

Biochemical and antibacterial activity of Jojoba Oil

'*Simmondsia chinensis*' in rats

By

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ABSTRACT

This study has evaluated the antibacterial effect of Jojoba oil (J.O) in vivo against *Staphylococcus aureus*. One hundred mature male Sprague Dawley rats were divided into four groups, including the control groups and the groups treated with J.O. as part of the diet mixture (2.5% of total body weight). Our findings suggest a significant increase in body weight gain (94.0 ± 5.404 g for J.O treated rats in comparison to 72.0 ± 3.077 g for untreated rats), final body weight (232.0 ± 7.277 g for J.O treated rats in comparison to 220.8 ± 9.71 g for untreated rats), and internal organs (0.31 ± 0.03 g, 0.802 ± 0.017 g and 0.160 ± 3.88 g for spleen, kidney and liver respectively in J.O treated rats in comparison to 0.268 ± 0.052 , 0.012 ± 0.786 g and 3.708 ± 0.125 g for spleen, kidney and liver respectively in untreated rats). On the other hand, rats infected with *S. aureus* and fed by ration mixed with J.O. showed insignificant increase in all studied parameters. Average body weight gain was 74.8 ± 3.673 g and internal organs weight were 0.055 ± 0.358 , 0.028 ± 0.826 and 4.124 ± 0.275 g for kidney, spleen and liver respectively. The positive control group showed acute septicemia. Blood samples were taken from diverse groups and were used for bacteriological tests and radial immune diffusion tests. Results have revealed that *S. aureus* was in pure form and identified biochemically.

Key words: Jojoba oil, antimicrobial activity, in vivo, *Staphylococcus aureus*.

INTRODUCTION

There are thousands of species of medicinal plants used globally for the cure of different infections (**Murgan,2012**). These plants are used as antimicrobial agents and several works has been carried out by scientists to find out its scientific basis.

Jojoba oil (*Simmondsia chinensis*) is a mixture of liquid esters with physical properties comparable to vegetable oil and contains certain quantities of sterols and different tocopherols (**El-Mallah et al,2009 and Tada et al, 2005**). It including Egypt and Saudi due to its high economic value (**Ashour et al,2013**). Alos, Jojoba seeds has been contain considerable amounts of tannins (**Wiseman ,1987 a&b**) and flavonoids which responsible for antibacterial activity.

The tested oil is used in many aspects including cosmetic material (**Tauguchi and Kunimoto, 1977 &1980 and Alexander, 1985**) and **Yaron,(1982)** who studied the effect of J.O. on cholesterol level in blood.

In recent years , many investigators initiating large screening efforts to find and use plant extract including vegetable oil, essential oils which have effect on nematodes and microbes (**Zanial et al,1994 and Hanan &Elham , 1997**).

In spite of many studies conducted on Jojoba especially on its agronomy **Fisher(1980)** , not many studies on the antimicrobial and other biologically active compounds of plant have been carried out except by **Maspero et al ,(1984), Zanial et al(1994) and Hanan et al (1998)**.

In view of the possible effect of J.O. on body weight , it must reduced the digestibility of Jojoba oil by changing the diet into iso-energetic (**Weber et al,1983**).

The aim of the present investigation is to study the effect of J.O. on body weight as well as the study of antibacterial activity in vivo and its immunological effect.

MATERIALS AND METHODS

Materials:

Jojoba oil: crude J.O. were obtained from National Oil Company.

Standard antibiotic solution: Amoxycillin 20% were obtained from Al-Arabia Pharmaceutical Comp. and used in dose 5.1 gm/ 10 L of water 3-5 days.

Laboratory animals:

Rats: Hundred mature male Spraguely Dawley rats of an average body weight of 130- 150 gm were used for the experiments .Rats were fed on standard ration and water supply was given ad-libitum.

Mice : mice were used for detection of pathogenicity of *Staphylococcus aureus* strain according to (Elaine et al,1991).

Detection of pathogenicity of tested organism: To insure the virulence of tested strain (*Staphylococcus aureus*) before induce in the infection in male rats , the virulence test, bactericidal assay were applied.

Virulence of *Staphylococcus aureus* in mice: Bacteria were grown over night in tryptic soya broth at 18°C to obtain broth culture of 1.5x10⁹ (C.F.U) using McFarland nephelometer . Ten fold dilution was made and 0.2 ml from the original inoculum and the dilution was used to inject mice I/P in groups , each of 5 mice. Mortality rate was recorded after 72 hrs (Liu, 1966).

Bactericidal assay: Using guidelines of Taylor (1983), 10 ul sample of a 24 hrs brain heart infusion broth culture was used to inoculate to 37°C , then incubated for 24 hrs at 37°C to produce bacteria, the bacterial suspension was centrifuged for 20 min.at 3000 xg and bacterial pellets were suspended in 10 ml gelatin veronal buffer containing 0.15 M CaCl₂ and Mg Cl₂ ,PH 7.4.

Reaction mixtures of 250 ul containing 25 ul of bacterial suspension (1x10⁷C.F.U./ml) by using McFarland nephelometer standards , and 225 ul of undiluted bovine serum were incubated at 37°C for 3 hrs.

50 ul samples collected initially at "0" and at the end of 6 hrs incubation were placed in 9 ml gelatin veronal buffer. Numbers of variable bacteria were determined by plating 10 fold dilutions of this sample on blood agar plate , after incubation the number of colonies was greater than that in "0"hr sample.

Experimental design: This was planned to study the effect of Jojoba Oil mixed with diet in ratio of 2.5% on body weight gain, final body weight ,weight of internal organs ,biochemical parameters in serum and measuring of some antioxidant enzymes in tissue. Also, to detect the antibacterial effect of Jojoba Oil in treatment of bacterial infection.

For this purpose , 100 mature male rats were divided into five equal groups of 20 rats each. The five groups were divided as follows:

Group1: Served as control negative

Group 2: Feed on basal diet and was challenged by virulent strain of *Staphylococcus aureus* and kept as control positive.

Group 3: Feed on basal diet mixed with J.O. 2.5 %

Group 4: Feed on basal diet and infected with virulent strain of *Staphylococcus aureus* and treated with amoxicillin 20%

Group 5: Feed on basal diet mixed with J.O. 2.5% and infected with virulent strain of *Staphylococcus aureus*

Bacteriological examination:

Re-I solation of the infective organism from all tested male rats (treated or not treated) by examination of blood samples collected from infected rats treated either by amoxicillin or J.O. and examined bacteriologically by culturing the all collected samples on blood agar and mannitol salt agar for the presence of infective organisms (*Staphylococcus aureus*). All Suspected growing colonies were studied morphologically and tested biochemically according to **Bailey and Scott (1990)** and *Staphylococcus aureus* organisms according to **Cruickshank et al (1975)**.

RID test:

Radial Immuno Diffusion (RID) is a technique used for the measuring the concentration of antigen according to (**Mancini and Vearman,1964**) through an antigen –antibody complex.

Characteristic changes:

Effect of Jojoba oil on body weight and biochemical analysis:

Rats were weighted weekly and at the end of the experiment (after 28 successive days). Two blood samples were collected one for bacterial examination and other left to clot and serum were separated for biochemical analysis. Estimation of serum total protein and electrophoretic pattern were carried out after **Sonnenwirth and Jaret(1980),Davis(1964)**. Catalyze activity ,lipid peroxidation as malonaldehyde (MDA) and reduced glutathione(GSH) in homogenate liver tissues were determined according to **Aebi(1974),Ohkawa et al (1979) and Ellman (1959)**.

Serum enzymatic activity as Aspartate aminotransferase (AST),Alanine aminotransferase (ALT) &Alkaline phosphatase (AP) were determined by the method described by **Reitman and Frankel (1957) and Roy (1970)**.Urea and creatinine

levels in serum were estimated as explained by **Wybenga et al (1971) and Faulkner and King (1976)**.

Statistical Analysis:

Parametric data were statistically analyzed using analysis of variance (ANOVA) test and comparative means were performed according to Duncan Multiple Range test for comparison of means according to **Snedecor (1982) using SPSS 14 (2006)**.

RESULTS

Effect of Jojoba oil on body weight and biochemical analysis:

The results tabulated in Table (1- 5)

Feeding on ration mixed with 2.5% for 28 successive days to rats showed a significant decrease in AST activity in serum (Table 3) , while infected rats fed on ration mixed with J.O(2.5%) showed insignificant increase in all studied parameters. On other hand infected rats(C+ ve) showed significant increase of AST and ALT.

Significantly increase in body weight gain ,final body weight and weight of internal organs in healthy and infected rats fed on basal ration mixed with J.O. (2.5%),while insignificant changes in food conversion when compared with other groups (Table 1-2) .

Total protein ,albumin and globulin in serum of rats feed on J.O. are presented in Table(4).Our results showed significantly decreased of total protein and A/G ratio in healthy and infected rats feed on J.O. when compared with control +ve group.

The group infected with *Staphylococcus aureus* significantly increase lipid peroxide while glutathione reductase showed significant decrease compared with control –ve group (Table 5).

Anti bacterial activity of J.O:

In the control positive group ,rats showing acute septicemia and death in some cases ,the other showing symptoms of infection . All collected samples from dead and diseased cases revealed the isolation of *Staphylococcus aureus* organism in pure form and identified biochemically.

On other hand, the infected rats and fed on J.O. showed no symptoms and the bacteriological examination of samples showed isolation of *Staphylococcus aureus* in a total colony count less than 10^4 which can not lead to any diseased condition.

The results of RID test:

All samples collected from infected rats fed on J.O. showing no zone of inhibition in agarose gel diffusion if compared with control samples which indicate the absence of *Staphylococcus aureus* organism in serum of infected rats treated with J.O. The same observation was noticed with the infected rats treated with amoxicillin. On the other hand, a detectable zone of inhibition was observed with the samples of infected non-treated rats.

DISCUSSION

Simmondsia chinensis, better known as Jojob oil is a hardy shrub that grows in arid regions of northern Mexico and south western USA (Farkas,1979). However, this plant has also been grown in south Asia and north Africa, especially in Thailand, Malaysia, United Arab Emirates, Saudi Arabia and Egypt. (Hani et al,2014 and Uthman et al,2002).

In spite of many studies conducted on J.O. especially on its agronomy (Belsby,1982 and Hogan and Palzkill,1982) not many studies on its anti-microbial and other biological active compounds of the plant have been carried out except by Ferial and Hayam, 2014, Osiris et al,2013, Hanan & Elham,1997 and Zaniel et al,1994). who mentioned that J.O. effective against some of the common bacteria, the second principle concerning the pathogenicity of the bacteria used and this point from the practical view takes place to differentiate between different strains of bacteria which was also sensitive to J.O.

Second question of concern in rats growth especially that group challenged by the bacteria is whether the rats eat more because they eat more, in the beginning it must be mentioned that healthy and infected rats for 28 days were fed on ration mixed with J.O. was significantly increased in body weight gain, final body weight, weight of some internal organs when compared with other groups fed on basal ration, these results agree with (Susan et al, 2010, Cokelaere et al, 2000, Hanan et al,1998, Yaron et al,1982).

The present study showed an increase in the level of creatinine, urea and on the activities of AST, ALT and AP in healthy and infected groups fed on ration mixed with J.O. This results in agreement with Halawa et al (2007), Verschuen (1989) and Ckolaere et al (2000) and Hanan et al (1998).

Rats fed on J.O. significantly decreased total protein level, attributed to the presence of simmondsin and trypsin inhibitor in J.O. This may induce reduction of

food intake and a loss of essential amino acid and so decrease in the protein synthesis (Ckolaere et al ,1993a,Verbiscar et al,1980 and Booth et al, 1974).

Data concerning that albumin/globulin ratio (A/G) showed significantly decreased which agree with Sobhy et al,2003 and Ckolaere et al ,1993a).

The group of rats infected with tested bacteria significantly increase lipid peroxide while glutathione reductase showed significant decrease that in agreement with Hanan et al(2015)reported that treatment with J.O. ameliorated these biomarkers .This significant improvement of the glutathione level was noticed when compared with C+ve group. Thus, the observed normalization of GSH level and catalase activities following J.O. treatment could be that oil caused decline of LPO accompanied by an increase in the activities /level of catalase and GSH in liver.

Jojoba oil proved many valuable uses ,it has immune stimulant and growth promoters (Youssef,2005) , also Jojoba protein contains high amounts of most essential amino acids(Motawe, 2005).

Table 1: Feed intake and body weight of control and infected animals

Groups	Initial body weight/g	Final body weight/g	Body weight gain/ g	Feed intake (g/ period)
Gr1 (ve)	144 ± 2.341 ^a	220.8 ± 9.71 ^c	72.0 ± 3.077 ^d	5000
Gr 3(J.O 2.5%)	138.2 ± 2.127 ^a	232.2 ± 7.277 ^c	94.0 ± 5.403 ^e	4.133
Gr 3 (inf)	139 ± 2.91 ^a	200.0 ± 5.846 ^c	68.8 ± 2.31 ^d	3.086
Gr 4 (J.O 2.5%)+inf	138.8 ± 1.39 ^a	211.6 ± 3.38 ^c	74.8 ± 3.673 ^d	4.511
Gr5 (Amox)+inf	131.8 ± 3.33 ^b	219.2 ± 9.737 ^c	87.4 ± 6.85 ^d	3.910

Table 2: Internal organ weight of control and infected animals

Groups	Kidney gm/ 100gm b.wt	Spleen gm/ 100gm b.wt	Liver gm/ 100gm b.wt
Gr1 (ve)	0.786 ± 0.012 ^a	0.268 ± 0.052 ^b	3.88 ± 0.160 ^c
Gr2 (J.O 2.5%)	0.802 ± 0.017 ^a	0.31 ± 0.03 ^b	3.708 ± 0.125 ^c
Gr 3 (inf)	0.788 ± 0.03 ^a	0.256 ± 0.04 ^b	3.844 ± 0.211 ^c
Gr 4 (J.O 2.5%)+inf	0.826 ± 0.028 ^a	0.358 ± 0.055 ^b	4.124 ± 0.275 ^c
Gr5 (Amox)+inf	0.764 ± 0.05 ^a	0.31 ± 0.017 ^b	3.984 ± 0.215 ^c

Values represent mean ± SE

a,b,,.....value with different letters at the same column are sig .diff at $P \leq 0.05$

Table(3): Effect of J.O on some serum biochemical parameters of control and infected animals

Groups	AST (u/l)	ALT (u/l)	AP (u/l)	Urea (%)	Creatinine (mg/dl)
Gr1 (ve)	22.6 ± 0.75 ^a	10.2 ± 1.56 ^a	342.4 ± 2.87 ^a	34.0 ± 0.55 ^a	0.604 ± 0.015 ^a
Gr2 (J.O 2.5%)	37.6 ± 0.98 ^b	19.0 ± 0.95 ^b	290.2 ± 14.3 ^b	27.6 ± 0.98 ^b	0.58 ± 0.013 ^a
Gr 3 (inf)	31.2 ± 1.02 ^{bc}	14.2 ± 0.86 ^a	333.6 ± 7.76 ^a	40.6 ± 1.03 ^c	0.668 ± 0.00014 ^b
Gr4 (J.O 2.5%)+inf	15.2 ± 1.11 ^d	12.6 ± 0.51 ^a	245.0 ± 4.11 ^c	29.2 ± 0.58 ^{bd}	0.624 ± 0.03 ^{ab}
Gr5 Amox)+inf	26.0 ± 2.47 ^e	12.2 ± 1.02 ^a	255.0 ± 11.6 ^{ce}	28.6 ± 0.81 ^{bde}	0.536 ± 0.0006 ^c

Values represent mean ± SE

a,b,,.....value with different letters at the same column are sig .diff at $P \leq 0.05$

Table(4): Effect of J.O , B.S and AF on serum total protein pattern of control and infected male rats

Groups	T.P	T. Alb	A/G ratio	T. Glo
Gr1 (ve)	6.784±0.11 ^a	2.082±0.08 ^a	0.450±0.02 ^a	4.656±0.09 ^a
Gr2 (J.O 2.5%)	6.35±0.1 ^b	2.02±0.04 ^a	0.475±0.0005 ^a	4.264±0.09 ^b
Gr 3 (inf)	5.732±0.11 ^c	1.928±0.07 ^a	0.514±0.03 ^a	3.792±0.13 ^c
Gr4 (J.O 2.5%)+inf	6.086±0.1 ^d	2.04±0.03 ^a	0.502±0.001 ^{ab}	4.05±0.08 ^d
Gr5 (Amox)+inf	5.88±0.05 ^{ce}	1.712±0.06 ^b	0.412±0.02 ^{ac}	4.168±0.04 ^{de}

Values represent mean ± SE

a,b,,.....value with different letters at the same column are sig .diff at P ≤ 0.05

Table 5: Mean values (±SE) of liver Malondialdehyde and GHS values in control and infected male rats fed J.O (n=5)

Groups	Malondialdehyde (mM/100g)	GHS (µmol/mg protein)
Gr1 (ve)	6.78 ± 0.25 ^a	16.01 ± 1.28 ^a
Gr 2(J.O 2.5%)	5.6 ± 0.152 ^b	12.096 ± 0.597 ^b
Gr 3 (inf)	8.078 ± 0.3 ^c	8.078 ± 0.36 ^c
Gr 4 (J.O 2.5%)+inf	6.15 ± 0.18 ^a	6.128 ± 0.09 ^d
Gr5 (Amox)+inf	5.2 ± 0.36 ^{bd}	6.024 ± 0.07 ^{de}

Values represent mean ± SE

a,b,,.....value with different letters at the same column are sig .diff at P ≤ 0.05

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