



EFFECTIVENESS OF *MATRICARIA CHMOMILLA* FLOWER EXTRACTS WITH *BACILLUS SUBTILIS*, *PSEUDOMONAS FLUORESCENS*, IN THE BIOLOGICAL CRITERIA OF THE BLOOD OF LABORATORY MALE RATS

Yousef Dakhil Rashed* and Najhah Mohammed Bari Ahmed

Technical College Al-Mussaib, Al-Furat Al-Awsat Technical University, Iraq.

Abstract

This study was conducted at the medicinal plants field and the animal house of Collage of Pharmacy / Karbala University. The aim was to investigate the effects of bio fertilization with *Bacillus subtilis*, *Pseudomonas fluoresceus* and *Bacillus subtilis* + *Pseudomonas fluoresceus* on the composition of active matte (Anti-Coagulant) inside the human body using alcoholic flower extracts, it is also aimed at asses the effects in some biochemical properties of rats males that was treated with these extracts.

Biochemical investigations showed that there were a significant decrease on the means of Albumin 2.01 gm / dicil compared to control that reached 3.69 gm / dicil for control treatment 1250 mg/kg. Results also showed that there was a highly significant increase on sugar and cholesterol concentration blood with alcoholic extract 90.06 mg / dicil, 51.62 mg / dicil respectively compared with control 130.00 mg / dicil, 71.23 mg / dicil respectively. On the other hand results also shown generally a significant decrease in the mean of calcium ion Ca^{++} in 1250 mg/kg dose 5.98 mg / dicil compared to control 10.99 mg/dicil.

Key words : *Matricaria chmomilla*, *Bacillus subtilis*, *Pseudomonas fluorescens*.

Introduction

Matricaria chamomile is an important medicinal plant known to humans since ancient times to treat many diseases. It has been isolated from many medicinal compounds such as phenols, flavonoids, turbocharged oils and calcicides, as well as high nutritional value for containing sugars and fats. Many of vitamins, aromatherapy and yellow lazulin (Darwish, 1983). Chamomile extract is an important drug commonly used as a treatment in many diseases. Qutb (1981) studies showed that *Matricaria chamomile* acts as an antioxidant and is beneficial in the prevention of skin cancer and that its cytotoxic effect has a inhibitory effect on the growth of the virus Polio virus and Herpis virus by inhibiting the cells that form the virus RNA. Bring *et al.*

(1995) recent research has found that chamomile extract contains Coumarin, which is similar to warfarin in preventing blood clotting and is associated with vitamin K, which is an important factor in blood clotting (Wingo *et al.*, 1995). Advanced biotechnologies are the addition of bio-fertilizers, which contribute to the processing of plants with a large number of nutrients such as metal elements and hormones to improve the quantity and quality of agricultural production (Abu Arqoub, 2000). To include the production of pharmaceutical and chemical compounds, the most important of which are antibiotics, energy hormones and bio-gas (Safiyazor *et al.*, 1995). *Bacillus subtilis* and *Pseudomomas fluorscens* were the leading field in this . for containing more of bio factor properties (AL-Mayah, 2001).

Due to the lack of studies in Iraq in the field of bio-fertilization of medicinal plants and their contents in blood

*Author of correspondence : E-mail : y-dr@tcm.edu.iq

qualities and treatment of blood diseases, this was followed by laboratory rats male, so the current study aimed at:

1. The effect of different concentrations of the chemical extract of the chamomile plant, which is biologically active in the bacterial species in the blood chemistry.
2. The effects of different doses of the extract of alcohol of the plant Chamomile oxidative biochemistry of the two types of bacteria in the qualities of the chemical vital blood.

Materials and Methods

Preparation of the extract of the flower of chamomile leaves in the two types of bacteria under study

The ethanolic extract was prepared according to Ladd *et al.* (1978). The weight of 10 g dry powder for chamomile flower was placed in the Soxhlet with a temperature of 54-40 m, with 200 mL of ethyl alcohol remaining 90% and the samples continued to be extracted for 24 hours. The process was repeated several times to obtain sufficient quantities for experimental purposes

Preparation of laboratory animals

The rats male of the Sprague-Dawley strain were obtained from the Drug Control Center, Baghdad. The animals in the research stages were subjected to similar laboratory conditions in terms of nutrition, ventilation, light and temperature.

Determination of lethal Dose LD₅₀ by subcutaneous Injection

In this study, isolated 16 rat were divided into four groups. Under the skin, one of the following doses of bio-fertilization extracts was studied, by dissolving a quantity of extract in the normal saline solution of the doses 5000, 6000, 7000, 8000, 9000, 10000 mg/kg body weight and then calculated the LD₅₀ (Karber, Behrens, 1953).

Study of the biochemical characteristics of the blood:

2ml of blood was withdrawn in a clean test tube placed in Centrifuge. The following tests were performed:

Estimation of the albumin concentration in the serum

The albumin concentration in the serum was calculated using the green bromo-cresol detector.

Bromocresol green reagent (Silverman *et al.*, 1970).

The serum albumin was estimated using three sterile and dry tubes for each sample of the serum samples. 3 ml of the bromocrystalline reagent was added to each

tube and 0.01 ml of distilled water was added to the first tube. It was used to emit the optical spectrometer (630 nm). Transfer the solution from the first test tube to the tube of the device and place the latter inside the device to extract the amount of absorption of the serum sample of light (A sample) and repeat the same process for the second test tube to extract. The amount of absorption of albumin standard solution. Then apply the following equation:

$$\text{Albumin Concentration (g / dl)} = A \text{ sample} / A \text{ standard} \times \text{standard concentration}$$

Standard concentration is the standard albumin concentration (4.5 g/dl) (Khafaji *et al.*, 2002).

Estimation of the glucose concentration in the serum

The concentration of glucose in the serum was enzymatically determined using GOD (Titeiz, 1982). Three test tubes were taken for each sample of the serum samples and each 2 ml of the reagent was added. The first tube (0.02) ml was added to the serum of the sample to be determined by the concentration of glucose in which (0.02) ml of the standard glucose solution was added to The third tube (the distillation tube) was injected with 0.02 ml of distilled water. The three tubes were well mixed and incubated at a temperature of 37°C for 5 minutes. The spectrometer was first filtered with distilled water and then by the yellowing tube., then transfer the solution from the first test tube to the tube of the spectrometer , and put the last into the (A sample). This process was repeated for the second test to extract the absorbance of the standard glucose solution. By applying the following equation, serum glucose concentration was extracted. Glucose concentration (mg / dl) = A sample / A standard × N

Where, N is the standard glucose concentration and equal to 100mg / dl.

Estimation of the total cholesterol in the serum (R: Chmond, 1973)

Serum concentration was determined using the biomerieay sa-france reagent (Richmond, 1993).

Three test tubes were taken for each sample of serum samples and each of 2 ml of the reagent was added to the first tube (0.02) ml of the sample serum to calculate the cholesterol concentration. In addition, (0.02) mL of the standard cholesterol solution was added to the second tube and the third tube (the yellowing tube) was added to it (0.02) ml of distilled water. The three tubes were well mixed and incubated at 37°C for 5 minutes. The spectrometer was then filtered with distilled water first

and then by the yellowing tube again at a wavelength of 500nm then the solution transfer from the first test tube to the tube of the spectrometer and put the last into the device Extract the amount of absorbance of the blood sample separation this process and repeated the second tube to the standard solution to extract the amount of cholesterol absorbance (A standard) or extract the concentration of cholesterol in the blood sample serum according to the following equation:

Cholesterol concentration (mg / dl) = A sample / A standard \times N

Where, N represents the concentration of standard cholesterol and is equal to 200 mg / dl.

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Estimation of the Calcium Concentration in the Serum

Add 1 ml of the RI reagent to a test tube and add 0.2 mL of the sample serum. Add 1 mL of the R2 detector and leave for 5 minutes at room temperature. The spectrometer is measured in the spectral wavelength spectrometer (570nm). The resulting reading was hit at a standard concentration of 10mg / dl which is the concentration of the calcium ion of the sample serum (Stern, 1957) (14 mg/dl).

Ethomolamin = RI reagent at 500 mmol / L concentration.

R₂ = 0 - Cresolphtaslein at 0.62 mmol/L concentration.

Hydroxi / quinolone at a concentration of 69 mmol / L

Statistical analysis

Random Design used random design as a C.R.D in the design of laboratory experiments and the design of the complete random sampling of field tests for CBRD. The least significant difference was obtained from LSD to ascertain the significance of the differences between the different parameters at the probability level 0.05 (Alrawy Khalafallah, 2000) The results were analyzed using the Statistical Analysis System (SAS).

Results and Discussion

The effect of the type of biological extract was evident in the concentration of albumin, where the ratio of biological fertilization (B. + Ps.) was the lowest with 2.59 g / dl which did not differ significantly from Ps. And B. and control (2.88, 2.67, and 3.69), respectively.

The results of blood glucose concentration showed the ability of all extracts to reduce sugar. The biological treatment of B + Ps was the lowest in this area, with blood sugar reaching 110.40 mg/dl while the effect of PS extract was almost equal. (B + Ps) showed a significant reduction of 59.01 mg / dl (11.85 mg/dl) and 118.99 mg / dl, respectively. The bio-fertilization extracts had an effective role in reducing cholesterol concentration. Compare with Ps. B and C treated with 63.91, 62.66 and 71.23 mg/dL, respectively.

The results of the interaction between the extracts and their doses showed that the tested extracts at the dose of 1250 mg/kg reduced the albumin ratio from 3.81 g / dl (control treatment) to 2.01 g / dl for the biological fertilization treatment (B. + Ps). For Ps. (2.15 and 2.35 g / dl respectively), while the effect of all extracts at 750 and 1000 mg / kg was compared. When reviewing the results of the effect of the extracts with different doses in the concentration of glucose, (B + Ps) at 1250 mg / kg was the most able to reduce the glucose content to 95.43 mg/dL compared to the control treatment of 13.11 mg/dl.

All bio extracts at 750 mg/kg were less efficient in lowering blood sugar levels.

As for the effect of bio-fertilization extracts and their different doses in reducing cholesterol levels, the effect was evident at the dose of 1250 mg / kg and the treatment of B. + Ps. Compared with the control treatment of 73.23

Table 1 : The LD₅₀ dose of the *Matricaria chamomile* extract for chamomile flowers is under study.

Dosage mg/kg	Total number of animals	Number of dead animals	A	b	C
5000	8	—	—		
6000	8	—	1000		
7000	8	—	1000		
8000	8	—	1000		
9000	8	1	1000	0	500
10000	8	4	1000	2.5	2500
11000	8	8	1000	6.0	6000

As:

a = Differences between potions.

b = Average number of animals killed between the first and second group

n = Average number of animals per group

LD₅₀ = Biggest dose – $\Sigma (a \times b) / n$

LD50 = 9875 mg/kg

which was 6.60 mg/dL compared to the control treatment of 10.83 mg/dL.

And reached 118.507 mg/dl and 118.990 mg/dl respectively. The bio-fertilization extracts were instrumental in lowering blood cholesterol concentrations, with the treatment of the extract B + Ps. A significant decrease of 59.01 mg / dL compared with Ps. And B and C (63.91, 62.66, 71.23 mg / dL), respectively.

The results of the study showed that the extracts of *Matricaria camomilla* flowers treated with bio-fertilization of B and Ps. And B. + Ps. , led to a decrease in the concentration of the blood protein, as the disorder or disorder in the functions of the gastrointestinal tract is the result of the containment of *Matricaria camomilla* on phenol compounds primarily, including compounds that act as protein complexes that are difficult to digest or decompose and thus decrease in serum protein concentration. The effect of *Matricaria camomilla*

Table 2 : Effect of the type of bio-fertilization extracts on *B. subtilis* (*Ps Fluorescens*) and *B. Subtilis* + *P. Fluorescens* in some biochemical characteristics of blood.

Type studied / type of abstract	Calcium concentration rate of mg / dl	Concentrate of glucose mg / dl	Concentrate of glucose mg / dl	Concentration rate of albumin g / dl
Control	21.11±0.127	72.23±1.111	130.00±0.200	3.69±0.271
Ps treatment	8.88±1.195	63.91±7.945	118.50±11.999	2.88±0.700
B. treatment	9.11±3.101	62.66±8.538	118.990±9.871	2.67±0.667
Treatment Ps + B	10.00±1.993	59.01±8.401	110.40±11.644	2.59±0.210
L.S.D (0.05)	0.220	1.034	0.502	0.110

Control 3. Type of Concentrate / Type of Extract Percentage of albumin concentration g / dl Concentrate of glucose mg / dl
Concentrate of glucose mg / dl Calcium concentration / mg / dl Values: The mean represents the standard error.

Table 3 : Effect of biopreparation extracts of *B. subtilis*, *P. fluorescens*, and *B. subtilis* + *P. Fluorescens* in some biochemical traits of blood

Attributes/Dosage (Mg / kg)	Calcium concentration rate (Mg / dL)	Cholesterol concentration (Mg/dL)	Glucose concentration (Mg/dL)	Albumin concentration rate (G / dL)
Control	10.99±0.136	71.23±1.111	130.00±0.200	3.69±0.271
750	10.00±0.177	68.79±1.372	128.45±0.403	3.11±0.271
1000	6.661±0.164	58.72±0.710	111.90±2.829	2.32±0.652
1250	5.89±0.182	51.62±2.894	90.06±2.510	2.10±0.105
L.S.D.	0.337	1.062	1.633	0.116

Attributes / Dosage

Mg / kg concentration of albumin
Mg / dL Calcium concentration rate

G / dL glucose concentration
Mg / dL

Mg / dL cholesterol concentration rate
Control 3.69

mg / dl., while the effect is almost equal to the bio extracts and at doses 750 and 1000 mg/kg.

The results of the cross-sectional interaction of the type of extract and the dose showed a clear effect on the concentration of calcium at the dose of 1250 mg / kg and the treatment of biological fertilization with B + Ps.,

extracts may be due to the inhibition of liver enzymes responsible for the production of proteins in the blood, leading to a negative reflection of proteins of blood (AL-Hindawi *et al.*, 1989). *Matricaria camomilla* extract and certain concentrations were used with some topical creams to help stimulate the absorption of hydrocortisone

Table 4 : The effect of the interaction between the two types of extract and the dose of *B. subtilis* and *P. fluorescens* (*B. subtilis* + *P. fluorescens*) in some of the biochemical characteristics of the blood.

Dosage (mg/kg)	Concentration Calcium (Mg / dL)	Concentration Cholesterol (Mg/dL)	Concentration Glucose (Mg / dL)	Concentration albumin (G / dL)	Type of Abstract
Control	10.99±0.554	73.23±1.21	130.00±0.200	3.69±0.271	Fertilization <u>B.</u>
	10.83±0.551	73.23±1.21	130.00±0.200	3.69±0.271	Fertilization <u>Ps.</u>
	10.83±0.551	73.23±1.21	130.11±0.200	3.81±0.211	Fertilization <u>B. +Ps.</u>
750	11.50±0.371	72.23±0.92	120.66±0.920	3.12±0.415	Fertilization <u>B.</u>
	10.93±0.711	72.59±0.81	120.56±0.891	3.16±0.149	Fertilization <u>Ps.</u>
	11.22±0.513	69.57±0.71	121.06±0.751	3.14±0.0783	Fertilization <u>B. +Ps.</u>
1000	7.63±0.173	60.57±0.873	102.63±5.688	2.50±0.087	Fertilization <u>B.</u>
	7.55±0.124	61.66±1.139	110.52±3.105	2.68±0.071	Fertilization <u>Ps.</u>
	7.53±0.137	59.32±1.003	106.25±1.684	2.55±0.081	Fertilization <u>B. +Ps.</u>
1250	6.78±0.279	60.18±0.032	100.81±2.909	2.35±0.510	Fertilization <u>B.</u>
	6.67±0.315	54.60±1.987	98.62±3.120	2.15±0.163	Fertilization <u>Ps.</u>
	6.60±0.241	59.75±0.600	95.43±0.612	2.01±0.135	Fertilization <u>B. +Ps.</u>
L.S.D.	0.072	0.210	0.400	0.029	

Dosage mg / kg Type of extract albumin concentration

Mg / dl cholesterol concentration

Mg / dL Control fertilization B. 3.69 0.271 ± 130.00

G / dL glucose concentration

Mg / dL concentration of calcium

in the creams (Vanketel, 1987). Which leads to low protein level for its role in the destruction of proteins, including blood proteins and thus low blood level (Guyton et al., 1987).

High concentrations at the dose of 1250 mg/kg may play an important role in causing renal tubular abnormalities, leading to the release of albumin protein molecules in the urin, there by reducing the concentration of albumin and thus lowering serum protein concentration (Schilcher, 1997).

The effect of *Matricaria camomilla* extracts on the cellular level, a reduction in RNA or DNA as a mRNA messenger, may cause a reduction in the amount of proteins in the body or the effect of these extracts may be caused by a negative nitrogen balance, which further undermines proteins and converts them into amino acids. As a result, the amount of proteins in the bloodstream decreases (Guyton Hall, 1996).

The treatment with *Matricaria camomilla* extracts resulted in a significant decrease in sugar concentration, as the extract was a stimulating action for insulin-producing cells (beta cells in the islands of Langerhans). Which reduced blood sugar because this hormone is the main function is to help increase the permeability of cell membranes of the body of sugar molecules and increase the transmission of sugar in the blood into the cells (Asher

and Al-alwgi, 1989).

The decrease in blood sugar may also be due to the negative effects that chamomile extract may have on the process of making sugar from non-carbohydrate sources by gluconeogenesis, such as fatty acids and amino acids (Al-Wadi *et al.*, 1991). The use of ointments, Metabolic effect such as the substance of the body with the presence of the extract (Laurence *et al.*, 1997). The substance increases the efficiency of all enzymes to convert amino acids into sugar in liver cells. This increase is due to the activation of the cortex for the process of cloning the DNA in the nuclei of the liver cells, and then the formation of the rRNA responsible for sugar formation. From non-carbohydrate sources, and the use of bio-oxidant extracts may lead to Ps. And B. and B. + Ps. To increase the absorption of hydrocortisone, but the biological effectiveness in the stimulation of beta cells in the islands of Langerhans to produce insulin, so the continuous secretion of insulin by stimulating the components of the extract, although a little in the rate it may always provide adequate levels of insulin hormone in the blood, which in turn increases the permeability of membranes. The cells of the sugar molecules there by reducing its blood level (Asher and Al-alwgi, 1989). As for the low concentration of serum cholesterol in the use of bio-oxidant extracts is due to the effect of anti-oxidant

extracts stored in body tissues (Safahy, 1994 and Pycrofi, 2003).

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