STUDIES ON ANTIBACTERIAL ACTIVITY OF WITHANIA SOMNIFERA EXTRACT IN GUINEA PIGS EXPERIMENTALLY INFECTED WITH E COLI

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ABSTRACT

In the present study, we evaluated and tested the antibacterial activity of both aqueous and ethanolic extracts of Withania somnifera (root and leaves), against pathogenic E coli in vitro and in vivo. One hundred guinea pigs of 1-2 months old were divided into 5 equal groups each (20). Control (Gp.1) did receive neither viable bacteria nor treatment. Each animal from the other groups (Gp.2-5) was challenged with (1-2_108) viable E coli in 200-μL normal saline (0.9%) through IP route. GP-2 infected group treated with 200-μL saline IP and kept as positive control group. (Gp.3-4) are infected treated with Withania somnifera (ethanol root extract) with a dose 50 and 100mg/400gm BW respectively.Gp-5 infected treated group with cephoperazone antibiotic at dose 35mg/400gm BW. The treatment by drug or the extracted medicinal plant was started 72h post- infection for 7 successive days. Serum and whole blood sample were collected from all groups 7^{th} and 14^{th} days post treatment to evaluate some hematological and biochemical changes as well as immunomodulatory cytokines TNF-α .

Oral treatment of the plant extract caused significant benefit results in infected guinea pigs appeared in the correction of some hematological and biochemical parameters also try to suppressed inflammatory cytokine response represent in TNF-α.

From the present work, it could be concluded that Withania somnifera extract has potent antibacterial activity, this appear in the correction of hematological, biochemical and immunological results.

INTRODUCTION

In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter **(Modak et al., 2007)**. Research work on medicinal plants and exchange of information obtained will go a long way in scientific exploration of medicinal plants for the benefit of man and is likely to decrease dependence on imported drugs **(Veerappan et al., 2007)**. The chemical diversity, structural complexity, lack of substantial tox-

ic effects, and broad spectrum of antiviral and antibacterial activity of natural products, make them ideal candidates for new therapeutics **(Mukhtar et al., 2008)**.

Withania somnifera is an important medicinal plant, a small, woody shrub 60-200 cm high, in the Solanaceae family, which is described under many commen names such as Ginseng and Ashwagandha. It can be found growing in Africa, the Mediterranean, and India. The roots are the main portions of the plant used therapeutically. **(Fawzy 1983; Girdhari and Rana 2007)**. Withanolides are the major active constituents of Withania somnifera that are isolated from its root and leaves **(Mishra et al., 2000)**. Recently, the plant was investigated to possess effective against treatment of some bacterial infection and tested for serious antibacterial properties **(Arora et al., 2004; Owais et al., 2005 and Teixeira et al., 2006)**. Beside it uses as antibacterial product several reports have demonstrated immunomodulator and tumor activity of this extract **(Ziauddin et al., 1996; Dhuley, 1997; Davis and Kuttan, 2000)**.

Cefoperazone is a commonly used broad spectrum semi-synthetic third-generation cephalosporin with a potent bactericidal activity against a wide range of gram-positive and gram-negative bacteria **(Mehta et al., 2005)**. Moreover, differs from other beta-lactams in the extent to which it is excreted in the bile and the stability of its elimination pharmacokinetics in the presence of renal impairment **(Shhnizu , 1980)**.

The objective of the present work to evaluate antibacterial activity of Withania somnifera in vitro and in vivo in Guinea pigs experimentally infected with pathogenic E coli.

MATERIAL AND METHODS

2.1. Preparation of extracts from root and leaves of W somnifera:

Withania somnifera root and leaves part were collected in month from August to October (2007) from Faculty of Agriculture, Mansoura University. The samples were taxonomically identified at the Botany Department, Faculty of Science, Mansoura University. Plant materials were dried in the dark at room temperature ,powdered and extracted following published procedure **(Malik et al., 2007)**. Air-dried,coarsely ground plant samples (500 grams), were percolated four times with ethanol at room temperature, the combined extracts were filtered, and concentrated under reduced pressure in a thin film evaporator at 50±^{o}C. (Removing the solvent under pressure). Finally the extract was completely dried under vacuum in the desiccators, which contain anhydrous calcium chloride. While in case of aqueous extracts, dried and powdered plant samples were soaked in sterile distilled water with constant stirring from 3 to 4 times till exhaustion of plant materials. The suspensions were further filtered through whatman (NO. 1) filter paper. Then the filtrates were concentrated in vacuo using a rotary evaporator at 70^{o}C then completely dried under vacuum in the desiccators.

2.2. Laboratory animals used :

Guinea pigs of 1-2 month old were obtained from Helwan farm of laboratory Animals. Animals were acclimatized to the animal house conditions (12:12 h light and dark cycle) for a week in galvanized zinc plante

cages under strict hygienic conditions. The daily requirement of ascorbic acid (vitamin C) (50 mg/ liter of drinking water) was supplied allover the experiment according to **(Sarah and Maggie, 2003)**.

2.3. Preparation of the inoculum:

The test organism (E coli) was obtained from culture, from the Animal Health Research Institute Gieza.. Serial dilutions were prepared from the stock culture, 1 ml from each dilution was IP administered into the Guinea pigs and watched for symptoms post infection. The dilution that established infection in the Guinea pigs which showed the symptoms and not high enough to cause rapid mortality was used as infective dosage for the Guinea pigs (1-2_108) throughout the experiment.

2.4. Determination of antibacterial activity of the extract:

The antibacterial activity of the extracts (from leaves and root) by different solvent was determined by using disc diffusion method following published procedure **(Akinyemi, et al 2005)**. The test organisms were added separately to nutrient agar. A sterile paper disc previously soaked in measured quantity of the sample (50 mg/ml and 100mg/ml) was placed on plate containing solid bacterial medium. The plates were incubated aerobically at 37^{o}C for 24 hrs and then examined for zones of inhibition with a ruler compared by +ve control (antibiotic disc) and -ve control (disc containing only physiological saline).

2.5. Infection and treatment:

The experiment was conducted with 100 Guinea pigs of 1-2 month old. The animals were divided into 5 equal groups each (20). Control (Gp.1) did receive neither viable bacteria nor treatment. On the other hand, each animal from the other groups (Gp.2-5) was challenged with (1-2_108) viable E coli in 200 μL normal saline (0.9%) through IP route. GP-2 infected group treated with 200-μL saline IP and kept as positive control group. (Gp.3-4) are infected treated with Withania somnifera (ethanol root extract) with a dose 50 and 100mg/400gm BW respectively. Gp-5 infected treated group with cephalosporin antibiotic (cephoperazone) at dose 35mg/400gm BW. The drug and medicinal plants doses change according to lab animals' body weight, which was calculated according to **(Paget and Barnes, 1964)**. The treatment by drug or the extracted medicinal plant was started 72h post- infection for 7 successive days. Serum and whole blood sample were collected from all groups 7th and 14th days post treatment.

2.6. Assessment of bacterial infection in the vital organs:

To assess the efficacy of Withania somnifera plant extract treatment on the establishment of infection, three animals from each group were sacrificed on day 3 post infection and after end of the experiment, the different organs were aseptically removed and homogenized thoroughly in 5 ml sterile PBS, then reisolation of the organism on solid media to observe the number of viable bacteria **(Owais et al., 2005).**

2.7. Haematological tests:

Erythrocytes (RBCs) and white blood cell count (WBC), calculation of blood indices according to **(Feldman et al., 2000)**, packed cell volume (PCV) **(Barbra, 1988)** haemoglo-

bin (Hb) levels **(Drabkin, 1949)**, and differential leukocytic count **(Coles 1986)**.

2.8. Serum biochemical analysis:

Prepared frozen samples were used and analyzed for some serum analysis including ALT, AST, AP, urea, creatinine, glucose, cholesterol, total and direct bilirubin, total protein, albumin, globulin and A/G ratio were determined with semi-automatic spectrophotometer (BM-Germany 5010) using commercial test kits (Randox Co. UK.) according to enclosed pamphlet.

2.9. Immunological studies:

Tumor necrosis factor - α (TNF-α) was assayed by Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microplate (Bio-Source, Co. Belgium). The assay uses monoclonal anti-bodies (MAbs) directed against distinct epitopes of TNF-α according to **(Aukrust et al., 1994)**.

2.10. Statistical analysis:

Our results were analyzed by (ANOVA) using SPSS software statistical program (SPSS for windows (ver.15.00, USA). Two groups were significantly different if P value was statistically lower than 0.05.

3. RESULTS & DISCUSSION

Our result (table-1) demonstrates the potential efficacy of both leaves and root extracts (alcoholic and aqueous solvent) of Withania somnifera plant as remarkable antibacterial activity in vitro. Our result agrees with **(Arora et al., 2004; Owais et al., 2005)**, but the result of the study in the vitro revealed that not all parts and solvent used give the same activity against the bacteria. This suggests that the antibacterial compound of Withania somnifera is polar in nature or at least exists either in the form of salt or glycoside under physical condition **(Mazzanti et al., 2000; Elaskka et al., 1990)**.

Administration of Withania somnifera in our work moderate doses did not lead to unfavorable hematological changes (table-2). The toxic manifestations of aqueous leaf extracts of Withania somnifera were demonstrated by incubating human erythrocytes with varying amount of crude extract (0-2mg/ml) comparing by cholramphenicol antibiotics, and the data show no lysis effect of the plant extract **(He et al., 1994; Owais et al., 2005)**. In the same line W somnifera did not show any direct/indirect hemolytic activity against human erythrocytes in vitro **(Girish et al., 2006)**. At the same time, Hb concentration increased at 14 days post treatment comparing with control, and this result may agree with **(Ziauddin et al., 1996)** who reported significant increase in hemoglobin concentration in Ashwagandha-treated mice as compared with untreated animals.

Transient regenerative anemia appear at 7 days then disappear at 14 days post treatment (table-3), this result may be an evidence on the side effect of cefoperazone antibiotic at which cause hemolysis of the RBCs and gastrointestinal hemorrhage **(Kumar and Rao 1999)**, also it was summarized that several second and third generation cephalosporins have been associated with hypoprothrombinaemia and bleeding. **(Schentag et al., 1987)**.

We found that the erythron mass of the infected untreated group with E coli revealed increase in the total RBCs, Hb concentration and PCV% in most of the experimental periods, this may be an indication of occurrence of polycythemia beside the significant increase of albumin and urea which confirm the dehydration occurrence (**Kaneko et al., 1997**).

In spite that Withania somnifera extract potential increase the total WBC count (table-3) and increase the bone marrow cellularity of animals. Moreover Withania somnifera root when administered orally at 25, 50, 100 and 200mg/kg doses for 14 consecutive days, showed a marked increase in the cell number of polymorphonuclear leucocytes cells (**Kuttan 1996 and Khan et al., 2006**). The results in this experiment revealed insignificantly changes in TLC count and heterophils count comparing with the control, but significantly decreased compared by infected untreated one, this is may be an indication on the antibacterial effect of this extract and it shows evidence that the E. coli was prevented from establishing infection. **Christina et al., (2004)** recorded correction in the hematological parameter as significant decrease in WBCs while RBCs count was elevated in the cancer mice treated by root extract Withania somnifera and normal control group. These observations are suggestive of the protective effect of Withania somnifera.

It cleared a slight significant transient leucopenia then reversible in drug treated group than control., but the decrease in heterophils count were not significantly with the control, that it was reported that this drug and other beta-lactam antibiotics caused reversible neutropenia and transient eosinophilia. .Also the mechanism of action of the drug, bind to both to neutrophils and bacteria in an individual manner and modify the binding and function of opsonins (**Strom et al., 1999**). Increase in the TLC, heterophils count and monocytes in infected untreated group which is a signal that an infection has been established (**Ogundare and Onifade, 2009**).

In Withania somnifera extract treated groups, transient elevation in ALT level with rapidly return to normal (tables 4-5), this may be agree with the result assayed the hepatoprotective effect of this extract. **Udayakumar et al., (2009)** reported that oral administration of W somnifera extract for eight days to experimentally diabetic rats return the liver transaminase enzyme to their normal level. Also **Bhattacharya et al., (2000)** reported the hepatoprotective action of W somnifera against iron induced oxidative stress in rats after, 10 days of oral administration of these active principles, in graded doses (10, 20 and 50 mg/kg).

The AP is significantly increased in infected non treated groups, this may indicative about the septicemic effect of bacteria and establishment of bacterial infection, this result agree with (**Nagano et al., 1994**) who found biliary infection with obstructive jaundice was induced in the animals by injecting E coli into the common bile duct .

No significance changed in biochemical parameters in Withania somnifera treated groups. Withania somnifera is without having any serious toxicity or side effects known and

thus can be safely used in humans for acute and chronic treatment regime **(Kulkarni and Dhir 2008)**. Total plasma proteins were insignificant differ in E. coli and Withania somnifera in compare with control one (tables 4-5). E. coli infection caused hypoalbuminemia could be due to fall in the levels of albumin mRNA in response to infection parallel to a decrease in intrahepatic albumin synthesis due to liver damage. Also, infection can lead to increased catabolic rate and/or redistribution of albumin from plasma to interstitial compartment **(Benoit et al., 2000)**.

In addition, Transient elevations of the BUN and serum creatinine have been noted in patients during treatment with cefoperazone in dose from 43 to 75 mg/kg body weight, also cefoperazone showed a low degree of nephrotoxicity. However, there was a decrease in glomerular filtration rate, which was seen in two patients that treated for 3 weeks with a relatively high dose **(Trollfors et al., 1982)**.

TNF-α is amplify, and coordinate proinflammatory sign also, resulting in the synchronized expression of effectors molecules that mediate diverse aspects of innate immunity. TNF is capable of eliciting expression of chemokines and adhesion molecules and thus may be critical to the recruitment of neutrophils from the blood and has severe damage to the target organs **(Isogai et al., 1998; Joseph et al., 2001)**. The transcription factor NFkB regulates the expression of cytokines, chemokines, adhesion factors, and inducible pro inflammatory receptors. The abnormal activation of NFkB has established for a series of inflammatory diseases and cancer. Withania somnifera is a medicinal plant that is widely used for the treatment of various inflammatory disorders. The leave extract of Withania somnifera, potently inhibits NFkB activation by preventing the tumor necrosis factor. Results indicate that pure Withania somnifera extracts can be considered as a novel class of NFkB inhibitors, which hold promise as novel anti-inflammatory agents **Kaileh et al., (2007)**. The effect of Withania somnifera on cytokine by I.P administration of 20mg of methanol root extract for 5 days and 10 days, significantly increase in both IL-2 and IFN-α while significant decrease in TNF-α was observed. **(Davis and Kuttan, 1999)**. TNF-α was significantly increased in E. coli infected group comparing with control one (table-6). This result goes with **(Theodore, 2000)** who recorded the most marked acute-phase reactions in responses to E. coli are TNF-α responses in blood plasma. This result also in accordance with **(Zimecki et al., 2004)** who reported that I.V administration of lethal dose of E. coli leading to increase of TNF-α level.

From the above result we can concluded that Withania somnifera extract is potent antibacterial compound by in vitro methods and also as therapeutic in Guinea pig model of Escherichia coli infection. These appear in the correction of immunological, hematological and biochemical alteration caused as response of infection.

Table (1): The antibacterial activity of (leaves &root) of *W. somnifera* against *E.coli* was determined by disc diffusion method.

Extract		Zone of inhibition (in mm)			
		Leaves extract		Root extract	
		50mg/ml	100mg/ml	50mg/ml	100mg/ml
Aqueous	1	4mm	6mm	5mm	11mm
	2	5mm	5mm	6mm	10mm
	3	5mm	7mm	5mm	10mm
	4	4mm	5mm	5mm	9mm
Ethanol	1	5mm	8mm	8mm	13mm
	2	6mm	7mm	6mm	12mm
	3	5mm	8mm	6mm	12mm
	4	6mm	6mm	8mm	11mm

Table (2): Some Hematological Parameters (mean ±S.E)) 7th Day Post Treatment with *Withania somnifera* Extract and Cephoperazone in Guinea pigs Experimentally Infected with *E.coli.*

Groups	RBC 10^6/mL	Hb g/dL	PCV %	MCV fl	MCH pg	MCHC %	TLC 10^3 / mL	Hetrophil 10^3 / mL	Esino. 10^3 /mL	Baso. 10^3 / mL	Lymph. 10^3 / mL	Monocy. 10^3 / mL
1	4.71^{b} ±0.13	13.36^{b} ±.25	37.50^{b} ±.64	79.58^{b} ±1.37	28.34^{b} ±.28	35.62^{b} ±.33	8.31^{b} ±0.37	2.86^{bc} ±0.23	0.46 ±0.13	0.02 ±0.02	4.41 ^{a} ±0.54	0.49^{bc} ±0.10
2	5.26^{a} .±14	16.99^{a} ±.41	41.50^{a} ±.64	78.97^{b} ±2.17	32.13^{a} ±.87	40.94^{a} ±.40	11.94^{a} ±0.78	6.13^{a} ±0.38	0.33 ±0.11	0.11 ±0.02	4.29^{a} ±0.63	1.05^{a} ±0.11
3	4.62^{b} ±.14	12.84^{bc} ±.41	37.50^{b} ±.64	81.24^{b} ±2.26	27.77^{b} ±.48	34.28^{bc} ±1.27	6.65^{bc} ±0.42	2.44^{c} ±0.28	0.34 ±0.16	0.02 ±0.02	3.51^{ab} ±0.33	0.42^{bc} ±0.10
4	4.55^{b} .±13	13.29^{b} ±.43	37.75^{b} ±.62	82.95^{ab} ±1.7	29.16^{ab} ±.56	35.17^{b} ±.65	7.89^{b} ±0.21	3.65^{bc} ±0.53	0.58 ±0.14	0.02 ±0.02	3.21^{ab} ±0.21	0.35^{bc} ±0.10
5	4.11^{c} ±.18	11.79^{c} ±.60	36.50^{b} ±.6	88.99^{a} ±2.70	28.62^{b} ±.52	32.26^{c} ±1.34	6.18^{c} ±0.47	2.34^{c} ±0.28	0.44 ±0.02	0.15 ±0.02	2.96^{b} ±0.45	0.28^{c} ±0.02

The same column not followed by the same letter differ significantly ($p < 0.05$)

Table (3): Some Hematological Parameters (mean ±S.E) 14th Day Post Treatment with *Withania somnifera* Extract and Cephoperazone in Guinea pigs Experimentally Infected with *E.coli.*

Groups	RBC 10^6/mL	Hb g/dL	PCV %	MCV fl	MCH pg	MCHC %	TLC 10^3 / mL	Hetrophil 10^3 / mL	Esino. 10^3 /mL	Basophil 10^3 / mL	Lymph. 10^3 / mL	Moncy. 10^3 / mL
1	4.87^{bc} ±.09	13.47^{c} ±.18	39.25^{bc} ±.47	80.60 ±1.35	27.94 ±.69	34.65 ±.65	9.50^{b} ±0.43	3.66^{b} ±0.35	0.13 ±0.04	0.02^{b} ±0.02	4.96^{ab} ±0.40	0.68^{b} ±0.14
2	5.30^{a} ±.12	14.89^{a} ±.75	43.00^{a} ±1.29	82.34 ±1.77	28.47 ±.94	33.37 ±2.31	11.77^{a} ±0.81	6.21^{a} ±.58	0.43 ±0.14	00.00^{b} ±0.00	4.02^{bc} ±0.18	1.09^{a} ±0.15
3	4.91^{bc} ±.065	13.62^{bc} ±.45	40.00^{b} ±.81	81.40 ±1.62	27.69 ±.57	34.07 ±1.11	8.87^{b} ±1.27	4.55^{b} ±0.53	0.24 ±0.24	0.13^{a} ±0.02	3.69^{c} ±0.55	0.23^{c} ±0.13
4	5.12^{ab} ±.084	14.97^{a} ±.35	41.00^{ab} ±.57	79.85 ±.28	29.14 ±.20	36.51 ±.37	9.28^{b} ±0.32	3.52^{b} ±0.35	0.27 ±0.09	00.00^{b} ±0.00	5.10^{a} ±0.02	0.36^{bc} ±0.13
5	4.65^{c} ±.062	12.94^{c} ±.41	37.75^{c} ±1.03	81.04 ±1.17	27.78 ±.62	34.28 ±.52	8.88^{b} ±0.29	3.86^{b} ±0.51	0.14 ±0.05	00.00^{b} ±0.00	4.41^{abc} ±0.32	0.47^{bc} ±0.14

Table (4): Some Serum Biochemical Profiles (Mean ± S.E), 7th Day Post Treatment with *Withania somnifera* and Cephoperazone Extract in Guinea pigs Experimentally Infected with *E.coli*

Groups	ALT U/L	AST U/L	AP U/L	T. Bili. mg/dl	D.Bil. mg/dl	I. Bil. mg/dl	T. P. gm/dl	Alb. gm/dl	Glob. gm/dl	A/G ratio	Chole. mg /dl	Glu. mg/dl	Urea mg/dl	Creat mg/dl
1	14.25^{c} ±1.7	31.5^{c} ±1.92	19.25 ±1.49	0.39 ±0.013	0.19 ±0.011	0.19 ±0.02	4.57 ±2.59	2.75^{b} ±0.29	1.80 ±0.29	1.26 ±0.15	33.75^{b} ±3.47	106.25^{a} ±6.25	39.50^{c} ±2.25	0.81^{b} ±0.04
2	32.00^{a} ± 1.58	50.50^{a} ±2.39	19.75 ±2.49	0.44 ±0.05	0.19 ±0.02	0.25 ±0.052	5.95 ±0.38	3.92^{a} ±0.22	2.02 ±0.26	2.03 ±0.34	43.75^{a} ±2.81	100.75^{a} ±5.4	57.50^{a} ±2.21	1.01^{a} 0±.08
3	25.50^{b} ±2.53	35.75^{cb} ±1.43	16.50 ±1.32	0.39 ±0.011	0.21 ±0.02	0.17 ±0.027	4.57 ±0.38	2.97^{b} ±0.26	1.60 ±0.29	2.04 ±0.39	28.50^{b} ±1.50	75.50^{b} ±6.06	37.00^{c} ±2.27	0.69^{b} ±0.031
4	24.50^{b} ±3.27	37.50^{b} ±2.02	19.00 ±1.87	0.36 ±0.033	0.18 ±0.008	0.17 ±0.027	4.87 ±0.49	3.1^{ab} ±0.32	1.77 ±0.42	2.1 ±0.61	26.75^{b} ±4.2	111.0^{a} ±4.49	37.75^{c} ±2.01	0.88^{ab} ±0.14
5	24.75^{b} ± 2.32	41.00^{b} ±1.87	16.50 ±1.70	0.35 ±0.02	0.18 ±0.01	0.16 ±0.025	4.77 ±0.82	2.77^{b} ±0.37	2.00 ±0.56	1.62 ±0.42	30.50^{b} ±2.32	98.25^{a} ± 4.64	48.75^{b} ±1.10	0.86^{ab} ±0.03

The same column not followed by the same letter differ significantly ($p < 0.05$).

Table (5): Some Serum Biochemical Profiles (Mean ± S.E), 14th Day Post Treatment with *Withania somnifera* and Cephoperazone Extract in Guinea pig Experimental Infected with *E .coli*

Groups	ALT U/L	AST U/L	AP U/L	T. Bili. mg/dl	D.Bil. mg/dl	I. Bil. mg/dl	T. P. gm/dl	Alb. gm/dl	Glob. gm/dl	A/G ratio	Chole. mg /dl	Gluco. mg/dl	Urea mg/dl	Creat mg/dl
1	19.75^{b} ±1.79	12.25^{b} ±1.37	13.75^{b} ±2.62	0.40 ±0.035	0.22^{a} ±0.027	0.18^{a} ±0.037	5.15 ±0.40	2.85^{b} ±0.27	2.29 ±0.13	1.23^{b} ±0.02	28.75^{ab} ±2.68	108.25 ±5.8	36.50^{b} ±3.17	0.84^{bc} ±0.05
2	47.00^{a} ±5.75	40.00^{a} ±3.16	22.75^{a} ±2.01	0.44 ±0.04	0.25^{a} ±0.042	0.19^{a} ±0.013	5.77 ±0.48	4.00^{a} ±0.26	1.77 ±0.33	2.49^{a} ±0.55	36.25^{a} ±2.52	106.5 ±9.13	52.50^{a} ±2.32	1.12^{a} ±0.08
3	20.25^{b} ±4.17	16.25^{b} ±3.03	13.00^{b} ±1.47	0.38 ±0.04	0.19^{a} ±0.009	0.18^{a} ±0.035	5.07 ±0.37	2.74^{b} ±0.31	2.32 ±0.02	1.17^{b} ±0.15	27.50^{b} ±2.50	95.00 ±2.97	39.50^{ab} ±7.59	0.82^{c} ±0.04
4	16.25^{b} ±1.25	18.50^{b} ±3.06	16.00^{ab} ±2.16	0.38 ±0.02	0.20^{a} ±0.011	0.18^{a} ±0.013	4.67 ±0.52	2.85^{b} ±0.18	1.82 ±0.37	1.73^{ab} ±0.31	33.25^{ab} ±3.14	113.25 ±7.49	40.75^{ab} ±3.06	0.83^{bc} ±0.09
5	15.50^{b} ±2.02	33.50^{a} ±3.09	16.25^{ab} ±1.10	0.30^{b} ±0.02	0.18^{b} ±0.02	0.12^{b} ±0.02	5.05 ±0.45	2.67^{b} ±0.31	2.37 ±0.23	1.14^{b} ±0.15	27.25^{b} ±2.75	101 ±8.13	42.75^{ab} ±6.38	1.03^{ab} ±0.08

The same column not followed by the same letter differ significantly ($p < 0.05$)

Table (6) : Serum TNF-α (Mean± S.E) in Guinea Pig Experimentally infected with *E coli* and Treated with *Withania somnifera* and Cephoperazone extract.

Groups	7th days pg/ml	14th day pg/ml
1	7.00^{b} ±0.52	12.33^{b} ±3.17
2	100^{a} ±15.39	57.33^{a} ±7.85
3	6.26^{b} ±0.02	14.66^{b} ±1.76
4	9.46^{b} ±1.63	16.00^{b} ±1.15
5	80^{a} ±11.54	9.50^{b} ±0.75

The same column not followed by the same letter differ significantly ($p < 0.05$)

4. REFERENCES

Akinyemi, K. O.; Mendie, U. E.; Smith, S. T.; Oyefolu, A. O. and Coker, A. O. (2005) : Screening of some medicinal plants used in south-west Nigerian traditional medicine for anti-Salmonella typhi activity. J Herb Pharmacother; 5(1):45-60.

Arora, S.; Dhillon, S.; Rani, G. and Nagpal, A. (2004) : The in vitro antibacterial/ synergistic activities of Withania somnifera extracts. Fitoterapia; 75: 385-388.

Aukrust, P.; Liabakk, N. B.; MÜller, F.; Lien, E.; Espevik, T. and Froland, S. S. (1994) : Serum level of tumor necrosis factor-α and soluble TNF receptors in human immunologic virus type1 infection correlation to clinical immunologic and virologic parameters. J.Inf.Dis;169:420-424.

Barbra, A. B. (1988) : Hematology Principles and Procedures, 5th ed. Lee and Febiger, Philadelphia, USA; pp: 81-83.

Benoit, R., Isabelle P., Fabienne B. and Christiane O. (2000) : Increased albumin plasma efflux contributes to hypoalbuminemia only during early phase of sepsis in rats. Am J Physiol., 284, PP 707-713.

Bhattacharya A., Ramanathan M., Ghosal S. and Bhattacharya S. K. (2000) : Effect of Withania somnifera glycowithanolides on Iron-induced hepatotoxicity in rats. Phytother Res; 14:568-570.

Christina, A. J. M.; Gladwin, J. D.; Packialakshmi, M.; Kothai, R.; Jerry, H. R. S.; Chidambaranathan, N. and Ramasamy, M. (2004) : Anticarcinogenic activity of Withania somnifera dunal against Dalton's Ascitic Lymphoma. Journal of Ethanopharmacology; 93 (2-3): 359-361.

Coles, E. H. (1986) : Veterinary Clinical Pathology. 4th ed, pp (279-97). WB Saunders Company, Philadelphia.

Davis, L. and kuttan, G. (1999): Effect of Withania somnifera on cytokine production in normal and cyclophosphamide treated mice. Immuno pharmacol Immunotoxicol; 21: 695-703.

Davis, L. and Kuttan, G. (2000) : Immunomodulatory avtivity of Withania somnifera. Journal of Ethanopharmacology; 71 : 193-200.

Dhuley, J. N. (1997) : Effect of some Indian herbs on macrophage functions in ochratoxin A treated mice. J Ethnopharmacol; 58 : 15-20.

Drabkin, D. L. (1949) : Standarization of hemoglobin measurement in "Fish Medicine " stoskopf K.M. (ed), (1993). 1st ed; W.B. Saunders Co.Philadelphia ,pp 114-115.

Elaskka, M.; Grigorescu, E. and Stanescu (1990): New data referring to chemistry of Withania somnifera species.Rev Med Chir Soc; 94:385-387.

Fawzy, T. K. H. (1983) : Medicinal plants in lybya. Arab Ecyclopedia House; 1st ed, PP: 820.

Feldman, B. F.; Zinkl, J. G. and Jain, V.

C. (2000) : Schalm's Veterinary Hematology 5th ed. Lippincott Williams and Wilkins Canada; pp: 1145-1146.

Girdhari, L. and Rana, A. (2007) : Withania somnifera (Ashwagandha): A Review. Pharmacognosy Reviews; 1(1):129-136.

Girish, K. S.; Machiah, K. D.; Ushanandini, S.; Harish, K. K.; Nagaraju, S.; Govindappa, M.; Vedavathi, M. and Kemparaju, K. (2006) : Antimicrobial properties of a nontoxic glycoprotein (WSG)from Withania somnifera (Ashwagandha). J. Basic Microbiol; 46 (5): 365-374.

He, X. G.; Mocek, U.; Floss, H. G.; Caceres, A.; Giron, L.; Buckley, H.; Cooney, G.; Manns, J. and Wilson, B. W. (1994) : An antifungal compound from Solamm nigrescens. J.Ethanopharmacol; 43(3):173-177.

Isogai, E.; Isogai, H.; Kimura, K.; Hayashi, S.; Kubota, T.; Fujii, N. and Takeshi, K. (1998) : Role of tumor necrosis factor alpha in gnotobiotic mice infected with an Escherichia coli O157:H7 strain.Infection and Immunity ;66:197-202.

Joseph, P. M.; Matt, R. S. and Claire, M. D. (2001) : Early response cytokines and innate immunity: Essential roles for TNF receptor 1 and Type I IL-1 receptor during Escherichia coli pneumonia in mice. The Journal of Immunology, 166: 4042-4048.

Kaneko, J. J.; John, W. H. and Micheal, L. L. B. (1997) : Clinical Biochemistry of domestic animals .5th ed . Academic Press, New York

Kaileh, M.; Berghe, W.; Heyerick, A.; Horion, J.; Piette, J.; Claude, L. C.; Keukeleire, D.; Essawi, T. and Haegeman, G. (2007) : Withaferin A strongly elicits IkB kinase_ hyperphosphorylation concomitant with potent inhibition of its kinase activity. The Journal of Biological Chemistry. (282)7; 4253-4264.

Khan, B.; Ahmad, S. F.; Bani, S.; Kaul, A.; Suri, K. A.; Satti, N. K.; Athar, M. and Qazi, G. N. (2006) : Augmentation and proliferation of T lymphocytes and Th-1 cytokines by Withania somnifera in stressed mice. International Immunopharmacology : 1394-1403.

Kulkarni, S. K. and Dhir, A. (2008) : Withania somnifera: An Indian ginseng .Neuro-Psychopharmacology & Biological Psychiatry; 32 (5): 1093-1105.

Kumar, A. and Rao, N. (1999) : Cefoperazone induced gastrointestinal haemorrhage. J Assoc Physic India; 47:840-841.

Kuttan, G. (1996) : Use of Withania somnifera Dunal as an adjuvant during radiation therapy.Indian Journal of Experimental Biology; 34 (9):854-856.

Malik, F.; Singh, J.; Khajuria, A.; Suri, K. A.; Satti, N. K.; Singh, S.; Kaul, M.; Kumar, A.; Bhatia, A. and Qazi, G. N. A. (2007) : Standardized root extract of Withania somnifera and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th 1-dominant polarization in BAIB/C mice. Life Sciences; 80:1525-1538.

Mazzanti, G.; Mascellino, M. T.; Battinelli, L.; Coluccia, D.; Manganaro, M. and Saso L. (2000) : Antimicrobial investigation of semipurified fractions of Ginkgo biloba. J. Ethanopharmacol; 17 (1-2):83-88.

Mehta, M.; Dutta, P. and Gupta, V. (2005) : Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in North India. Jpn J Infect Dis; 58:174-176.

Mishra, L. C.; Singh, B. B. and Dagenais, S. (2000) : Scientific basis for the therapeutic use of Withania somnifera (ashwagandha) a review. Altern Med Rev; 5: 334-346.

Modak, M.; Dixit, P.; Londhe, J.; Ghaskadbi, S. and Devasagayam, T. (2007) : Indian Herbs and herbal drugs used for the treatment of diabetes. Clin Biochem Nutr; 40 (3):163-173.

Mukhtar, M.; Arshad, M.; Ahmad, M.; Pomerantz, R.; Wigdahl, B. and Parveen, Z. (2008) : Antiviral potentials of medicinal plants. Virus Res; 131: 111-120.

Nagano, I.; Kato, S.; Nimura, Y. and Wakabayashi, T. (1994) : Hepatic mitochondrial changes in experimental obstructive jaundice complicated by biliary infection. hepatogastroenterology;41(5):432-437.

Ogundare, A. O. and Onifade, A. K. (2009) : The antimicrobial activity of Morinda lucida leaf extract on Escherichia coli.Journal of Medicinal Research;3(4):319-323.

Owais, M.; Sharad, K. S., Shehba, A. and Saleemuddin, M. (2005) : Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine Salmonellosis. Phytomedicine; 12 (3): 229-235.

Paget, G. E. and Barnes, J. M. (1964) : Evaluation of Drug Activities Pharmacometrics, Laurence and Bacharach, Vol 1, Academic Press, New York; 133-166.

Sarah, W. and Maggie, L. (2003) : Handbook of Laboratory Animal Management and Welfare.3rd ed.USA Iwa State Press.

Schentag, J. J.; Welage, L. S.; Grasela, T. H. and Adelman, M. H. (1987) : Determinants of antibiotic-associated hypoprothrombinemia. Pharmacotherapy; 7: 80-86.

Shhnizu, K. (1980) : Cefoperazone: absorption, excretion, distribution and metabolism. Clin. Ther; 3: 60-79.

Strom, B. L., Schinnar, R.; Gibson, G. A.; Brennan, P. J. and Jesse, A. (1999) : Risk of bleeding and hypoprothrombinaemia associated with NMIT side chain antibiotics: using cefoperazone as a test case. Pharmacoepidemiology and drug safety, 8: 81-94.

Teixeira, S. T.; Valadares, M. C.; Gonçalves, S. A.; Melo, A. D. and Queiroz, M. L. S. (2006) : Prophylactic administration of Withania somnifera extract increases host resistance in Listeria monocytogenes infected mice. International Immunopharmacology; 6 (10): 1535-1542.

Theodore, J. S. (2000) : Anti-inflammatory cytokines and cytokine antago-

nists. Current Pharmaceutical Design. 6: 633-649.

Trollfors, B.; Ahlmé, J. and Alestig, K. (1982) : Renal function during cefoperazone treatment. J of Antimicrobial chemotherapy; 9: 485-487.

Udayakumar, R.; Kasthurirengan, S.; Mariashibu T. S.; Rajesh, M.; Vasudevan, Ramesh Anbazhagan V. R.; Kim S. C.; Ganapathi, A. and Choi, C. W. (2009) : Hypoglycaemic and Hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. Int J Mol Sci.; 10(5): 2367-2382.

Veerappan, A.; Miyazaki, S.; Kadarkaraisamy, M. and Ranganathan, D. (2007) : Acute and subacute toxicity studies of Aeglo marmelos Corr., an Indian medicinal plants. Phytomedicine.14; 209-215.

Ziauddin, M.; Phansalkar, N.; Patki, P.; Diwanay, S. and Patwardhan, B. (1996) : Studies on the immunomodulatory effects of Ashwagandha. Journal of Ethnopharmacology; 50 (2):69-76.

Zimecki, M.; Artym, J.; Chodaczek, G.; Kocieba, M. and Kruzel, M. L. (2004) : Protective effects of lactoferrin in Escherichia coli-induced bacteremia in mice: relationship to reduced serum TNF alpha level and increased turnover of neutrophils. Inflamm Res., 53(7):292-296.

الملخص العربى

دراسات على التأثير المضاد للبكتريا لمستخلص نبات فراخ أم على فى خنازير غينيا المصابة تجريبياً بالميكروب القولونى

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تمت دراسة تأثير نبات فراخ أم على فى خنازير غينيا المصابة تجريبياً الميكروب القولونى عن طريق الدراسة على الأطباق وأيضاً على حيوانات التجارب المعدية بالميكروب.

قد تم إستخلاص المادة الفعالة لنبات بطريقتى الكحول الإيثيلى والماء المقطر من جذور وأوراق النبات، ثم إستخدام المادة الفعالة لرؤية مدى فاعلية النبات على الميكروب القولونى ومن ثم تحديد الجزء الفعال من النبات والمستخلص الفعال والجرعة الفعالة ورؤية مدى تأثير المادة على خنازير غينيا، وقد تم عدوى الحيوانات وعلاجها بجرعتين من مستخلص جذور النبات ٥٠ و ١٠٠ ملج/ ٤٠٠ جرام من وزن الحيوان، ومقارنة النتائج بحيوانات أخرى أخذت العدوى دون علاج وحيوانات معدية ومعالجة بمضاد حيوى سيفابرزون وقد أظهرت الدراسة مدى إيجابية النتائج للنبات ومدى قدرته على التخلص أو تثبيط عمل الميكروب وزيادة مناعة الجسم.