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Noura Al-Kattan

Appendix I

Questionnaire

١- التسلسل:

الاسم:

العمر: الجنس:

المحافظة: الحالة الإجتماعية:

٢- هل يوجد في العائلة مرض داء الملوك اوإلتهاب المفاصل لحد القرابة من الدرجة الاولى والثانية؟

٣- هل يستخدم المريض العلاج؟

Xyloric ◯

Hyporic ◯

مدة العلاج / الجرعة:

٤-هل المريض داخل نظام تغذية Regime ؟

Test	Value	
	Before	**After**
ASOT		
Anti-ds-DNA		
Latex		
ESR		
Uric acid		

Appendix II : Results of 80 patients before and after treatment with black seed

No.	Gender	ESR (mm/1hr.West.)		Latex (RF) (I.U./ml)		ASOT (I.U./ml)		Anti-ds-DNA (I.U./ml)		Uric acid (mg/dl)	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	Female	25	13	+Ve	+Ve						
2	Female	17	8	+Ve	-Ve						
3	Female	22	10	+Ve	-Ve						
4	Female	11	4	+Ve	+Ve						
5	Male	28	15	+Ve	-ve						
6	Female	10	3	+Ve	-Ve						
7	Female	34	22	+Ve	+Ve						
8	Female	18	7	+Ve	-Ve						
9	Male	11	5	+Ve	-ve						
10	Female	19	10	+Ve	+Ve						
11	Male	9	4	+Ve	+ve						
12	Female	7	2	+Ve	-Ve						
13	Female	15	7	+Ve	-Ve						
14	Male	18	7	+Ve	-ve						
15	Male	10	3	+Ve	-ve						
16	Female	11	5	+Ve	+Ve						
17	Female	22	13	+Ve	+Ve						
18	Female	18	8	+Ve	+Ve						
19	Male	12	3	+Ve	+ve						
20	Male	8	2	+Ve	+Ve						
21	Female	41	30			+Ve 400	-Ve 200				
22	Female	24	16			+Ve 800	+Ve 300				
23	Male	20	9			+Ve 400	+Ve 300				
24	Female	16	7			+Ve 400	-Ve 200				
25	Male	18	9			+Ve 500	+Ve 300				
26	Male	13	5			+Ve 600	+Ve 300				
27	Female	15	7			+Ve 300	-Ve 200				
28	Female	9	3			+Ve 300	-Ve 200				
29	Male	9	4			+Ve 500	+Ve 300				
30	Female	19	8			+Ve 400	-Ve 200				
31	Female	13	8			+Ve 400	-Ve 200				
32	Male	20	3			+Ve 300	-Ve 200				
33	Female	17	5			+Ve 300	-Ve 200				
34	Male	74	58			+Ve 300	-Ve 200				
35	Female	25	12			+Ve 400	-Ve 200				
36	Male	22	17			+Ve 500	+Ve 300				
37	Female	15	7			+Ve 400	+Ve 300				
38	Male	17	6			+Ve 400	+Ve 300				

39	Female	10	4			+Ve 500	+Ve 300				
40	Male	13	5			+Ve 600	+Ve 300				
41	Female	9	3					18.8	13		
42	Female	22	11					27.6	16		
43	Female	24	10					18.8	12		
44	Male	40	28					15	10		
45	Female	11	3					24.3	13		
46	Female	10	4					27.6	14		
47	Male	22	10					21	13.3		
48	Female	25	12					26	16		
49	Female	21	9					18	12.1		
50	Male	14	4					20	13.3		
51	Female	12	6					23	16		
52	Male	28	12					15	9.7		
53	Female	33	20					17	11		
54	Female	18	8					38	30		
55	Female	50	38					15	9.4		
56	Male	52	43					20.5	14		
57	Female	64	50					6.4	3.2		
58	Male	48	35					20.5	14		
59	Female	55	40					18	13		
60	Female	138	130					18	12		
61	Male	20	8							6.8	4
62	Female	32	20							8	6.8
63	Male	30	22							9	8.8
64	Female	22	10							7.8	5.6
65	Female	27	20							7.2	6.5
66	Male	30	24							6.9	6
67	Female	26	19							6.8	6
68	Female	35	24							6.5	6
69	Female	25	13							7.8	7
70	Male	35	21							5.1	4.2
71	Female	27	16							5.1	4.2
72	Male	22	10							6	4
73	Female	32	19							6.4	5
74	Male	45	30							7.6	5
75	Male	27	18							6	4.2
76	Male	32	20							6	6
77	Female	41	24							6	5.4
78	Male	45	32							6	3.2
79	Male	48	33							5.2	5
80	Female	32	21							6	5.7

Chapter One

Introduction and Literature review

1.1 Introduction

Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value (Barens, 2002). An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Internet, 2003 a).

Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator

used to decrease respiratory congestion) were all originally discovered through research on plants (Internet, 2003).

Early humans recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Physical evidence of use of herbal remedies goes back some 60,000 years to a burial site of a Neanderthal man uncovered in 1960 (Solecki, 1975). In a cave in northern Iraq, scientists found what appeared to be ordinary human bones. An analysis of the soil around the bones revealed extraordinary quantities of plant pollen that could not have been introduced accidentally at the burial site. Someone in the small cave community had consciously gathered eight species of plants to surround the dead man. Seven of these are medicinal plants still used throughout the herbal world (Bensky and Gamble, 1993).

Herbs have long held an important place in the treatment and the prevention of illnesses and they are becoming even more popular than ever. They have been used since the cave days to treat many illnesses and the American Indians and Egyptians were well known for their use of the plants they found around them. Most herbal remedies were compounds extracted and blended by apothecaries from many plants, each of which was known on its own as a ‘simple’. Apothecaries realised that there were differences

in the purity and potency between the substances found in similar species, and that the effects also depended on factors such as the site where they were gathered, or the time of the month or year (Internet, 2000).

1.2 Aims of the study

This work was an attempt for studying the effect of *Nigella sativa* in humans who have Arthritis and Gout diseases by treatment with black seed powder (*Nigella sativa*) as a curing agent.

1.3 History of black seed

The historical tradition of black seed in medicine is substantial. It is the black seed referred to by the Prophet Muhammad (PBUH) as having healing powers; black seed is also identified as the curative black cumin in the Holy Bible, and is described as the Melanthion of Hippocrates and Discoredes, and as the Gith of Pliny (Worthen *et al.*, 1998).

The Prophet Muhammad (PBUH) said, "Hold onto the use of the black seed for it has a remedy for every illness except death".

Black cumin (*N. sativa*) was discovered in Tutankhamen's tomb, implying that it played an important role in ancient Egyptian practices. Although its exact role in Egyptian culture is not known, we do know that items entombed with a king were carefully selected to assist him in the afterlife. The earliest written reference

to black seed is found in the book of Isaiah (28:25, 27) in the Old Testament. Isaiah contrasts the reaping of black cumin with wheat: for the black cumin is not threshed with a threshing sledge, nor is a cart wheel rolled over the cumin, but the black cumin is beaten out with a stick, and the cumin with a rod (Nooruddine, 2003).

The Greek physician Disckoredes used black seed to treat headaches, nasal congestion, toothache, and intestinal worms. Hippocrates, the grandfather of today scientific medicine regarded black seed as a valuable remedy in hepatic and digestive disorders. Black seed was also to be found in the tombs of Pharoahs, including that of Tutenkhamens tomb, believing that they could use it to fight sicknesses in the afterlife. It is known to have been used by Cleopatra for her health and beauty giving qualities (Nooruddine, 2003).

Al-Biruni (973-1048 Hijri) Muslim scholar, who composed a treatise on the early origins of Indian and Chinese drugs, mentioned that the black seed is a kind of grain called alwanak in the Sigzi dialect. This reference to black seed as grains points to the seed's possible nutritional use during the tenth and eleventh centuries.

Ibn Sina (980-1037 Hijri) in describing the black seed as that which "stimulates the body's energy and helps recovery from fatigue or dispiritedness", still holds true to "Tibb" (the Islamic Medicine) health practitioner today. The rich nutritional value contained in

black seed as outlined by scientific analysis of black seed, also points it as a great source of energy (Philippe, 2005).

Black seed is also included in the list of natural drugs of al-Tibb al-Nabawi, and, according to tradition, "hold onto the use of the black seed for in it is healing for all illnesses except death". This prophetic reference in describing black seed as having a healing for all illnesses is not exaggerated as it at first appears. Black seed has been traditionally used in the Middle and Far East countries for centuries to treat ailments including bronchial asthma and bronchitis, rheumatism and related inflammatory diseases, to increase milk production in nursing mothers, to treat digestive disturbances, to support the body's immune system, to promote digestion and elimination, and to fight parasitic infestation. Its oil has been used to treat skin conditions such as eczema and boils and is used topically to treat cold symptoms. The many uses of black seed have earned for this ancient herb the Arabic approbation habbatul barakah, meaning "the seed of blessing" (Philippe, 2005).

1.4 Description of plant

Black seed is tiny and hairy, being no more than 3mm in length. Black seed originates from the black cumin plant. As seen in the Figure (1.1). The plant has finely divided foliage and pale bluish purple or white flowers. The flowers grow terminally on its branches while the leaves grow opposite each other in pairs, on

either side of the stem. Its lower leaves are small and petioled, and the upper leaves are long 6-10cm. (Mukerji, 1953; Townsend, 1980). The stalk of the plant reaches a height of 12 to 18 cm as its fruit, the black seed, matures. (*N. sativa)* is bisexual and forms a fruit capsule which consists of many white triagonal seeds. Once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (black seeds) (El-Dakhakhany, 1965).

Figure (1.1) Black seed (*Nigella sativa*) plant. (Internet, 2005).

1.5 Where black seed grows

Native to western Asia, southeastern Europe and the Middle East, *N. sativa* is cultivated around the world and grows well in most gardens but doesn't sustain frost well. All of the nigellas grow well in Zones 3 through 10, and this annual can be added to the gardens, sprinkle seeds thinly in late spring over a prepared bed of light garden loam in a sunny spot. Pat the seeds in gently and keep moist but not wet. Although they're more successfully sown in the ground, black seed may start indoors and transplanted when threat of frost has passed. To do so, sow seeds in a container of moistened soil less mix, such as perlite or vermiculite, in early spring. Barely cover the seeds with the mix. Cover the container with plastic wrap and store at 65 to 70 F^{o}. When you begin to see growth, move the container to a sunny window, pot plants up as they grow, gradually getting them accustomed to arrangement (Rachel *et al*., 2000).

Black cumin seed planted in the winter produces attractive flowers in the spring and is harvested in the early summer for its jet-black seeds. These have a very distinct flavor are used to flavor bread and other backed goods. The seeds are still threshed from the fruits by beating the dried plants with a stick (Isaiah 28:28).At least one case of dermatitis has been reported from contact with the oil of black cumin seed (Steinmann *et al*., 1997).

1.6 *Nigella sativa*

Name: black seed.

English name: black cumin (kalounji).

Origin: Iraq, Syria, Egypt, Turkey and India.

Scientific name: *Nigella sativa*.

Other names, varying between places: Black caraway, "Habbat al baraka", "Habbat Sawada", Shonaiz, Schwarzeummel, Sinouj, Nutmeg flower and Fennel flower.

Black seed is an annual herbaceous plant, believed to be indigenous to the Mediterranean region but has been cultivated into other parts of the world including the Arabian Peninsula, northern Africa and parts of Asia (El-Dakhakhany, 1965).

Classification of *Nigella sativa* is:

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Magnoliidae –
Order	Ranunculales –
Family	Ranunculaceae – Buttercup family
Genus	*Nigella* L. – nigella
Species	*Nigella sativa* L. – black cumin

Cited from (Kartesz, 1998)

1.7 Chemical composition of black seed

The specific seed constituents that have been identified and investigated include:

1.7.1 Fixed oils:

The fixed oils constitute (32-40 %) of the seed involving saturated and unsaturated fatty acids. Black seed contains a high rate of essential fatty acids such as oleic, linoleic, and linolenic, these are important for healthness, because they are not produced by the body but eaten in food. These are just as vital to our body as a vitamin and mineral intake, as well as for hormone production (Gad *et al*., 1963; Babayan *et al*., 1978).

1.7.2 Volatile oils:

The volatile oils are about (0.4-0.45 %) in the black seed, showed anti-bacterial properties.

Volatile oil like: nigellone, thymoquinone and dithymoquinone were detected in black seed. Crystalline nigellone was first isolated and identified as providing many health benefits (Badry *et a*l., 2000).

Nigellone is known to be an important factor in the treatment of asthma and it also causes inhibition of histamine release. Nigellone has a suppressive effect that inhibits protein kinase C, a substance known to trigger the release of histamine. Nigellone also,

decreased the uptake of calcium in mast cells which also inhibits histamine release. The other active compound of black seed is thymoquinone which is considered as anti- inflammatory agent. These above studies are mentioned by (Nooruddine, 2003).

1.7.3 Alkaloids:

Alkaloids are plant constituents which are capable of strongly affecting human physiology. Examples are atropine and caffeine. Three alkaloids were isolated of which two were identified, namely: nigellicine and nigellamines (Bhikha, 1990).In pharmacology experiments; the isolated alkaloids have been shown to lower cholesterol and triglycerides (Kumara and Huat, 2001).

1.7.4 Amino-acids:

Amino acids are constituents of protein (16-19.9%) including 15 amino acids like arginine, leucine, lysine …etc. It was reported that arginine is essential for infant growth (Babyan *et al*., 1978).

1.7.5 Minerals:

Minerals (1.79- 3.74 %) like: Calcium, potassium, sodium, phosphorous, and iron. They are required only in small amounts by the body; these elements main function is to act as essential cofactors in various enzymes functions. The elements known to be

toxic to animals such as uranium. Thus black seed is considered as safe food ingredient (Kumara and Huat, 2001).

1.7.6 Other components:

Black seed contains carbohydrates (33.9 %), fibers (5.5%); it was mentioned that black seed contains a non- starch polysaccharide component which is considered as a useful source of dietary fiber, and finally 6% water (Randhawa and Al-Ghamdi, 2002).

Other components include Enzyme: like, lipase (Korchagina and Rudyuk, 1979), vitamins: like, Vit. A, B1, B2, B6, C, Niacin, and Folacin (Nergiz and Otles 1993), and Lectins (Sharon, 1993).

1.8 Types of black seed

The three most important species of black cumin are: ***N. damascene***, ***N. arvensis,*** and ***Nigella sativa***. (Rachel *et al.*, 2000).

Nigella sativa True black cumin grows to a height of 6 to 12 inches; bears milky white apical blossoms that turn bluish-green near the tip, containing a coarse ball-like fruit capsule that develops after the plant blossoms; and a crown of five protruding beak-like spikes. Ground *Nigella* seeds emit a fragrance vaguely similar to fennel, anise or nutmeg, which some compare to camphor or cajaput. They taste slightly bitter, spicy and piquant and have been

used as a substitute for caraway and black pepper in bread making and at the table (Rachel *et al.*, 2000).

1.9 Medicinal uses of *Nigella sativa*

Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health and treatment of many ailments including fever, common cold, headache, various microbial infections and to expel worms from the intestines. It is also used for scorpion and spider stings and bites of snake, cat and dog. In addition, it is used as a flavoring additive to bread and pickles (El-Kadi and Kandil, 1986; Al-Jishi, 2000).

The multiple uses *N. sativa* in the folk medicine encouraged many investigators to isolate the possible active components and to conduct *in vivo* and *in vitro* studies on laboratory animals and human beings in order to understand toxicological and pharmacological properties (Muhammad and Mastoor, 2002).

1.9.1 Toxicological properties

The seed extract and its constituents appear to have a low level of toxicity. The administration of *N. sativa* seed extract (50 mg/kg) intraperitoneally to rats for 5 days did not significantly affect the activities of several enzymes and metabolites indicative of hepatic and renal function (El- Daly, 1998).

Oral administration of the seed oil at doses up to 10 ml /kg in rats and mice did not cause any mortality or overt toxicity during the observation period of 48 hr. (Khanna *et al.*, 1993).

This was recently confirmed when it was shown that oral administration of the fixed oil of *N. sativa* at a dose of 10 ml /kg, for up to 12 weeks did not cause any mortality or significant alteration of the key hepatic enzymes in rats (Zaoui *et al*., 2002).

1.9.2 Pharmacological properties

Pharmacological properties are as follow:

1.9.2.1 Anti-microbial and Anti-parasitic actions:

The anti-bacterial effect of the phenolic fraction of *N. sativa* oil was first reported by (Topozada *et al.*, 1965). Thymohydroquinone was isolated from the volatile oil of *N. sativa* and found to have high activity against gram-positive bacteria (El-Fatatry, 1975).One study of the antimicrobial effect of diethyl-ether extract of *N. sativa* reported that it had a concentration- dependent inhibition of gram-positive bacteria represented by *Staphylococcus aureus* and gram-negative bacteria represented by *Pseudomonas aerugenosa* and *Escherichia coli* (Hanafi and Hatem, 1991).

Black seed has anti-parasitic properties (Akhtar and Riffat, 1991).It was found that black seeds can prevent liver damage

induced by *Schistosoma mansoni* infection in mice (Mahmoud *et al.*, 2002).

The activity of the black seed was compared with five antibiotics: ampicillin, tetracycline, cotrimoxazole, gentmicin, and nalidixic acid. The oil proved to be more effective against many strains of bacteria including: *V. cholera*, *E. coli*, and all strains of *Shigella* species except *S. dysenteriae*. These results suggest that black seed would probably be a good therapeutic agent in the treatment of diarrhea (Nooruddine, 2003).

1.9.2.2 Immune System effect:

As a natural remedy people take black seed (*N. sativa)* seed which can play an important role to enhance human immunity, particularly in immunocompromise proteins (El-Kadi and Kandil, 1986).Black seed could be also suitable for diseases of the immune system it self, e.g. allergies; tuberculosis (TB); Cancer; AIDS, etc…the immune system is strengthened with regular doses, and as scientists at the Immuno Biology Laboratories in South Carolina have reported, regular doses of this black seed extract actually stimulate bone marrow and immune cells. Cell damaging viruses are reduced as the black seed increases the number of anti-bodies which produce the B-cells lymphocytes in the body. All these function make black seed an ideal supplement to human every body in order of well –being (Randhawa and Al-Ghamidi, 2002).

Moreover, one scientist noticed that *N.sativa* enhanced the production of interleukin-3 by human lymphocytes when cultured with pooled allogenic cells or without any added stimulator. They also observed an increase in interleukin-1 beta (IL-1b) suggesting that *N. sativa* has an effect on macrophages as well (Haq *et al*., 1995).

1.9.2.3 Anti-inflammatory and Analgesic effect:

A group of scientists decided to test the effectiveness of the fixed oil of black seed and its derivative, thymoquinine, as an anti-inflammatory agent, and in the traditional medicine to treat a wide range of diseases including diarrhea and asthma.Their study found that the oil inhibited eicosanoid generation and demonstrated anti-oxidant activity in cells (Houghton *et a*l., 1995).

Analgesic effect of aqueous suspension of *N. sativa* seeds, comparable to aspirin, as measured by hot plate test conducted in rats. However the suspension did not relieve yeast-induced pyrexia in rats (Al-Ghamidi, 2001).

1.9.2.4 Anti-cancer effect:

The anticancer activity of *(N. sativa)* was first revealed by one scientist who observed enhancement of (NK) cell activity ranging from 200-300 % in advanced cancer patients receiving multimodality immunotherapy programme in which *N. sativa* was

one of the components (El-Kadi and Kandil, 1986). Later on, the anti-cancer effect of black seed was investigated both *in vitro* using cancer cell lines and *in vivo* using animal models (Randhawa and Al-Ghamidi, 2002).

Topical application of *N. sativa* and *Crocus sativus* extracts inhibited two-stage initiation/promotion of skin carcinogenesis in mice by delaying the onset of papilloma formation and reducing the number of papillomas per mouse (Salomi *et al*., 1991).

Moreover, an active principle of *(N. sativa)* containing fatty acids demonstrated, *in vitro*, 50% cytotoxic activities against Ehrilch ascites carcinoma (EAC), Dalton's lymphoma ascites and sarcoma-180 cells and, *in vivo*, completely inhibited EAC tumour development in mice (Salomi *et al*., 1992).

Thymoquinone and dithymoquinone, active principles of (*N. sativa)*, had cytotoxic effect against parental and multi-drug resistant human tumors cell lines which were over 10-fold more resistant to doxorubicin and etoposide (Worthen *et al*., 1998).

1.9.2.5 Anti-oxidant activity:

(*N. sativa*) extracts and some of its active principles, like thymoquinone, have been shown to possess protective effect against haematological, hepatic, renal and other toxicities induced by anti-cancer drugs and some toxins (Nair *et al.*, 1991).

More recently, the protective action of *N. sativa* for carbon tetrachloride- induced liver fibrosis and cirrhosis in rabbits (Turkdogan *et al.*, 2001). As well as the protective effect of *N. sativa* against the genotoxic action of an herbicide, 2, 4-D (El-Sherbeny, 2001).

1.9.2.6 Anti-histaminic action:

Histamine is a substance released by bodily tissues, sometimes creating allergic reactions and is associated with conditions such as bronchial asthma. Scientists found that dimer dithymoquinone isolated from black seed's volatile oil, under the name of "Nigellone," and given by mouth to some patients suffering from bronchial asthma, suppressed the symptoms of the condition in the majority of patients. Following the results of this early study, crystalline nigellone was administered to children and adults in the treatment of bronchial asthma with effective results and no sign of toxicity. It was observed, however, that although effective, crystalline nigellone displayed a delayed reaction (Badar El- Din, 1960; Mahfouz and El- Dakhakhany, 1960).

One scientist found that the actual mechanism behind the suppressive effect of crystalline nigellone on histamine is that crystalline nigellone inhibits protein kinase C, a substance known to trigger the release of histamine. In addition, his study showed that crystalline nigellone decreased the uptake of calcium in mast cells,

which also inhibits histamine release. The importance of these results is that people who suffer from bronchial asthma and other allergic diseases may benefit from taking crystalline Nigellone (Chakravarti, 1993).

1.9.2.7 Respiratory system effect:

In Saudi Arabia and neighboring countries *N. sativa* seeds and oil are commonly used for the treatment of asthma. Nigellone (a carbonyl polymer of thymoquinone) proved to be an excellent prophylactic agent for both bronchial asthma and asthmatic bronchitis and was more effective in children than adults (Badar El-Din, 1960; Mahfouz and El-Dakhakhany, 1960).One scientist has also reported the use of *N. sativa* in asthma in the traditional medicine (EL- Sayed *et al*., 1994).

However, the *N. sativa* volatile oil induced dose- dependent increase in the respiratory rate and the intra-tracheal pressure, which were antagonized by mepyramine, atropine and reserpine but not by indomethacin, diethyl-carbamazine or hydrcortisone. A central mechanism was suggested for these effects (El-Tahir *et al*., 1993).

1.9.2.8 Genito-urinary system effect:

In Unani medicie *N. sativa* is promoted for the treatment of oligomenorrhoea, to induce menstruation and to treat infertility (AL-Jishi, 2000).One study reported that *N. sativa* crude oil

induced uterine contractions both *in vivo* in pregnant rabbits and *in vitro* of non-pregnant rat uteri (El-Naggar and El-Deib, 1992). One scientist found that the hexane extract of *N. sativa* exhibited mild uterotropic activity and prevented pregnancy in rats when given on day 1-10 post-coitum (Keshri *et al*., 1995).

1.9.2.9 Gastro-intestinal tract effect:

In Unani medicine *N. sativa* is used for stomachache and as a digestive, carminative, laxative and anti-jaundice (El- Kadi and Kandil, 1986). Oral *N. sativa* powder was reported to relieve flatulence while nigellone, an active principle of *N. sativa* was found to antagonize histamine induced contractions of guinea pig intestine, cited by (Chopra *et al*., 1956). In addition, a choleretic effect of *N. sativa* oil and its active principles (thymoquinone, thymohydroquinone and dithymoquinone), respectively (Mahfouz and El-Dakhakhany, 1960; El-Dakhakhany, 1965).

One scientist investigated the effect of *N. sativa* oil on gastric secretion and ethanol-induced ulcer in rats. Significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation, with a protection ratio of 53.56 %, was found in the *N. sativa* oil pretreated group (El-Dakhakhany *et al*., 2000). More recently, the crude extract of *N. sativa* was shown to cause a dose –dependent (0.1 – 3.0 mg/ml) relaxation of spontaneous contractions of rabbit jejunum as well as

inhibition of K^+ induced contractions in a similar dose range, suggestive of calcium channel blockade (Gilani *et al*., 2001).

1.9.2.10 Hypoglycemic effect:

The use of a plant mixture containing *N. sativa*, Myrr, Gum *Olybanum*, Gum *Asafoetida* and *Aloe* by diabetics in Kuwait. They confirmed the blood glucose lowering effect of *N. sativa*, in combination with other herbs in rats (Al-Awadi and Gumma, 1987). The mechanism of action was later investigated and appeared to be due to the inhibition of hepatic gluconeogenesis (Al-Awadi *et al*., 1991).

The volatile oil of *N. sativa* alone also produced a significant hypoglycemic effect on normal and alloxan-induced diabetic rabbits without changes in insulin levels (Al-Hader *et al*., 1993). The hypoglycemic effect of *N. sativa* in combination with other herbs on alloxan-induced diabetic rats (Eskander *et al*., 1995; El-Shabrawy and Nada, 1996). Furthermore, reported a significant decrease in blood sugar of healthy human volunteers treated with 1 gram of *N. sativa* capsules twice daily (Bamosa *et al*.,1997).

1.9.2.11 Anti- hepato and nephrotoxic action:

In some countries *N. sativa* seeds are sold to treat conditions that include liver diseases. Using isolated rat hepatocytes, to investigate the protective action of thymoquinone isolated from *N.*

sativa against the hepatotoxicity of tert-butyl hydroperoxide (TBHP). Thymoquinone showed protective actions against TBHP. The hepatoprotection of thymoquinone was compared with that of silybin, a known hepatoprotective agent. On the whole, thymoquinone was as effective as silybin in protecting certain aspects of hepatic function (Daba and Abdel-Rahman, 1998).

1.9.2.12 Anti- ulcer action:

A single report in rats has suggested that the aqueous extract of *N. sativa* seeds was effective in reducing the ulcer index (induced by aspirin) by about 36% (Akhtar *et al*., 1996).

The treatment reduced the peptic activity and acid production, but did not change mucin activity. These results seem to suggest that the folkloric use of the plant to treat peptic ulcer may not be founded. However, more recently one scientist obtained opposite results and reported that administration of *N. sativa* oil (0.88 g/kg/day) for 2 weeks increased gastric mucin and glutathione content, reduced histamine content, but did not affect the free acidity and peptic activity of the gastric juice (El-Dakhakhany *et al.,* 2000).

The above report is in line with earlier ones that have suggested a cytoprotective action of *N. sativa* oil and lends some credence to the folkloric use of the oil as an antiulcer agent. However, further work on the action of the oil and extract of the

seeds on experimental gastric and duodenal ulcer is warranted (El-Kadi *et al.*, 1987).

1.9.2.13 Promote lactation:

A literature search by the University of Potchefstroom (1989) revealed that black seed's capacity to increase the milk flow of nursing mothers could be attributed to a combination of lipid portion and hormonal structures found in the black seed (Tierra,2004).

1.9.2.14 Estrogenic activity:

Very few studies have focused on the medicinal properties of *N. damascen*e, some phenol compounds of *N. damascen*e seeds have estrogenic activity (Agradi *et al*., 2001).

1.9.2.15 Anthelmintic activity:

The essential oil from *N. sativa* seeds showed *in vitro* anthelmintic activity against earthworm and tape worm, the action being comparable with that of piperazine phosphate (Agarwal *et al*., 1979).

1.9.2.16 Effect on Arthritis disease:

The pharmacological properties of the oil support the traditional use of *N. sativa* and its derived products as a treatment

for rheumatism and related inflammatory disease (Houghton *et al*., 1995).

1.10 Arthritis

Although the term literally means joint inflammation, arthritis really refers to a group of more than 100 rheumatic diseases and conditions that can cause pain, stiffness and swelling in the joints. Certain conditions may affect other parts of the body such as the muscles, bones, and some internal organs and can result in debilitating, and sometimes life-threatening, complications. (Doherty, 2002). If left undiagnosed and untreated, arthritis can cause irreversible damage to the joints (Doherty, 2002); types of arthritis include:

1- Rheumatoid arthritis (RA) which affects around 2-3% of the population, again with greater incidence in women. It is a progressive disease, with onset most likely between 25-50 at a time when people are active in the workplace or family care roles. RA is characterized by inflammation within joints that serves no evidently useful purpose and which damages joint structures. The synovial membrane that lines joints is thickened and an over-production of synovial (joint) fluid occurs, symptoms: The joints become painful, swollen, and stiff and, as the process continues, deformed from damage to the cartilage and other soft tissue (Shipley, 1993).

2- Osteoarthritis (OA) is the most common form of arthritis, affecting around 5-10% of the total population, particularly women. It develops when articular cartilage (the smooth covering over bones in the joints) starts to break down, usually as a result of trauma, ageing or failure of joint repair and maintenance mechanisms. Degradation of the cartilage can be associated with underlying bone damage, thickening and bone-on-bone friction. Symptoms: include stiffness, pain and tenderness in the joints and surrounding muscles and ligaments, possibly with fatigue, reduction in motor skills, and deformities (Shipley, 1993).

3- Systemic Lupus Erythematosus is also type of arthritis disease. (SLE) is a chronic inflammatory autoimmune disease of the connective tissues. It affects the skin, especially in sun exposed areas such as the cheeks, which become red and scaly and various internal organs (kidneys, heart, lungs and brain can all be affected by inflammation and subsequent scar tissue). Lupus often causes:

1. General fatigue.
2. Tiredness.
3. Loss of concentration and memory.

Internal organ involvement can lead to organ failure and death (Blech and Zurier, 1995).

4- Gout caused by the reaction of defense cells in joints to the presence of uric acid crystals. Uric acid (urate) is a by-product of the breakdown of the purines in the body. (Purines are components of the genetic template (DNA) and of certain messenger substances within cells.) Gout is characterized by severe acute attacks of joint pain and swelling, which typically affect joints such as the big toe, the ankle, knee and elbow. An excess of urates can also cause kidney damage, including the formation of stones (Andres, 1981).

5- Fibromyalgia (previously known as fibrositis) is a condition in which discomfort is widespread and felt within the muscles and ligaments, which may be tender. Damage to joints or other tissues is not a feature. A common association with sleep dysfunction and irritable bowel symptoms suggests an underlying neural irritability. Fatigue, feelings of demoralisation and seemingly insoluble life stresses may be part of the picture. Fibromyalgia is to be distinguished from 'soft tissue rheumatism' which refers to irritation or inflammation of structures such as ligaments and the synovial sacs that lubricate tendon movement. And other type is Infectious arthritis, also known as septic arthritis, is produced by an infection. Unlike other types of arthritis, infectious arthritis is generally curable if treated promptly and properly. Without proper treatment, infectious arthritis can result in serious joint damage and may spread to other parts of the body. Any age group, including

newborns and children, can contract infectious arthritis. Infectious arthritis is not contagious (Internet, 2003 b).

Anyone can get infectious arthritis. People who have a suppressed immune system caused by other diseases such as diabetes, sickle cell anemia, or severe kidney disease, or who have jobs involving contact with animals, plants, soil, or marine life have a greater chance of developing the condition, symptoms include: joint swelling, soreness, tissue fluid leakage, and the joints feeling warm to the touch. In most cases, the patient will have fever and chills. Children sometimes develop nausea and vomiting (Internet, 2003 b).

1.11 Clinical diagnosis

The clinical diagnosis of RA can only be established by an accurate and careful history and physical examination. Only limited help is proved by laboratory tests (Doherty *et al*., 2002). The clinical hallmark of inflammatory joint disease is persistent synovitis. In patients with isolated small joint synovitis the acute phase response may be normal, because the magnitude of this response is correlated with the amount of inflammatory activity (synovitis bulk). A set of classification criteria for diagnosis of (RA) are:

1-Morning stiffness (>1 hr).

2-Arthritis of three or more joint areas.

3- Arthritis of hand joints.

4-Symmetrical arthritis.

5-Rheumatoid nodules.

6-Rheumatoid factor.

7- Radiological changes.

8-Duration of 6 weeks or more.

These criteria were designed to distinguish patients with RA from those with other arthropathies in a clinical population, and for comparative epidemiological studies (Doherty *et al*., 2002).

The diagnosis of gout, in almost all first attacks a single distal joint is affected. Common sites affected are the ankle, knee, small joints of hands, wrist and elbow. The axial skeleton and large proximal joints are rarely involved and never as the first site. Typical attacks have the following characteristics:

1- Extremely rapid onset, reaching maximum severity in just 2-6 hrs, often waking the patient in the early morning.

2- Severe pain, often described as the "worst pain ever".

3- Extreme tenderness- the patient is unable to wear a sock or to let bedding rest on the joint.

4- Marked swelling with overlying red, shiny skin.

5- Self-limiting over 5-14 days, with complete return to normality.

During the attack the joint shows signs of marked synovitis but also periarticular swelling and erythema. The attack may be accompanied by fever, malaise and even confusion, especially if a large joint such as the knee is involved. As the attack subsides pruritus and desquamation of overlying skin are common. The main differential diagnosis is septic arthritis, infective cellulites or another crystal disease. Sepsis, however, is usually more subacute in onset and progresses in severity until treated (Doherty *et al*., 2002).

1.12 Lab. diagnosis

1.12.1 ESR test:

The erythrocyte sedimentation rate is a test that involves placing a blood sample in a tube and determining how far the red blood cells settle in one hour. When inflammation is present in the body, certain proteins cause red blood cells to stick together and fall more quickly than normal to the bottom of the tube. The more red cells that fall to the bottom of a special test tube in one hour, the higher the ESR. These proteins are produced by the liver and the immune system under many abnormal conditions, such as an infection, an autoimmune disease, or cancer. There are many possible causes of an elevated sedimentation rate. For this reason, an ESR test is done with other tests to confirm a diagnosis. Once a diagnosis has been made, an ESR test can be done to help monitor

the course of the disease or the effectiveness of treatment (Renee, 2004).

1.12.2 Latex (Rh.F) test:

Rh.F. is an antibody found in many patients with RA. It is one of several criteria used in diagnosing RA, as 80 percent of RA patients have Rh.F. in their blood. Rh.Fs is antibodies directed against antigenic sites in the Fc fragment of human and animal IgG. Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease. A Rh.F. test can be positive in response to other inflammatory or infectious diseases other than RA (Tarborn, 1979; Dornerm, 1987).

1.12.3 ASOT test:

The ASO-latex is used for detection of anti-streptolysin O antibodies. Latex particles coated with streptolysin O are agglutinated when mixed with samples containing ASO. Streptolysin O is a toxic immunogenic exoenzyme produced by β-hemolytic streptococci of groups A,C,G. Measuring the ASO antibodies is useful for the diagnostic of rheumatoid fever, acute glomerulonephritis and streptococcal infection (Kalien *et al*., 1979).

1.12.4 Anti-ds-DNA test:

Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA's) that have been observed in several autoimmune diseases. Anti-bodies reacting with native (ds) DNA are regarded as being specific for (SLE) and have been observed in approximately (50-80 %) of the patients (Tan *et al*., 1982).

The detection of (ANA's) has long been an important tool in the diagnosis of systemic rheumatic diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei; this has led to them being termed Extractable Nuclear Antigens (ENA's). The most commonly measured ENA specifications are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1 and anti-Scl-70. The intracellular antigens SS-A/Ro, SS-B/La, Sm, Sm/RNP, Jo-1 and Scl-70 are targets for autoimmune responses in many patients with rheumatic diseases. Anti-SS-A/Ro antibodies are found in 30-50 % of patients with (SLE), but most significantly in around 95% of patients with primary or secondary Sjogren's Syndrome (SS). Anti-SS-B/La antibodies are also found in SLE and SS patients. Anti-Sm antibodies are considered highly specific for SLE and approximately 30-40% of patients show their presence. Anti-RNP antibodies are predominantly found in patients with Mixed Connective Tissue Disease (MCTD) but are also associated with SLE, SS and Scleroderma. Anti-Jo-l antibodies are considered

specific for Polymyositis and Dermatomyositis. Anti-Scl-70 antibodies are recognized as specific markers for Primary Systemic Sclerosis (PSS or Scleroderma) (Wilson and Nitsche, 1986).

An antibody to ENA was depending on disease state. The following are estimated incidences of ENA antibodies in various diseases:

Antibody	**SLE**	**SS**	**PSS**	**MCTD**	**PM**	**DM**
SS-A/Ro	30-50%	~95%	-	-	-	-
SS-B/La	>15%	~87%	-	-	-	-
Sm	30-40%	-	-	-	-	-
Sm/RNP	35-45%	>30%	>20%	95-100%	-	-
Jo-l	-	-	-	-	>25%	>25%
Scl-70	-	-	20-30%	-	-	-

(Wilson and Nitsche, 1986)

1.12.5 Uric acid test:

Uric acid tests are tests that are done to measure the levels of uric acid in blood serum or in urine.

The uric acid tests are used to evaluate the blood levels of uric acid for gout and to assess uric acid levels in the urine for kidney stone formation. The urine test is used most often to monitor patients already diagnosed with kidney stones, but it can also be used to detect disorders that affect the body's production of uric acid and to help measure the level of kidney functioning (Joseph, 2005).

Chapter Two

Materials and Methods

2.1 subjects

One hundred fifty (150) patients suspected have arthritis diseases are send from Clinician to the 14th Ramadan Clinical Lab.

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2.2 Sample collection

All samples were taken from patients attending 14th Ramadan Clinical Lab. In addition to miscellaneous source and the work is done in this lab. (From October 2003 to January 2005). Venous blood samples were taken from (80) patients suspected to have arthritis disease by means of disposable syringe (5ml). (2.5 ml) of blood was layered in anti- coagulant tube with (EDTA) and tested for ESR and other (2.5ml) of blood was centrifuged after coagulant for obtaining serum used for estimation of latex (Rh.F.), ASOT, Anti-ds- DNA, and uric acids.

2.3 Preparing

N. sativa seed is grind to powder by grinder (1000 r.p.m.) and (1g) by (El-Kadi and Kandil, 1986), packed in capsule was administrated to patients with 2 categories first one: patients have arthritis disease, second including patients afflicted with gout

disease (2 times daily for 21 days), then blood was drown again and the above parameters were retested after treatment by *N. sativa*.

2.4 Materials

2.4.1 Equipments and apparatus:

The following equipments and apparatus were used through-out the study:

Table of Equipments

No.	Equipment	Company	Origin
1	Centrifuge	Gallen Kamp	England
2	Incubator	Gallen Kamp	England
3	Spectrophotometer	Aurora instrument ltd. UV 201	England
4	Sensitive Balance	Mettler	Switzerland
5	ELISA system	Metertech	Korea
6	Micropipette	Gelason	France
7	Pipette	Gelason	France
8	ESR rack	Gelason	France
9	Micro wells	Metertech	Korea

2.4.2 Kits and Reagents:

No.	Reagent	Company	Origin
1	Latex(Rh.F) reagent kits	*GmbH*	Germany
2	ASOT reagent kits	LABKIT	Germany
3	Anti –ds-DNA reagent kits	AESKU	Germany
4	Uric acid reagent kits	Linear Chemicals	Spain

2.5 Methods

2.5.1 ESR (Wistergreen Method):

1. In small test tube 0.4 ml of tri-sodium citrate (3.8% TSC) **"prepared by Institute of Vaccines and Serology / Ministry of Health Baghdad"** was taken.

2. Using sterile syringe (1.6 ml) of patient's blood was drawn and mixed with anti coagulant in test tube.

3. The mixture was drawn by using ESR pipette to the number 0.

4. Hanging this pipette in ESR Holder in vertical position at room temperature.

5. After one hr. reading of column of plasma were recorded (Renee, 2004).

2.5.2 Latex (Rh.F.) (By agglutination technique):

1. Temperature of reagent and specimen (serum) was equalized to room temperature before use. Then the Rh.F. latex reagent was shakn gently to obtain uniform suspension.
2. One drop (50 µl) of the serum was added on successive field of the reaction slide using serological pipette.
3. One drop of Rh.F. latex reagent was added to the specimen (serum). Using the stirring stick, mixing and spreading were made on the reaction mixture over entire test field. Then the slide was rotated for 2 minutes either by hand or with a rotator (80-100 r.p.m.) and read immediately under indirect oblique light if agglutination appears. According to leaflet kit from (GmbH company) procedure.

2.5.3 ASOT (By agglutination technique):

1. The temperature of reagent and samples (serum) was equalized to room temperature.
2. One drop (50 µl) of the samples was added into circles on the slide test using serological pipette.
3. One drop of ASOT reagent was added to the specimen (serum) then both drops were mixed with a stirrer, spreading them over the entire surface of the circle.
4. The slide was rotated with a mechanical rotator at (80-100 r.p.m.) for 2 minutes. False positive result could appear if the test is read

later than two minutes. According to (LABKIT) procedure in leaflet information of the kit.

2.5.4 Anti- ds –DNA (Estimated by ELISA technique):

1. Twenty ml of concentrated sample buffer was diluted with D.W. 80ml.
2. Two ml of concentrated wash buffer was diluted with distilled water 98ml.
3. Ten µl of serum samples was diluted with 1ml of sample buffer.
4. Hundred µl of each diluted serum was pipette into the designated micro wells.
5. Hundred µl of calibrator's cut-off control, negative and positive controls were pipetted into designated wells.
6. Then Incubated for 30 minutes at room temperature.
7. Washing 3 x with 300 µl washing buffer.
8. Hundred µl of conjugate was pipetted into each well.
9. Then Incubated for 15 minutes at room temperature.
10. Washing 3x with 300 µl washing buffer.
11. Hundred µl of substrate was pipetted into each well.
12. Then Incubated for 15 minutes at room temperature, in the dark.
13. Hundred µl of stop solution was pipetted into each well.
14. Incubated for 5 minutes minimum.
15. The plate carefully agitated for 5 sec.

16. And reading absorbance at 450 nm (optionally 450/620nm) within 30 minutes. According to leaflet of kit from (AESKU) procedure.

2.5.5 Uric acid (Enzymatic technique):

1. Samples were pipetted into a labeled tub.

Tubes	Blank	Sample	Standard
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	-	25µL	-
Standard	-	-	25µL

2. Then mixed and put for 10 minutes at room temperature.

3. The absorbance of the samples and standard were read at 550nm against the reagent blank.

4. The color is stable for at least 30 minutes protected from light. According to (Linear Chemicals) procedure in the leaflet information of the kit provided.

Calculations:

$$\frac{\mathrm{A}_{(\text{absorbant of sample})}}{\mathrm{A}_{(\text{absorbant of stander})}} \times C_{conc.} = \text{mg/dl uric acid}$$

2.6 The statistical analysis

The usual statistical methods were used in order to analyse and assess our results, these included:

I- Descriptive Statistics:

a- Statistical Tables.

b- Arithmetic mean (X).

c- Standard deviation (Sd).

d- Cross Tabulation (Correlation values).

e- Percentage.

f- Graphical Presentation by (Bar-Charts).

II- Inferential Statistics:

These were used in order to accept or reject the statistical hypothesis, these included:

a- Student- test.

b- Analysis of variance (Anova).

c- Least Sig.Diff. (LSD).

d- Kolmergrove- Simanove for one sample test.

e- Binomial test.

III- Pentium III system: All computer results were obtained through using SPSS under windows ver. 10.0 (SPSS, 1993).

Chapter Three

Results and Discussion

3.1 Age

Eighty patients suspected to have arthritis disease were divided into 60 patients for arthritis disease which subdivided into 20 for 3 tests includes: Latex, ASOT, Anti-ds-DNA, and 20 patients for gout disease. Depending on the evaluation of five parameters (Latex, ASOT, Anti-ds-DNA, Uric acid and ESR), in the serum and peripheral blood of patients before and after treatment with *N. sativa* as show in appendix II, And according to the results, observed in Figure (3.1) the number and percentage of patients with age range group (40-54 years) was higher (37 and 46.25%) than other age range groups.

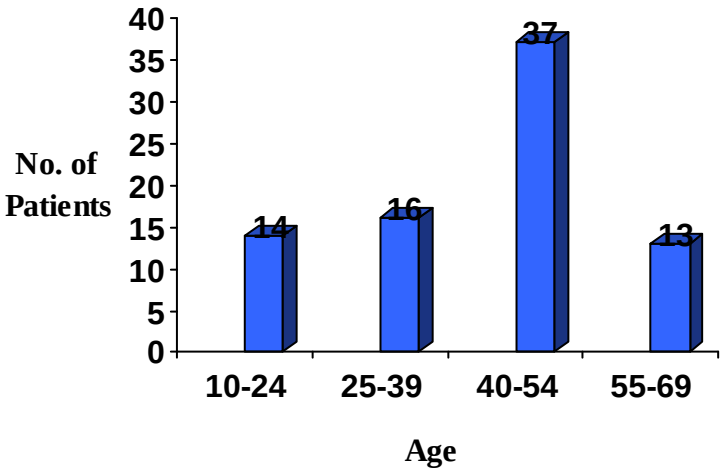

Figure (3.1) Distribution of patients according to age range group

There are different risk factors that can cause arthritis including age, body weight, environmental factor and other factors:

Cartilage becomes more brittle with age and has less capacity to repair itself. So as people grow older they are more likely to develop arthritis. Because joint damage is partly dependent on the load the joint has to support, excess body weight can lead to arthritis. This is especially true of the hips and knees that can be worn quickly in heavier patients (Jonathan, 2005).

Many scientists think that something must occur to trigger the disease process in people whose genetic makeup makes them susceptible to RA. A viral or bacterial infection appears likely, but the exact agent is not yet known. This does not mean that RA is contagious: a person cannot catch it from someone else (Internet, 1998).

3.2 Gender

Figure (3.2) showed that the number and percentage of female patients were higher (48 and 60%) when compared with the number and percentage of male patients (32 and 40%).

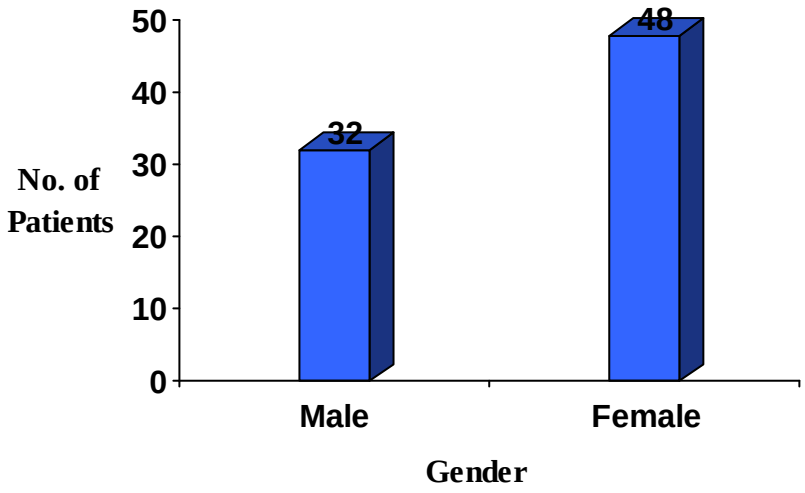

Figure (3.2) Distribution of patients according to gender

A variety of hormonal factors may be involved. Women are more likely to develop rheumatoid arthritis than men, pregnancy may improve the disease, and the disease may flare after a pregnancy. Breastfeeding may also aggravate the disease. Contraceptive uses may alter a person's likelihood of developing RA. Levels of the immune system molecules interleukin 12 (IL-12) and tumor necrosis factor-alpha (TNF-α) may change along with the changing hormone levels seen in pregnant women (Internet, 1998). This change may contribute to the swelling and tissue destruction seen in RA. These hormones, or possibly deficiencies or changes in certain hormones, may promote the development of rheumatoid arthritis in a genetically susceptible person who has been exposed to a triggering agent from the environment (Internet, 1998)

It is well known that these joints become less stiff, and osteoid is laid down in bone under the influence of estrogen. Estrogen appears to control the function of both osteoclasts and osteoblasts in bone and thus influences the rate of absorption and deposition of calcium. Remodeling of bone continues throughout life, but after estrogen deprivation, the osteoclastic activity far exceeds the osteoblasts ability to lay down calcium. Under these conditions. Osteopenia and finally osteoporosis occur (Barry, 1992).

Results agree with the work done by (Doherty *et al*., 2002 d) who reported that 5% of women and 2% of men over 55 years being affected. RA is uncommon in men under the age of 45, where there is a 6:1 female excess.

3.3 Evaluation of ESR

Table (3.1) Mean level of ESR (mm/1 hr.West.) in peripheral blood of patients before and after treatment with *N. sativa.*

Treatment with *Nigella Sativa*	**No. of patients**	**Mean level of ESR (mm/1 hr.west.)**	**S.D. ±**	**C.S.**
Before	80	26	18.61	(S)
After	80	15	13.58	
Mean normal value of ESR		15	13.58	

S=P<0.05 HS=P<0.01 NS=P>0.05

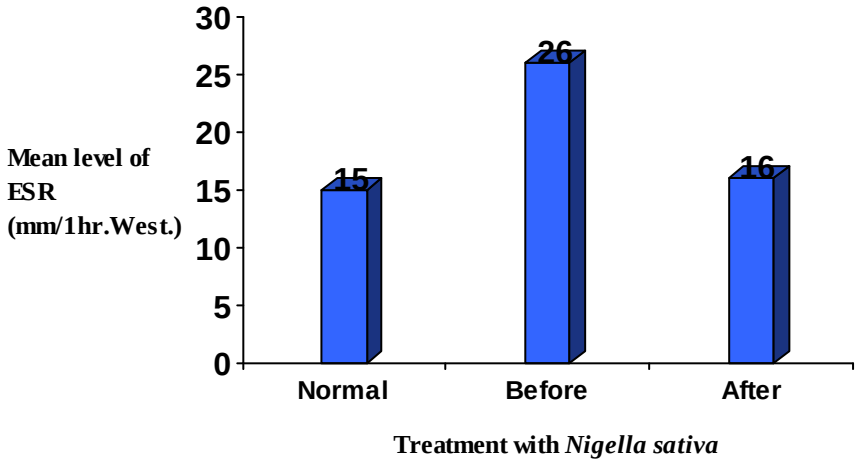

Figure (3.3): Mean level of ESR (mm/1 hr.West.) in peripheral blood of patients before and after treatment with *N. sativa.*

The results represented in Table(3.1) and Figure (3.3) showed that the mean level of ESR in peripheral blood of patients after treatment with *N. sativa* was (15 mm/1hr.West.) with significant decrease ($P< 0.05$) when compared with its mean level in peripheral blood of patients before treatment (26 mm/1hr.West.).

The above results are in agreement with the results obtained from many studies. One of them is done by AL-Okbi *et al*., they evaluated that the ESR level of RA patients before and after 2 months supplementation of *N. sativa*, showed significant reduction in ESR level (AL- Okbi *et al*., 2000).

Many studies stated that β-sitosterol found in *N. sativa* is considered to have anti-inflammatory activity (Gupta *et al*., 1980) and (Nergize and Otlis, 1993)**.**

One of the most important steroidal compounds is β- sitosterol, which is known to be used in replacement therapy and similar compound structure with similar function in the body by adrenal cortex are used in the treatment of inflammatory diseases after they are synthesized in the body, and in the treatment and management of arthritis disease such as RA (Mary *et al*., 2000 a).

The ESR is the best parameter for evaluating the efficacy of new anti-inflammatory agents in RA (Arvidsson *et al.*, 1998).

3.4 Patients with arthritis disease:

3.4.1 Latex (Rh.F.):

Depending on the results demonstrated in table (3.2) and Figure (3.4), from (80) patients select (20) patients who have (positive latex (Rh.F.) test) and out of (20) patients only (10) patients have shown (-ve) results of latex test after treatment with *N. sativa*, the significant difference is ($P<0.05$) as compared with that before treatment.

Table (3.2) Distribution of patients according to positive results of latex test (Rh.F.) before and after treatment with *N. sativa*.

Treatment with *Nigella sativa*	No. of patients	No. of patients		S.D. ±	C.S.
		+ve	%		
Before	20	20	100	0.44	(S)
After	20	10	50	0.41	

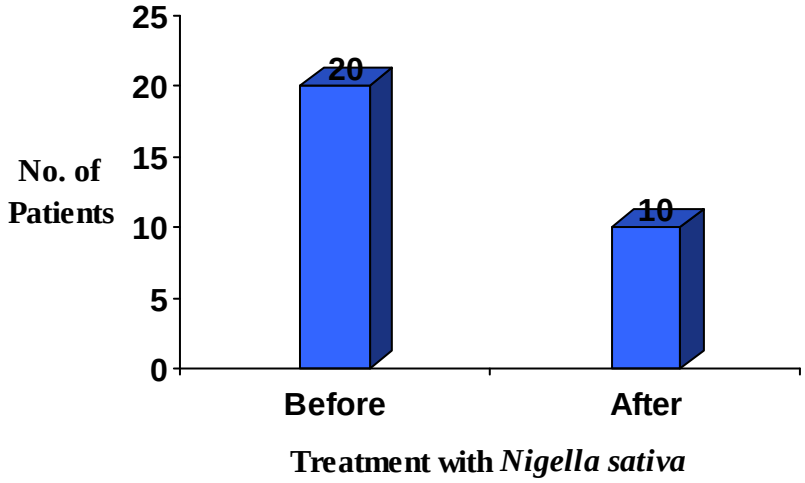

Figure (3.4) Distribution of patients according to positive results of latex test (Rh.F.) before and after treatment with *N. sativa*.

Certainly Rh.F. is one of the parameter used for diagnosis of RA (Tarborn, 1979). High levels of Rh.F. are associated with more severe rheumatoid disease. This factor is also associated with a higher tendency to develop non-joint manifestations of rheumatoid disease, such as rheumatoid nodules and rheumatoid lung disease (William, 1996). RA is an autoimmune disease, so-called because a person's

immune system, which normally helps protect the body from infection and disease. White blood cells, the agents of the immune system, travel to the synovium and cause inflammation (synovitis). During the inflammation process, the normally thin synovium becomes thick and makes the joint swollen and puffy to the touch (Michelson *et al*., 1994).

The activity of *N. sativa*, may be attributed to the (Linoleic and Linolenic acids) found in the seeds. These two compounds also were demonstrated in the treatment of RA and that's why *N. sativa* has been used extensively for the treatment of this disease (Bhikha, 1990).

Also long chains of fatty acids such as palmitic and stearic found in *N. sativa* have been reported to have anti-denaturant activity, so protect against protein denaturation which might result in beneficial effects in rheumatoid arthritis and other rheumatic conditions (Saso *et al*., 1999).

3.4.2 ASOT:

According to the results of our study, from (80) patients select (20) who have ASOT. Table (3.3) and Figure (3.5) showed that the mean conc. of ASOT in serum of patients after treatment with *N. sativa* was changed significantly ($P<0.05$) from (400 to 200 I.U/ml).

Table (3.3) Mean conc. of ASOT (I.U. /ml) in serum of patients before and after treatment with *N. sativa*

Treatment with *Nigella sativa*	**No. of patients**	**Mean conc. Of ASOT (I.U./ml)**	**S.D. ±**	**C.S.**
Before	20	400	149.32	(S)
After	20	200	231.67	
Mean normal value of ASOT		200	231.67	

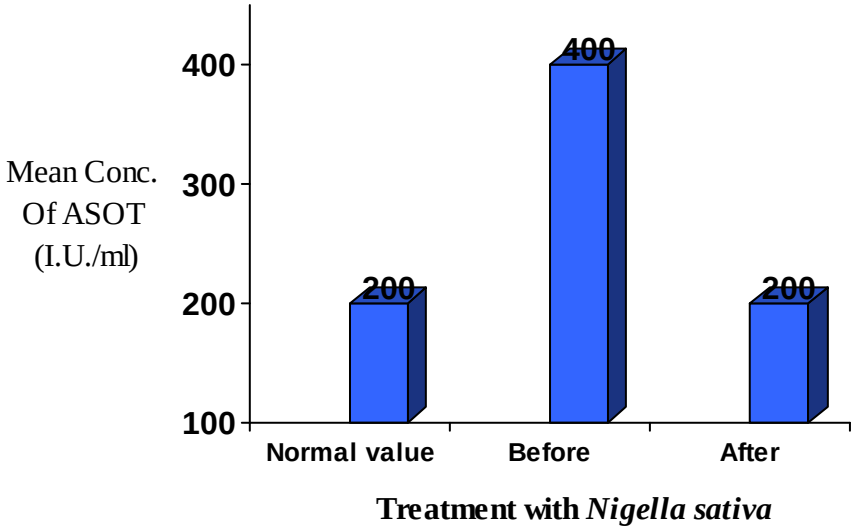

Figure (3.5): Mean conc. of ASOT (I.U. /ml) in serum of patients before and after treatment with *N. sativa*

The carbonyl and phenol components present in the black seed are responsible for the antiseptic and anti-microbial properties of the seed. Extensive microbiological tests have confirmed its action

against gram-positive as well as gram- negative organisms (Bhikha, 1990).

Streptolysin O is a toxic immunogenic exoenzyme produced by β-hemolytic streptococci (+ve) bacteria of groups A, C, G. The effect of a *N. sativa* could be attributed to the thymohydroquinone found in it which has high activity against gram-positive bacteria (EL-Fatatry, 1975).

3.4.3 Anti-ds-DNA:

Table (3.4) Mean conc. of Anti-ds-DNA (I.U. /ml) in serum of patients before and after treatment with *N .sativa*.

Treatment with *Nigella sativa*	**No. of patients**	**Mean conc. of Anti-ds-DNA Abs (I.U./ml)**	**S.D. ±**	**C.S.**
Before	20	20	7.05	(HS)
After	20	10	7.34	
Mean normal value of Anti-ds-DNA		10	7.34	

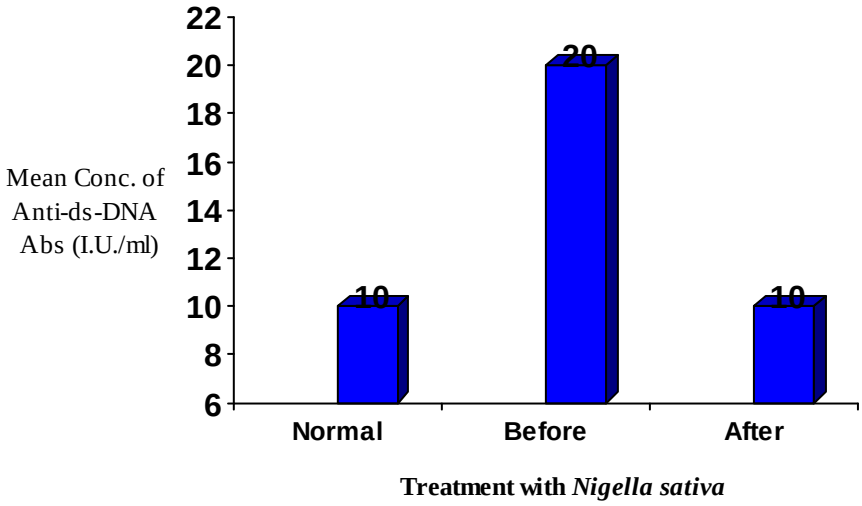

Figure (3.6) Mean conc. of Anti-ds-DNA (I.U. /ml) in serum of patients before and after treatment with *N. sativa*

Data demonstrated in table (3.4) and figure (3.6), select (20) patients have Anti-ds- DNA from (80) patients, showed that the mean conc. of Anti-ds- DNA in serum of patients after treatment with *N. sativa* was decreased from (20 to 10 I.U. /ml) with highly significant difference ($P<0.01$).

Anti-ds-DNA is one of parameters used for diagnosis of SLE. SLE is an autoimmune disease, which causes multi-system inflammation. Musculoskeletal system is one of the systems that could be affected by SLE via the inflammation of muscles tendons and joints (Blech and Zurier, 1995).

These results confirmed previous studies which reported that the administration of (1g) of black seed twice daily in human volunteers enhanced immune functions as manifested by improved helper T-cell (T4) to suppressor T-cell (T8) ratio and improved natural killer cell

activity. However, there was a decrease in the immunoglobulin (IgA, IgG, and IgM) levels. These immunoglobulins are parameters established in immune system study (El-Kadi and Kandil, 1986).

(Haq *et al*., 1995) noticed that *N. sativa* enhanced the production of interleukin-3 by human lymphocytes when cultured with pooled allogenic cells or without any added stimulator. They also observed an increase in interlukin-1 beta (IL-β) suggesting that *N. sativa* has an effect on macrophages as well.

Interlukin-1 is a biological response by the immune system to inflammation and it is considered an important part of the promotion and perpetuation of RA pathogenesis (Klippel, 2001).When part of the body become inflamed the immune system responds by producing (IL-1). The IL-1 is a specific inflammatory mediator that is produced in areas of inflammation. Because of RA inflammation, production of IL-1 is increased beyond normal levels (Klippel, 2001).

3.5 Patients with gout disease

3.5.1 Uric acid:

Data represented in table (3.5) and figure (3.7), (20) patients which have high level of uric acid were selected from (80) patients, showed that the mean conc. of uric acid in serum of patients was decreased to (5mg/dl with a highly significant difference ($P<0.01$) after treatment with *N. sativa* when compared with its mean conc. in

serum of patients before treatment and its mean normal value (7 and 5 mg/dl) respectively.

Table (3.5) Mean conc. of uric acid (mg/dl) in serum of patients before and after treatment with *N. sativa*.

Treatment with *Nigella sativa*	**No. of patients**	**Mean conc. of uric acid (mg/dl)**	**S.D. ±**	**C.S.**
Before	20	7	1.43	(HS)
After	20	5	1.30	
Mean normal value of Uric acid		5	1.30	

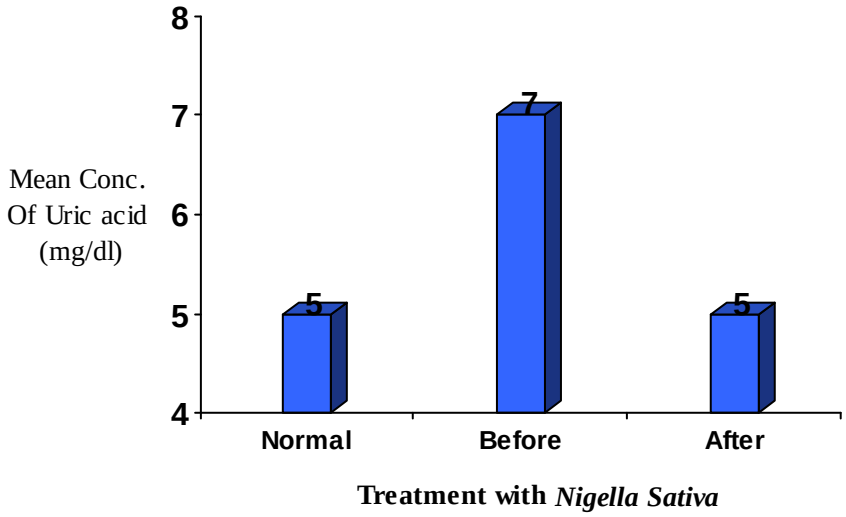

Figure (3.7) Mean conc. of uric acid (mg/dl) in serum of patients before and after treatment with *N. sativa*.

Different active compounds are considered as drugs affecting uric acid conc. in blood such as fish oil, primrose oil, and flavnoid (Situnayake *et a*l., 1991).

Effects noticed in our experimental work may be attributed to thymoquinone, thymohydroquinine, and polythymoquinone found in black seed. All these data were in agreement with (El-Dakhakhany, 1965) in his work on the effect of black seed on uricsuric activity in male rats.

The cause of hyperuricemia is an overproduction of uric acid relative to the patient's ability to excrete it. Most therapeutic strategies for gout involve lowering the uric acid level below the saturation point, thus preventing the deposition of urate crystals. As a result the elimination of uric acid increased and so these drugs can relieve the pain of gout. This can be accomplished by:

1. Interfering of uric acid synthesis with *allopurinol.*
2. Increasing uric acid excretion with *probenecid* or *sulfinpyrazone*.
3. Inhibiting leucocytes entry into the affected joint with *colchicines* (Mary *et al.*, 2000 b).

Chapter one

Introduction & Literature Review

Chapter Two

Materials & Methods

Chapter Three

Results & Discussion

Appendices

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Conclusions & Recommendations

Conclusions:

1. Black seed could be considered as analgesic, anti-inflammatory, anti-microbial and as an immunity stimulant.
2. *Nigella sativa* and its components may be used as a natural remedy for many ailments.
3. *Nigella sativa* powder have an anti-inflammatory activity and that's why they could be used with successful results in rheumatoid arthritis.

Recommendations:

1. Isolation, identification and characterization of the main active compounds responsible for the effect of black seed as drug.
2. Further experimental work is needed on the accurate dose of black seed used to treat arthritis disease.
3. Further study on different pharmacological effects of black seed.

Examining Committee Certification

We the examining committee certifies that we have read this thesis and examined the student in its contents and that according to our opining is accept as a thesis for the degree of Master of Science in Biotechnology.

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

تأثيرات الحبة السوداء في امراض
التهاب المفاصل و داء النقرس

رسالة
مقدمة الى كلية العلوم في جامعة النهرين كجزء من متطلبات نيل
درجة ماجستير علوم في التقانة الإحيائية

من قبل
نورا فخري سعيد القطان
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جمادي الثاني ١٤٢٧
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Effect of *Nigella sativa* (Black Seed) *On* Arthritis & Gout diseases

A Thesis
Submitted to the College of Science of
Al-Nahrain University in Partial Fulfillment of the
Requirements for the Degree of Master of Science
in Biotechnology

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Summary

This study was planned to evaluate the effect of black seed *(N. sativa)* powder (1g) on Arthritis and Gout diseases in 80 Iraqi patients using (Latex, ASOT, Anti-ds-DNA, Uric acid and ESR) as parameters in the serum and peripheral blood. The results are summarized as following:

1- The highest percentage of patients was belonging to the group with age range (40-54 years) it was (46.25%). Also the percentage of female patients was higher than that of male patients; it was (60% and 40%), respectively.

2- Positive results were obtained under the effect of treatment with black seed, data showed that the mean level of ESR in peripheral blood of patients after treatment with *N. sativa* was (15 mm/1hr.West.) with significant decrease ($P<0.05$) when compared with its mean level in peripheral blood of patients before treatment (26mm/1hr.West.).

3- The results also indicated, from (80) patients select (20) patients who have (positive latex (Rh.F.) test) and out of (20) patients, only (10) patients have (-ve) results of Latex test in serum of patients after treatment with *N. sativa*. A significant difference of ($P<0.05$) was estimated.

4- There was a significant decrease ($P<0.05$) in the mean concentration (conc.) of ASOT in patients serum under the effect of treatment with *N. sativa*. The value decreased from (400 I.U. /ml) to (200 I.U. /ml).

5- Results indicated that the mean conc. of Anti-ds- DNA in serum of patients after treatment with *N. sativa* was changed from (20 to 10 I.U./ml) with highly significant decrease ($P<0.01$).

6- The mean conc. of uric acid in serum of patients was decreased from (7 to 5 mg/dl) after treatment with *N. sativa* with highly significant differences ($P<0.01$).

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List of Abbreviations

Code	Word
ANA's	Anti- nuclear antibodies
ASOT	Anti-Streptolysine O Titer
Conc.	Concentration
Dl	Deci liter
DM	Dermato myositis
Ds DNA	Double stranded DNA
D.W.	Distilled water
EAC	Ehrilch ascites carcinoma
ENA's	Extractable Nuclear Antigens
ESR	Erythrocyte Sedimentation Rate
Hr	Hour
Ig	Immunoglobulin
IL	Interleukin
MCTD	Mixed connective tissue disease
Min	Minutes
ml	Milliliter
NK cell	Natural killer cell
OA	Osteoarthritis
PM	Poly myositis
PSS	Primary systemic sclerosis
RA	Rheumatoid Arthritis
Rh.Fs	Rheumatoid Factors
RPM	Revolution per minute
Sec	Second
SLE	Systemic Lupus Erythematosus
SS	Sjorgen's Syndrome
TB	Tuberculosis
TBHP	Tert-butyl hydroperoxide
TNF-α	Tumor necrosis factor-alpha
TSC	Tri sodium citrate
µl	Micro liter
WEST	Westergreen

Supervisor Certification

We certify that this thesis was prepared under our supervision in Al-Nahrian University, College of Science as a partial fulfillment of the requirement for the degree of Master of Science in Biotechnology.

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Supervisor: Dr.Nabel E. Waheda
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Date:

In view of the available recommendations, I forward this thesis for debate by the Examining Committee.

Signature:
Name: Dr.Nabel Al-Ani
Date:

Head of Biotechnology Department

الإهداء

إ لى الذي يعلم تفاصيل الأمور ، ودقائق الإشياء و خفايا الضمائر

و النفوس ... ربي

إلى الحبيب الذي ينزف دما لأجلي .... و طني

من غرز في نفسي العراقة .... أ بي وأمي

الى من ساندني من الألف الى الياء .... زوجي رامي

الى من علمني حرفا فملكني عبدا .... أساتذتي

الى من سارو معي الدرب .... أخوتي

أهدي جهدي المتواضع

نورا

الخلاصة

أجريت هذه الدراسه لبيان تأثيرات الحبة السوداء في أمراض التهاب المفاصل و داء النقرس على ٨٠ مريض بإستخدام الاختبارات التالية:
(Latex, ASOT, Anti-ds DNA, Uric acid, & ESR) في مصل الدم وخلايا الدم المحيطية قبل و بعد العلاج بالحبة السوداء. و يمكن تلخيص النتائج بما يأتي:

١- اظهرت النتائج ان اعلى نسبة عدد للمرضى كان ضمن الفئة العمرية (٤٠ – ٤٥ سنة) حيث بلغت (٤٦,٢٥%). و كان عدد المرضى الأناث اعلى من المرضى الذكور (٦٠% و٤٠%) على التوالي.

٢- حصلت التأثيرات الأيجابيه نتيجة أستخدام بذور الحبه السوداء و اوضحت البيانات ان معدل مستوى ESR في خلايا الدم المحيطيه للمرضى بعد العلاج بالحبة السوداء كانت اقل (١٥mm/1hr.West.) مع فارق معنوي (P<0.05) مقارنة مع معدل مستوى ESR قبل العلاج (٢٦mm/1hr.West.) ومع المعدل الطبيعي.

٣- كما تبين ان من اصل (٨٠) مريض تم إختيار (٢٠) مريض نتائجهم موجبة لتحاليل الأختبار Latex في مصل الدم قبل العلاج بالحبة السوداء، مقارنة مع النتائج بعد العلاج فقط (١٠) مرضى نتائجهم سالبة و بفارق معنوي (P<0.05).

٤- اوضحت البيانات ان معدل تركيز ASOT في مصل الدم للمرضى بعد العلاج بالحبة السوداء كانت بفارق معنوي (P< 0.05) حيث انخفضت من (٤٠٠ I.U./ml) قبل العلاج الى (٢٠٠ I.U./ml) بعد العلاج.

٥- كما تبين ان معدل تركيز Anti-ds-DNA في مصل الدم للمرضى بعد العلاج بالحبة السوداء كان ذا فرق معنوي عالي (P<0.01) حيث انخفض من (20 الى 10 I.U./ml).

٦- اما تركيز Uric acid فقد تأثر بالمعالجة بالحبة السوداء حيث انخفض من (7 الى 5 mg/dl) و بفرق معنوي عالي (P<0.01).

بسم الله الرحمن الرحيم

وَآيَةٌ لَّهُمُ الْأَرْضُ الْمَيْتَةُ أَحْيَيْنَاهَا وَأَخْرَجْنَا مِنْهَا حَبًّا فَمِنْهُ يَأْكُلُونَ (٣٣) وَجَعَلْنَا فِيهَا جَنَّاتٍ مِن نَّخِيلٍ وَ أَعْنَابٍ وَ فَجَّرْنَا فِيهَا مِنْ الْعُيُونِ (٣٤) ِليأكُلُوا مِن ثَمَرِهِ وَمَا عَمِلَتْهُ أَيْدِيهِمْ أَفَلَا يَشْكُرُونَ (٣٥) سُبْحَانَ الَّذِي خَلَقَ الْأَزْوَاجَ كُلَّهَا مِمَّا تُنبِتُ الْأَرْضُ وَ مِنْ أَنفُسِهِمْ وَ مِمَّا لَا يَعْلَمُونَ (٣٦).

صدق الله العظيم

سورة يس، الاية(٣٣-٣٦)

In the name of Allah, Most Gracious, Most Merciful

(33) A Sign for them is the earth that is dead: We do give it life, and produce grain there from, of which ye do eat (34) And we produce therein Orchards with date-palms and vines, and we cause springs to gush forth therein (35) That they may enjoy the fruits of this (artistry) it was not their hands that made this will they not then give thanks (36) Glory to God, who created In pairs all things that the earth produces, as well as their own (human) kind and (other) things of which they have no knowledge.

Allah Almighty has spoken the truth.

Sora Ya- Sin (33- 36)