine, hyaluronidase and lecithinase were the most important.

Shigair (1992) detected formic acid, hydrochloric acid, histamine, coline, tryptophan, sulphur and other substances like magnesium phosphate, which makes 0.4% of the dry weight of the bee venom.

O'connor (1978) mentioned that melittin is the main constituent of the bee venom and constituted about 50% of the dry venom. Its LD₅₀ was 3-4 mg / kg (mouse. I V). And contains 26 amino acids.

As general the honey bee venom contains over 78 different components. However, not all these components are consistently present in each bee's venom. There are six major components that are thought to provide the most important therapeutic effects at the application of bee venom therapy that include: melittin, phospholipase A₁ and A₂, hyaluronidase, apamin, histamine and mast cell degranulating peptide (American Apitherapy Society. 2001).
According to Dotimas and Hider (1987) the bee venom contains many enzymes, proteins and peptides, physiologically active amines, amino acids, sugars, phospholipids and volatile compounds (Table 1).
Table (1): The chemical composition of the honey bee venom from worker bee according to Dotimas and Hider (1987):

<table>
<thead>
<tr>
<th>Class of Molecules</th>
<th>Component</th>
<th>% of dry venom</th>
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<tbody>
<tr>
<td>Enzymes</td>
<td>Phospholipasee A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10-12</td>
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<td></td>
<td>Hyalauronidase</td>
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<td>AcidPhosphomonooesterase</td>
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<td>Lyso phospho lipase</td>
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<td>α- glucosiolase</td>
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<td>Other proteins and peptides</td>
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<td></td>
<td>Apamin</td>
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<td>Mast cell degranulating peptide (MCD)</td>
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<td>Secapin</td>
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<td></td>
<td>Procamine</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>Adolapin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protease inhibitor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small peptide (with less than 5 amino acid)</td>
<td>13-15</td>
</tr>
<tr>
<td>Physiologically Active amines</td>
<td>Histamine</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td></td>
<td>Dopamine</td>
<td>0.2-100</td>
</tr>
<tr>
<td></td>
<td>Noradrenaline</td>
<td>0.5</td>
</tr>
<tr>
<td>Amino acids</td>
<td>α - amino butyric acid</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>α- amino acid</td>
<td>1</td>
</tr>
<tr>
<td>Sugars</td>
<td>Glucose and fructose</td>
<td>2</td>
</tr>
<tr>
<td>Phospholipids</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Volatile compounds</td>
<td></td>
<td>4-8</td>
</tr>
</tbody>
</table>
1.4.1.2. Bee venom therapy:

The Bee venom therapy can be useful in a wide variety of medicinal situations. It is very old practice especially for rheumatism and arthritis treatment (Ryan, 1954).

Krell (1996) mentioned that during the last seven decades, over 1700 scientific publications on the composition and various effects of bee venom on animals and humans have been published. An overwhelming proportion comes from Eastern Europe and Asia. Most of them concentrated on demonstrating the side effects, physiological effects of the individual component such as membrane destruction, toxicity and the stimulation or blocking of enzymatic reactions. This has largely increased the understanding of the process occurring after the sting, the physiological effects of isolated venom component and the substances responsible for most of the allergic reactions.

Studies using bee venom in dogs (Vick and Brooks, 1972) and rats (Dunn, 1984) showed that melittin and apamin venom compounds increase plasma cortisol, together with various other arguments, this suggests that many of the curative effects of bee venom may work through stimulation of the body enzymes and the modification of the immune system in away similar to the common drug cortisone which has been used in the treatment of many diseases, but it is also shown to have strong, undesirable side effects.
Melittin was also shown to have toxic side effects as do some of the other individual components in the venom, when the whole venom is applied however, no side effects have been shown, other than that in allergic patients (Krell, 1996).

Melittin, the polypeptide venom component was found to be mainly responsible for the elevation of plasma cortisol level and it has paralytic and stimulatory effects on heart functions and cause blood pressure to fall (American Apitherapy Society, 2001).

The anti-inflammatory effects of the bee venom were abundantly studied and the various mechanisms have been repeatedly described in the literature (Kim, 1989, Rekka and Kourounakis, 1990).

Preliminary experiments on animals have indicated that apamin which is one of the three active components of the bee venom (melittin, apamin and phospholipase) increased both the heart beating and forces of pumping blood in the body (Root, 1975).

The influence of the bee venom administration on the blood pressure and the heart rate in rats have been examined by Kamal, et al. (2002). They found that the intravenous administration of the bee venom in a dose of 0.2 - 2 mg/kg body weight induce a dose dependant decrease in the blood pressure and the heart rate.
As certain peptide components of honey bee venom possess anti-inflammatory properties their effects on the complement system were investigated by Gencheva and Shakenderov (1986), they reported that the subcutaneous injection of rat every day for two weeks with peptide fraction and apamin caused a significant decrease in serum complement system activity, the action of the apamin and peptide fraction were slow and dose-dependant which could have therapeutic use. It was therefore, suggested that the decrease of complement activity produced by the peptide fraction and apamin could have anti-inflammatory action. Maschultz and Kloft (1971) reported that the melittin had a hemolytic activity, he added that it has a stimulating effects on the heart rate.

The bee venom therapy has been particularly successful in individual suffering from rheumatoid arthritis, gout and multiple sclerosis, but a variety of immune disorder disease like asthma are also treated (Personal communication, 2002).
CHAPTER TWO

Materials and Methods

2.1. The experimental design:

This experiment was designed to examine the effect of honey bee venom on serum thyroid hormones and thyroid stimulating hormone level in hyperthyroid women. The study was solely conducted in El-khalia Investment Company in Khartoum, and it had been carried out during September to December 2003.

2.2. Subjects:

Fifteen women suffering from hyperthyroidism were included in this study. Their ages range between 30-60 years, all patients were from Khartoum State. These patients were all diagnosed to have hyperthyroidism according to the levels of thyroid hormones and thyroid stimulating hormone.

The frequent symptoms of all patients included fine hair, weakness, nervousness, worm and moist skin, fine tremor of the outstretched fingers and sweating. The eye signs of Graves’ disease and the presence of goiter was not a constant sign. For comparison fifteen healthy women were used.

The main view of the study was to start treatment with bee venom daily for two weeks after the measurement of the serum thyroid hormones
T₄, T₃ and thyroid stimulating hormone TSH levels so as to confirm biochemically the presence of hyperthyroidism. By the end of the venom treatment the serum thyroid hormones and TSH level were measured and the results were compared with the control.

2.3. The method of bee venom treatment:

The Sudanese type of honey bee which is called Apis mellifera was used.

The active honey bee was hold at the thoracic by forcep, and then it was put on the arterial aspect of the patient’s neck. The bee was pressed and excited by the forcep and started to sting the patient at the proper site, after that the sting was left for about 5-10 minutes and then removed according to El -Sarrag, (2002).

The number of stings was two stings on the first day, then the number was increased to four stings from the next day and repeated daily for two weeks (El-Sarrag, 2002). It was found that after evaporation each one sting comprise 0.1mg of pure dried venom (American Apitherapy Society.2001).

2.4. Blood collection:

5 ml of venous blood from the cubical vein were obtained from each patient, this was done under medical supervision. Serum was separated after
centrifugation at (3000 rpm) for 3 minutes and it was stored frozen at -20°C until the determination of hormones was carried out.

2.5. Hormonal determination:

It was performed at the Sudanese Atomic Energy Commission (SAEC) in Khartoum, using the radioimmunoassay (RIA) technique (Laryson, 1978) for thyroid hormones determination and immunoradiometric assay (IRMA) for thyroid stimulating hormone determination. The normal values in the laboratory are 50-150 nmol/L for T₄, 0.8-3.0 nmol/L for T₃ and 0.4-4.0 mIU/L for TSH.

2.5.1. Determination of serum total thyroxine (T₄) concentration:

Principle of the method:

The T₄ radioimmunoassay method (RIA) depends on the competition between ¹²⁵I- T₄ and T₄ contained in standards or in the samples to be assayed, for a fixed and limited number of T₄ antibody binding sites.

After the incubation, the amount of ¹²⁵I- T₄ bound to the antibody is inversely related to the amount of T₄ present in the sample.

By measuring the proportion of ¹²⁵I- T₄ bound in the presence of reference standards containing various amount of T₄, the concentration of T₄ present in the unknown samples can be interpolated.

Materials and equipments:
- Micropipettes with disposable tips.
- Vortex type mixer.
- Multi sample centrifuge.
- Tube rack.
- Water bath.
- Gamma counter for measuring $^{125}$I.

**Assay procedure:**

1. The assay tubes were labelled and arranged in the assay rack.
2. 50 µl of the standards and of the samples were pipetted into the tubes.
3. 100 µl of $^{125}$I -T$_4$ solution were dispensed into each tube.
4. 100 µl of T$_4$ antibody suspension or NSB reagent were dispensed into each tube.
5. The tubes were mixed thoroughly and incubated at 37°C for 60 minutes.
6. The tubes were placed on the magnetic separator for 10 minutes.
7. The supernatant was decanted and it was counted for 60 seconds in a gamma counter.

**Calculations:**

Result was calculated as follows:
1. The counts (B) for each of the standards and unknowns as a percentage of the mean counts of the zero standard \( (B_0) \) were expressed as:

\[
\frac{B}{B_0} \times 100\% = \frac{\text{B of standard or unknown}}{B_0} \times 100\%
\]

\( B \) = Count per minute (CPM) of sample, standard, quality control.

\( B_0 \) = CPM standard zero.

2. The percentage values obtained for \( T_4 \) standards were plotted against \( T_4 \) concentration and the standard curve was constructed.

2.5.2. Determination of serum total triiodothyronine (\( T_3 \)):

**Principle of the method:**
The T₃ radioimmunoassay principle is the same as that mentioned for T₄ determination.

**Assay procedure:**

1. The assay tubes were labelled and arranged in the assay rack.
2. 25 µl of the standards or samples were pipetted into each tube.
3. 100 µl of ¹²⁵I-T₃ solution were dispensed into the tubes.
4. 100 µl of T₃ antibody were dispensed into each tube.
5. All tubes were vortexed and incubated for one hour at 37°C.
6. 250 µl of precipitant were dispensed into each tube.
7. All tubes were centrifuged at 3000 r pm for five minutes at room temperature.
8. The supernatant was decanted and counted for 60 seconds in a gamma counter.

**Calculations:**

Result was calculated as follows:

1. The count (B) for each of the mean standards and unknown samples as a percentage of the mean counts of the zero standards (Bo) were expressed as:
   \[
   \frac{B\%}{\%} = \frac{\text{B of standard or unknown}}{\text{Bo}} \times 100\%
   \]

2. The percentage values obtained for the T₃ standards were plotted and the standard curve was constructed.
$B/B_0\%$

$T_3 \text{ nmol/L}$
2.5.3. **Determination of serum thyroid stimulating hormone (TSH):**

**Principle of the method:**

The immunoradiometric assay (IRMA) method in the measurement of TSH in the serum involves the reaction of TSH present in the serum with monoclonal and polyclonal antibody.

The monoclonal antibody is labelled with $^{125}$I as tracer ($^{125}$I-McAb) and the polyclonal antibody is coupled to magnetic iron oxide particle (PcAb<M>).

The formed $^{125}$I McAb-TSH-PcAb<M> complex (sandwich) is separated from unbound tracer by placing the assay tubes in the magnetic separator and decanting supernatant.

The radioactivity of the tracer in the tubes is directly proportional to the concentration of TSH in the sample.

**Reagents:**

1. Wash buffer.
2. $^{125}$I-anti-TSH (monoclonal).
3. Magnetic antibody (polyclonal).
4. TSH standards.
**Assay procedure:**

1. 100 µl of standards, control and unknown samples were added to the labelled tubes.
2. 100 µl of $^{125}\text{I}$-anti-TSH solution were added to each tube, and mixed.
3. The tubes were incubated for one hour at 37°C.
4. 200 µl of well mixed antibody suspension were added and mixed gently.
5. The tubes were incubated for one hour at 37°C.
6. The tubes were placed on magnetic separator and allowed to stand for 15 minutes.
7. 1 ml of wash buffer was added and vortexed.
8. The supernatant was decanteted.
9. All tubes were counted for one minute in gamma counter.

**Calculations:**

1. The count rates for each standard tube against the TSH concentration were plotted.
2. The mean count rate for each unknown sample was calculated and TSH concentration was read from the standard curve.
2.6. Statistical analysis:

The data was analysed by analysis of variance (ANOVA) and mean separation according to Gomez and Gomez (1984) with aid of SAS computer programme (SAS, 1988).
CHAPTER THREE

Results and Discussion

Apitherapy is used widely in the Sudanese folkloric medicine to treat different disease conditions including hyperthyroidism.

The present study was conducted to investigate the effect of bee venom on the level of serum thyroid hormones (T₄, T₃) and thyroid stimulating hormone (TSH) in hyperthyroid women.

3.1. The effect of bee venom on serum thyroxine (T₄):

In this study hyperthyroidism was diagnosed by the elevation in serum thyroid hormones which is commonly resulted from the overactivity of the thyroid gland which may be due to Graves’ disease or toxic multinodular goiter or toxic adenoma (Edwards, et al. 1995). The effect of bee venom treatment on serum T₄ was presented in Table (2) and Fig. (3). The level of T₄ in hyperthyroid women was significantly higher compared to the control group. Bee venom treatment resulted in a significant (P>0.05) reduction in T₄ level compared to the pretreatment level. Ganong, (2001) reported that the aim of all therapy for hyperthyroidism is to diminish the excessive secretion of the thyroid hormones either by interfering with the iodine trapping mechanism or by blocking the organic binding of iodine and/or destroy the thyroid cells.

Table (2): The effect of bee venom on serum T₄, T₃ and TSH levels in
hyperthyroid women:

<table>
<thead>
<tr>
<th>Groups</th>
<th>T4 (nmol/L)</th>
<th>T3 (nmol/L)</th>
<th>TSH (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>96.6±1.4a</td>
<td>1.5±0.8a</td>
<td>1.4±0.1a</td>
</tr>
<tr>
<td>B</td>
<td>205±5.2b</td>
<td>3.3±0.5b</td>
<td>0.3±0.09b</td>
</tr>
<tr>
<td>C</td>
<td>115±2.8a</td>
<td>2.5±0.3a</td>
<td>1.2±0.1a</td>
</tr>
</tbody>
</table>

Means within columns followed by different letters are significantly different (P<0.05).

Values are means ± SE.

Group A = control.

Group B = Hyperthyroid patients before treatment.

Group C = Hyperthyroid Patients after treatment.
Fig. (3): The effect of bee venom on serum \( T_4 \) level in hyperthyroid women

A: Control.

B: Hyperthyroid patients before treatment.

C: Hyperthyroid patients after treatment.
Salvador (1998) studied the effect of radioactive iodine on the treatment of hyperthyroidism, he found that there was a significant reduction in serum thyroxine. The author reported that this reduction was due to the destruction of the thyroid cells which was induced by radiation.

Thiocyanate which is one of the goitrogenic drugs exerts a direct effect on the thyroid gland through the inhibition of iodine trapping mechanism. Capin, (1994), Rao and Lakshmy (1995) mentioned that the addition of thiocynate to food deprived of KI caused a significant decrease in the circulating level of T₄ in rats.

Also administration of sodium thiocyanate in camels resulted in a significant increase in serum thiocyanate concentration which was accompanied by a significant decrease in serum T₄ and T₃ levels. The authors reported that the reduction in the thyroid hormones level might be attributed to thiocyanate overload, as it competes with iodine at entry into the thyroid gland, increasing its urinary losses and produce a relative or absolute iodine deficiency, hence inhibiting the hormone synthesis (Bourdoux, et al. 1978., Thilly, et al. 1991 and Rao and Lakshmy, 1995).

Boutin, et al. (1994) reported that the bee venom had immunoregulatory role on the production of IgG antibodies, therefore, the reduction in thyroxine level after bee venom treatment may be due to the
reduction in IgG antibodies which is the main pathogenesis in graves' disease which is a common cause of hyperthyroidism where the IgG antibodies bind to the thyroid TSH receptor stimulating thyroid hormones production (Alter and Moshang, 1991).

Although the mechanism of bee venom goitrogenesis is not understood, but according to the above mentioned studies the goitrogenic substance (s) in the venom may be concentrated in the thyroid gland and inhibits the hormone synthesis through one of the mentioned mechanisms.

3.2. The effect of bee venom on serum T₃ level:

The effect of bee venom on serum T₃ level was presented in Table (2) and Fig. (4). The level of T₃ in hyperthyroid women was significantly higher compared to the control group. Bee venom treatment significantly reduced T₃ level.

Approximately 85% of T₃ is produced by monodeiodination of T₄ in other tissues such as the liver, muscle and the kidney and only small amount is produced by the thyroid gland (Edwards, et al. 1995).
Fig. (4): The effect of bee venom on serum $T_3$ level in hyperthyroid women

A: Control

B: Hyperthyroid patients before treatment.

C: Hyperthyroid patients after treatment.
Ganong (2001) reported that propylthiouracil which is an effective drug in the treatment of hyperthyroidism acts by inhibiting the enzyme 5-deiodinase and hence preventing the peripheral conversion of $T_4$ to $T_3$. According to this study the reduction in $T_3$ level after bee venom treatment may be due to the inhibition of the conversion of $T_4$ to $T_3$ in the peripheral tissues which may result from one or more active ingredients of the bee venom or it may be due to the reduced amount of serum $T_4$ which also decreases after the bee venom treatment.

3.3. The effect of bee venom on serum TSH level:

The level of TSH was presented in Table (1) and Fig. (5). In this study the level of TSH in the hyperthyroid patients was significantly lower than the normal range, this lower level is due to increased concentrations of the thyroid hormones which suppress the pituitary secretion of TSH (Michalkiewiez, 1987 and Brabant et al. 1991).

Cascedo et al. (1976) reported that the injection of $T_4$ in rats resulted in a decline in the serum level of TSH. In line with that the level of TSH in patients with hypothyroidism was found to be raised above the normal range (Selwa, 1999).

By the end of the bee venom treatment the level of serum TSH was significantly ($P>0.05$) increased when compared to the pretreatment level.
The elevation in TSH level after venom treatment may be due to the reduction in serum thyroid hormones as the pituitary secretion of TSH is more affected by the circulating thyroid hormones. This is because of the rapid deiodination of T₄ to T₃ by the pituitary cells. Also the increased level of TSH after bee venom treatment may be due to one or more active ingredients of the bee venom as Dunn and Killion (1988) reported that the injection of 0.5 mg/kg body weight of melittin venom compound in rats cause an increase in the pituitary function.
A: Control

B: Hyperthyroid patients before treatment

C: Hyperthyroid patients after treatment.
Conclusion

It is concluded from this study that the bee venom treatment in a dose of four stings daily for two weeks had a significant effect on hyperthyroid patients.

Further work is suggested for the isolation of the goitrogenic constituent(s) of the bee venom.
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