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Study of cellular immune response in mice Balb /c teated with LPS of *Klebsiella pneumonia* antigen and *Glycyrrhiza glabra* extract.

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Abstract . In this study , lipopolysaccharides of *Klebsiella pneumonia* and concentrate of *Glycyrrhiza glabra* were utilized to examine the cellular immunity in mice Balb/c (in vivo). Conversely, little is thought about cell invulnerability prompted by lipopolysaccharides and *Glycyrrhiza glabra* extract. A few parameters were utilized to accomplish this investigation, are neutrophil WBC (PMNs) rate, phagocytosis coefficient of PMNs at various eras 30, 60, 90 and 120 minutes, Formazan granules arrangement in them, and movement restraint factor (MIF).

Keywords: LPS, immunology, herbal extract, in vivo.

Introduction

Glycyrrhiza, involving in excess of 28 species having a place with the Fabaceae family, is one of the most seasoned and generally utilized natural prescriptions on the planet, and is recorded in different Asian and European pharmacopeias[(1,2.3).] Glycyrrhiza comprises of huge amounts of optional mixes, especially triterpene saponins, and phenolic mixes, for example, flavones, isoflavones flavanones, and chalcones, which are viewed as in charge of the bioactivities of licorice[(4,5,6)]. Glycyrrhiza separate is basically insoluble in water. Licorice remove hence can be considered to have a place with class II or IV of biopharmaceutical order framework (BCS[)] [(7,8)]. In this study the Glycyrrhiza glabra, commonly known as licorice, is

used as a green source of corrosion inhibitors for mild steel inhibition fromcorrosion in acidic solution. Formany years the Glycyrrhiza glabra, which is an herbaceous perennial, has been used in foods and medicinal remedies[(9,10)].

Materials and Methods

Nitroblue tetrazolium (NBT) Preperation

0.1 g of stain powder was broken up in 2 ml methanol and afterward included 50 ml PBS arrangement. This stain arrangement is put away in 4 ºC to use in NBT test [(11)].

Phosphate buffer saline (pH 7.2)

This cushion was set up concurring technique for Hudson and Hay (1980). Just 0.85 g KH2PO4, 0.795 g Na2HPO4 and 9 g NaCl are broken down in 1000 ml D.W and disinfected utilizing Autoclave for 15 min. this puffer utilized as control in this test [(12)]

Preparing migration medium

This medium was set up by dissolving 1.5 g agarose in 100 ml D.W and afterward disinfected utilizing the bubbling. A while later, the medium is cooled to 45 ºC and included Hanks adjusted Salt (pH 7.2)(prepared from Flow Laboratories by v:v) and latent sera to acquire the last fixation 10% in medium. All fixings were totally blended and put away in 4 ºC until the utilization[(13).]

Growth of the bacterial isolate

The bacterium *Klebsiella pneumonia* was gotten from College of Science, University of Iraq. This disconnect was subculture in 10 ml Nutrient stock and hatched for 48 hr at 37 ºC. At that point, the insulation of microscopic organisms was achieved by taking 4 ml of the way of life soup in 2000 ml of disinfected supplement juices and incubated in similar conditions in shaker hatchery at 150 cycle/min for 48 hr centrifugation of culture stock at 4000 cycle/min for 20 min at 4 ºC and the dregs was suspended in Phosphate cradle saline (pH 7.2) and centrifuged by same condition three times until getting the unadulterated silt and put away in 4 ºC .

Extraction and purification of LPS

LPS of *Klebsiella pneumonia* was extracted using ([14)]

Laboratory animals

Males of Swiss white mice Balb/c with old 5-6 weeks in this study .

Results

Effect of the immunization by using lipopolysaccharides (LPS) of *Klebsiella pneumonia* and extract of *Glycyrrhiza glabra* on survival of PMNs is reported in table 1. The results of the statistical analysis showed that there was no significant effect of immunization of the LPS antigen for *Klebsiella pneumonia* in the PMNs. That PMNs and all parameters had no significant differences compared to control and that the percentage of PMNs was high indicating the safety of their isolation. These results were agreed upon([15)]

Table 1 Effect of immunization of mice using LPS antigen of *Klebsiella pneumonia* and *Glycyrrhiza glabra* extract on PMNs

Treatments	Percentage of PMNs
LPS of *K. pneumonia*	89.0±0.71[a]
Glycyrrhiza glabra extract	89.2±0.42[a]
LPS & *Glycyrrhiza glabra*	88.6 ±0.84[a]
Phosphate buffer (control)	87.2±0.42[a]

Legend: The different letters in the same column refers to the significant differences (p<0.05).

Effect of LPS and *Glycyrrhiza glabra* extract on phagocytosis of the killed *Candida albicans*, as exhibited in table 2. LPS and *Glycyrrhiza glabra* indicated best phagocytosis coefficient with huge contrasts ($p<0.05$) reach to 71.3, pursued 66.3 by LPS in correlation with the control (62.1). *Glycyrrhiza glabra* extricate alone gave lower phagocytosis coefficient 62.1. The varied day and age from 30 to 120 minutes for every treatment were examined on phagocytosis coefficient. Following 30 and a hour, LPS and the plant extricate displayed together higher phagocytosis coefficient 71.3±0.36 and 66.7.5±0.31 altogether ($p<0.05$) and afterward diminished with expanding the time after 90 and 120 min to 70.5±0.33 and 68.1±0.37 individually. The bacterial LPS showed higher phagocytosis coefficient after 30 min (71.3±0.34) . This study coincides with other studies([16,17]).

Table 2 Study influence of injection using LPS of *Klebsiella pneumonia* and *Glycyrrhiza glabra* extract on phagocytosis of *Candida albicans* killed by the heat

Treatments	Phagocytosis coefficient of PMNs at different time periods (minutes)				**Mean**
	30	60	90	120	
LPS of *K. pneumonia*	66.7±0.34a	66.0±0.13a	66.0±0.38a	63.7±0.43b	**65.6B**
Glycyrrhiza glabra extract	62.1±0.62a	62±0.66a	60.1±0.57b	58.6±0.63c	**60.7C**
LPS & *Glycyrrhiza glabra*	71.3±0.36a	70.5±0.31a	69.7±0.33a	68.1±0.37b	**69.9A**
Phosphate buffer (control)	62.1±0.62a	62.0±0.66a	60.1±0.57b	58.6±0.63c	**60.7C**

Legend: The different small letters in the same row refers to significant differences ($p<0.05$) in the period for each treatment. The different capital letters in the last column refers to significant differences ($p<0.05$) among average of treatments

LPS and *Glycyrrhiza glabra* were used to investigate zone of migration and migration inhibition factor (MIF) of PMNs, table 3. LPS and *Glycyrrhiza glabra* extract jointly showed lower zone of PMNs migration reached to 10.05±0.12 mm significantly ($p<0.05$), while LPS antigen individually exhibited migration zone of 14.3±0.10 mm compared with the control (19.34±0.04 mm). Also, LPS and *Glycyrrhiza glabra* extract showed higher migration inhibition factor (MIF) 0.52, followed 0.74 by LPS individually compared with the control which reached to 1.00. Using

Glycyrrhiza glabra individually did not record any significant differences ($p<0.05$). This study coincides with other studies([18,19]).

Table 3 Influence of injection of mice using LPS and *Glycyrrhiza glabra* on the migration of PMNs

Treatments	Zone of migration (mm)	Migration inhibition factor (MIF)
LPS of *K. pneumonia*	14.3 ±0.10 c	0.74
Glycyrrhiza glabra extract	19.3±0.04 a	1.00
LPS & *Glycyrrhiza glabra*	10.05±0.12 d	0.52
Phosphate buffer (control	19.34±0.04 a	1.00

Legend: The different letters in the same column refers to the significant differences ($p<0.05$).

Treatment of LPS and *Glycyrrhiza glabra* extract showed the delayed type hypersensitivity (DTH) by increase of foot thickness of mice 3.40±0.101 mm, 3.33±0.113 mm and 2.44 0.084 mm respectively in comparison with the mice before the treating (1.49±0.034 mm) significantly ($p<0.05$), .Furthermore, the delayed type hypersensitivity of *Glycyrrhiza glabra* extract individually reached to 2.11±0.028 mm before the treating and that declined to 0.45±0.028 mm after 72 hr in which is similar to the control 0.44±0.024), table 4.

Table 4 The delayed type hypersensitivity test after various periods (mm

The injected antigens	Foot thickness before the treating (mm)	Foot thickness after the treating (mm)		
		After 24 hr	After 48 hr	After 72 hr
Phosphate buffer (control	1.20±0.024a	2.15±0.023b	2.0±0.025b	0.44±0.024b
Glycyrrhiza glabra extract	1.38±0.027a	2.11±0.028b	2.08±0.026b	0.45±0.028b
LPS & *Glycyrrhiza glabra*	1.49±0.034a	3.40±0.101a	3.33±0.113a	2.44 0.084a

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