Some toxicological impacts and invitro antibacterial activity of *Datura innoxia* extract in rats

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Abstract:

The study was carried out to investigate some toxicological effects and in-vitro antibacterial activity of *Datura innoxia* dried leaves.The yield percentage of *Datura innoxia* 95% ethyl alcohol leaves extract was 19%. Phytochemicals analysis of both *Datura innoxia* leaves powder and alcoholic extract, indicated the presence of alkaloids, phenols, glycosides, tannins, resins, saponins, flavonoids and steroids which are responsible for toxic and therapeutic effects. The acute toxicological effect of *Datura innoxia* ethanolic extract in male rats was determined by estimation the oral median lethal dose (LD_{50}) by up and down method was 1214 mg/kg. BW. The in-vitro antibacterial activity of *Datura innoxia* ethanolic extract and its minimum inhibitory concentration activity (MIC) showed that *E. coli* was more sensitive to *D.I* ethanolic extract than *Staph. aureu*s, the MIC for *D.I* extract against *E. coli* was less than MIC against *Staph. aureus* which was 5 and 10 mg/ml respectively, also *E. coli* was more sensitive to ciprofloxacin .

بعض التأثيرات السمية والمضادة للبكتيريا في الزجاج لمستخلص الداتورا انوكسيا في الجرذان

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الخلاصة:

أجريت هذه الدراسة لتحديد بعض التأثيرات السمية والتأثيرالمضاد للبكتريا في الزجاج لمستخلص الداتورا أنوكسيا في الجرذان . لقد أعطى المستخلص الكحولي 95% نسبة استخلاص قدرها 19%.في حين أظهرت نتائج التحليل الكيمياوي لأوراق النبات وجود القلويات والفينولات والكلايكوسيدات والتاتينيات والراتنجيات والصابونيات والستيرويدات المسؤولة عن التأثيرات العلاجية والسمية. أما الدراسة السمية الحادة لنبات الداتورا أنوكسيا في ذكور الجرذان المهقاء ,فقد سجلت جرعة فموية وسطية مميتة (LD_{50}) قدرها 1214ملغم/كغم من وزن الجسم للمستخلص الكحولي 95%.في حين أظهرت

نتائج دراسة التأثير المضاد للبكتريا في الزجاج للمستخلص الكحولي لنبات الداتورا أنوكسيا بأن الأشريشيا القولونية كانت أكثر حساسية من المكورات العنقودية ,حيث كان التركيز الأدنى المثبط لنمو الأشريشيا القولونية 5ملغم/مل أقل مقارنة بالمكورات العنقودية التي سجلت 10 ملعم / مل , إضافة الى أن الأشريشيا القولونية كانت أكثر حساسية للسايبروفلوكساسين.

Introduction:

The most common causes of acute diarrhea are bacterial and viral infections (1)and(2).Bacterial causes like; *Escherichea coli* , *Staphylococcus aureus*, *Sallmonella* and *Vibrio cholera*, also parasitic infection especially protozoa can lead to sever acute diarrhea which is responsible for the high levels of mortality and morbidity in humans and animals(3)and(2)). Therapeutic value of plants used in trade medicine derives from the presence of phytochemicals principles (secondary metabolites) which are found in all parts of plant such as alkaloids, tannins, flavonoid, saponins and phenols (4). At this time, resistance to the anti-microbial agents is recognized as a major global public health problem, it is possible that anti-microbial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have a clinical value in treatment of resistant strains (5). One of the most important medicinal plants is *Datura innoxia* (thorn apple), which is an annual plant belongs to the family of Solanaceae. *Datura* plant is an important medicinal plant as it is a well known source of different phytochemicals (secondary metabolites), it is distributed throughout most of the part of the world and it is abundant in Iraq, This plant is rich in alkaloids which induce to stimulation of C.N.S and depression of the peripheral nerves (6), also *Datura* plant is rich in secondary metabolites which have antibacterial activity by a different mechanisms (7). Acute toxic effect of *Datura* alkaloid include; nausea, confusion, incoherence, dizziness, agitation, and loss of motor coordination. Overdose symptoms include convulsions, coma, intoxication lasting days (up to twenty days in some case), permanent damage to the eyes, heart and brain, and death(8).

For all these facts, steps are taken to do study our local plant *Datura innoxia* aiming the following targets ,So our study targeted for

1- Preparation of ethanolic extract of *Datura innoxia* plant.

2- Identification of *Datura innoxia* active phytochemicals (secondary metabolites) which may have toxicological and chemotherapeutic properties .

3- Study of oral acute toxic effect (LD_{50}) of *Datura innoxia* ethanolic extract in rat.

4- In-vitro study of the antibacterial activity of *Datura innoxia* ethanolic extract .

Materials and Methods:

Datura innoxia leaves were collected from Al-Kut city-Iraq during August after 4 week of germination . Alcoholic extraction of the *Datura innoxia* leaves by Soxhlet apparatus was obtained by ethanol 95% according to method described by (9).

Phytochemicals detection of the Active Components of *Datura* innoxia carried out on the plant powder and its ethanolic extract.

-Detection of Tannins dependent on the appearance of gelatinous white precipitant as described by Harborne, (10).

-**Detection of Saponins** dependent on the appearance of big foam,and was carried out according to Harborne (10).

Detection of Coumarins Components dependent on the exposure to UV light and appearance of green –yellow color as described by (11).

-Detection of Flavonoids Components dependent on the appearances of yellow color ,and was carried out according to (12) .

-Detection of Phenolic Components dependent on the appearance of blue green color referred to the phenolic according procedure of Harborne,(13).

-Detection of Steroids and Terpenoids Components was conducted according to (14), and dependent on the appearance of a brown color referred to terpenes , while blue green color is indicator of steroids .

-Detection of Resins Components carried out according to (10).

- **Detection of Alkaloids Components** dependent on the presence of white , orange ,and green precipitate according to procedure described by (15).

- Detection of Glycosides Components dependent on the red precipitate according to (10).

- **Acute Toxicity Study of *Datura innoxia* Ethanolic Leaves Extract in male rat** The estimation of oral LD_{50} in male albino rats was done according to (16) by using the Up and Down method, eight adult male rats were used . The range doses that used orally was 800-1300 mg/kg B.W, while the difference between doses was 100 mg/kg B.W., LD_{50} of plant extract was calculated according to survival or death outcome of the performed toxicity study by using the following equation :

LD_{50} = xf + kd

LD_{50} = Median Lethal Dose

xf=Last dose used in the experiment

d = Difference between doses

k = Factor of change from the table **(appendix 1)**

O = Symbol of survival animal after 24 of hours of dosing

X = Symbol of dead animal after 24 hours of dosing

Preparation of Different Concentration of *Datura innoxia* -

Stock solutions were prepared by mixing 2 g of dried extract with 20 ml of 90% ethyl acetate, that was sterilized with Millipore membrane filter (0.20µm). The concentrations of 2.5 , 5 , 10 ,20 , 40 , 60 , 80 and

100 mg/ml were prepared from stock solution.

-Bacterial spp :- Pathogenic isolates of ***Escherichia coli*** and ***Staphylococcus aureus*** were obtained from the College of Veterinary Medicine/ Department of Internal and Preventive Medicine/University of Baghdad.

- Preparation of Standard Bacterial Suspension

The average number of viable, *Escherichia coli*, *Staphylococcus aureus* organisms per ml of the stock suspensions was prepared by means of the Standard McFarland solution No.0.5. (17).

-In-vitro Antibacterial Test of Ethanolic Extract of *Datura Innoxia* Plant

The agar well diffusion method was adopted according to (18(19)), for assessing the antibacterial activity of the prepared extract. 0.2 ml of standardized bacterial stock suspensions (1.5×10^8) cell/ml of *E. coli* and *Staph aurous* was thoroughly mixed with each 20 ml of sterile nutrient agar. This 20 ml of the inoculated nutrient agar was distributed into sterile petri dishes. The agar was left to set, and in each of these plates 4 well, 8 mm in diameter were performed by sterile cork borer and the agar discs were removed, wells were filled with 0.1ml of each concentration of 2.5, 5, 10, 20, 40, 60, 80 and 100 mg/ml of *Datura innoxia* extract using microtiter-pipette that allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 Ċ for 24 hours. Three replicates for each concentration extract were performed, and the activity of plant extract was determined by measuring the diameter of inhibition zone around each well by millimeter against each of the tested organism. Simultaneously, addition of the respective solvent (90% ethyl acetate) instead of extract was carried out as controls.

Ciprofloxacin was used as a reference antibiotic to determine sensitivity of each bacterial species tested (20)(21), by using the concentrations of 2.5, 5, 10 and 20 mcg/ml., 0.1 ml of sterilized distilled water was served as a control.

-Determination of Minimum Inhibitory Concentration (MIC) of *Datura* Extract and Ciprofloxacin Against Tested Bacteria

MIC was determined by using agar plating method for the concentrations (5, 10, 20, 40, 60) mg/ml for *Datura* extract and the concentrations 2.5, 5, 10 and 20 mcg/ml for ciprofloxacin.

The lowest concentration of extract and antibiotic that inhibits the visible growth of the bacteria in cultured agars was considered as MIC of plant extract and ciprofloxacin (22)(23)(24).

Results and Discussion:

1- Extraction of *Datura innoxia*:- Extraction of *Datura innoxia* leaves with 95% ethanol gave a deep green color extract with plant powder yield percentage of 19% .

This result is nearly similar to the results of(25) who found that the percentage recovery of 60% ethanolic extract was 13% w/w from fine *Datura* leaves powder . The small different in yield percentage may be attributed to the high concentration of ethanol which had been used in our extraction. Deep green crude extract color is similar with what found by (26)(27).

2-Phytochemicals Detection of the Active Components in *Datura innoxia* Leaves Powder and Extract:- Active components in *Datura innoxia* plant leaves powder , and in its 95% ethanolic extract and their method of detection were listed in table 1. The plant leaves powder screening showed the presence of the alkaloids, phenol, steroids, terpenoids, resins, saponins, flavonoids, tannins and glycosides, while coumarins was absent . The detection of phytochemicals in ethanolic extract gave an evidence of exist the alkaloid, phenol, steroids, resins, saponins, flavonoids, tannins and glycosides, while coumarins and terpenoids were absent . Alkaloid, phenol and tannins were seemed to be found in a high level in crude extract. (28)reported that nearly all of the identified components of the plant which are known by their activity against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol or methanol extraction.

The presence of alkaloids, phenol, steroids, resins, saponins, flavonoids, tannins and glycosides in *Datura* 95% ethanolic extract were confirmed by finding of Uma Reddy (9).

(29) supported phytochemical screening of this study when they have found the presence of alkaloids, saponins, tannins and glycosides in *Datura stramonium* 95% ethanolic extract of leaves powder.

Table -1*:* Phytochemical analysis of *Datura innoxia* leaves .

Test	*D.I* leaves	*D.I* 95%ethanolic	Results

	powder	extract	
Alkaloid			
Mayer´s	++	++	White Precipitate
Dragendroff	++	++	Orange Precipitate
Pecric acid	++	++	Green Precipitate
Flavonoid	+	+	Yellow Color
Steroid	++	+	Blue-Green Color
Terpenoid	+	–	Brown Color
Resins	+	+	Appearance of Turbidity
Coumarins	–	–	
Phenol	++	++	Blue –Green Color
Saponnins			
Stirring	++	+	Big Foam
Silver Nitrate	++	+	Silver Mirror
Mercuric Chloride	+	–	Silver Color
Tannins			
Lead Acetate	++	++	Gelatin-White Color
Frrric hloride	+	+	Blue –Green Color
Glycosides			
Fehling .T.	++	+	Red Precipate
Bendect´s T.	++	+	Red Precipate

+ = positive , ++ = good present, – = not detected

3-Acute Toxicity Study of *Datura innoxia* Ethanolic Extract in male Rat

3-1-Acute Toxicity Symptoms:- The toxic symptoms and mortality outcome listed in table 2:

Table -2:: The toxic symptoms and mortality outcome that developed according to different doses of *Datura innoxia* 95% ethanolic leaves extract in rat according to up and down method.

Dose (mg/Kg. B.W)	Clinical signs during 24 hours	Results (O or X)
800	- - -	**O**
900	Some depression	**O**
1000	Depression ,shallow breathing.	**O**
1100	Depression , shallow breathing, restlessness , convulsion ….	**O**
1200	Depression , shallow breathing, restlessness , convulsion , paralysis ,coma ,death through 24hours .	**X**
1100	Depression ,shallow breathing …	**O**
1200	Depression ,shallow breathing, restlessness ….	**O**
1300	Depression ,shallow breathing, restlessness , convulsion, paralysis ,coma ,death through 24hours .	**X**

O : survival , X : death

Datura plants may contain a large number of different alkaloids, the major symptoms of intoxications in animals are consistent with the well-known anti-cholinergic effects of tropane alkaloids that include; hyposalivation, tachycardia, hyperventilation, pupil dilation, restlessness, nervousness, muscle tremor, convulsions, delirium and death from asphyxia. Tropane alkaloids in the plant leave extract include atropine , hyoscyamine,and scopolaminewhich act as competitive blockers at muscarinic cholinergic receptors with different specificity for individual receptor subclasses. (30).

3-2- Determination of acute toxicity by median lethal dose (LD_{50}) in male albino rat (table 3), the value of oral LD_{50} was 1214 mg/kg B.W, it was calculated according to the following equation :

LD_{50} = xf + kd = 1300 + (–0.860) × 100 = 1214 mg/kg B.W

Table 3 : calculate of LD_{50} for *Datura innoxia* ethanolic extract leaves by up and down method-orally (Dixon 1980 method).

Decrease or increase in dose	Death or survival of animal after 24 hours.	Value of (K) table	Last used dose (xf)	Value of LD_{50}
100 mg /kg B.W	**OOOOXOOX**	**–0.860**	**1300 mg /kg**	**1214 mg/kg B.W**

O:survival , X : death

This results are in disagreement with (31) who found the oral LD_{50} of *Datura innoxia* leaves petroleum ether extract in mice 500mg/kg, These differences in LD_{50} values may be attributed to the great ability of petroleum ether to dissolve tropane alkaloid (atropine ,hyocymine and scopolamine) which is responsible for anticholenergic toxicity effects (28).

-In-vitro Antibacterial Activity of 95% ethanolic Extract of *Datura Innoxia* Leaves Powder

The zones of inhibition were different according to bacterial spp. and concentration of extract (table 4). The results showed that *E. coli* was more sensitive to antibacterial effect of *Datura* extract than *Staph. aureus* in all the concentrations . In all concentrations there was a significant increase ($P<0.05$) in diameter of zone of inhibition in *E. coli* growth when it compared with zone of inhibition of *Staph. aureus,* figures; 1, 2 , and 3 .

Table (4): In-vitro antibacterial activity of *D.innoxia* extract in different concentrations on *E. coli* and *Staph. aureus* growths (diameter of inhibition zone in mm.)

Concentration mg/ml	*E. coli* (inhibition zone-mm) Mean ±SE	*Staph. aureus* (inhibition zone-mm) Mean ±SE
2.5	0.00±0.00	0.00±0.00
5	10.33±0.33 **Ga**	0.00±0.00 **Fb**
10	14.00 ±0.57 **Fa**	9.00±0.00 **Eb**
20	18.33 ±0.33 **Ea**	12.00±0.00 **Db**
40	21.00±0.57 **Da**	15.00±0.57 **Cb**
60	25.33±0.33 **Ca**	16.00±0.66 **Cb**
80	28.66±0.33 **Ba**	21.00±0.33 **Bb**
100	31.33±0.66 **Aa**	28.00 ±0.66 **Ab**
90%Eathyl acetate	0.00±0.00	0.00±0.00

Values represent mean ±S.E -

Different capital letters mean significant (P<0.05) results between different concentrations .-
Different small letters mean significant (P< 0.05) results between bacterial spp. growths .-

The results of *Datura* antibacterial activity against *E .coli* and *Staph. aureus* growths were in agreement with (9 ;31 32 ;34) They attributed the antibacterial activity of *Datura* composition of secondary metabolites like; alkaloid, flavonoids, phenol, tannins, saponins, steroids, glycosides and resins which have antibacterial activity with different mechanisms of action (28). Iranbakash *(35)* connected antibacterial activity according to plant part, age, growth stage and solvent that was used in extraction of *D.innoxia* or *D.stramonium*, it seems that young leaves had high ratio of phytochemicals , that have antibacterial activity especially alkaloids compounds which oppose *E. coli* growth in more value when the extraction done on this leaves during its tissue formation and separation (29) estimated the antimicrobial activity of *Datura stramonium* 95% ethanolic crude extract against *E. coli* and attributed its activity to saponins soapy characteristics, also they referred to precipitation of the microbial protein and making the nutritional proteins unavailable for bacteria by tannins. Classes of alkaloids are among the major powerful chemical known, some alkaloids have been proved also to be useful in correcting renal infections; this means that the alkaloids of *Datura* may be a poison for both lower or higher organisms.

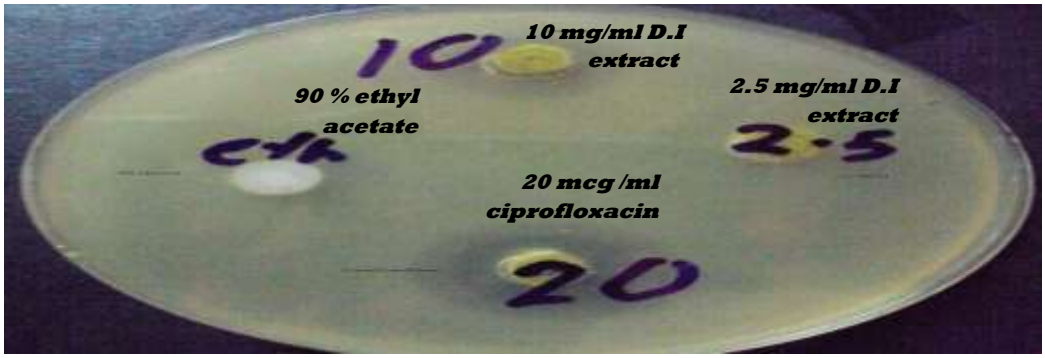
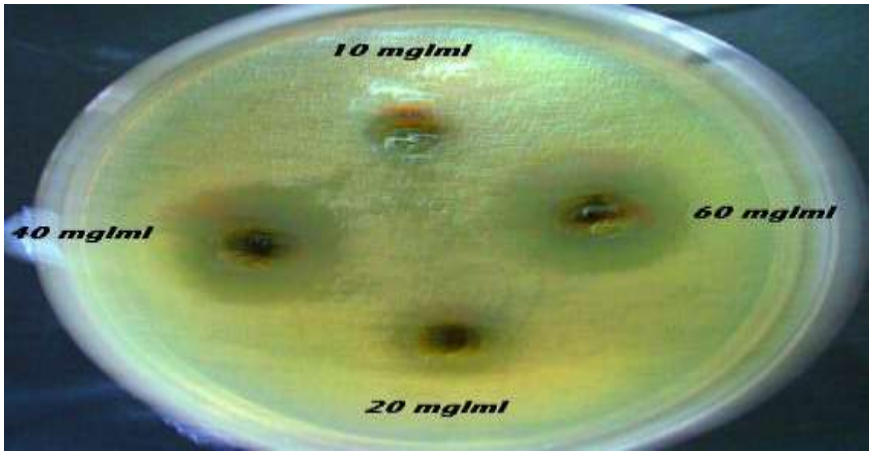


Figure (1) : **Sensitivity of *E. coli* to D.I extract in compared to ciprofloxacin and ethyl acetate.**



Figure(2): **sensitivity *Staph. aureus* of to *Datura innoxia* extract(mg /ml).**

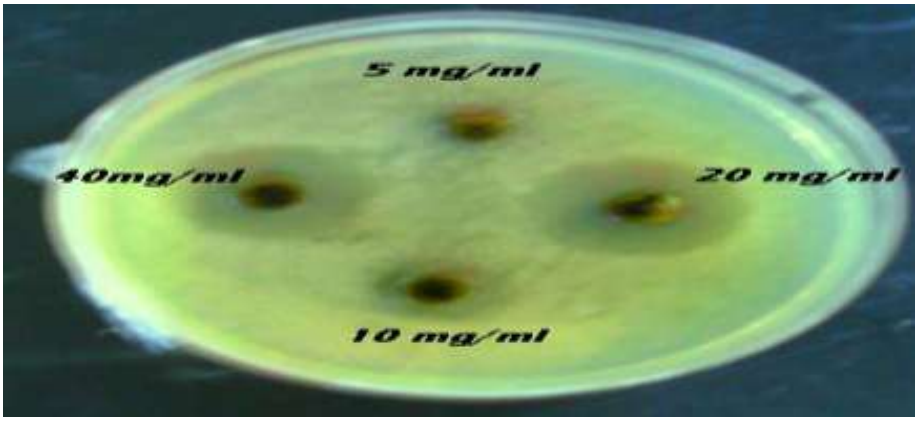


Figure (3): **Sensitivity of *E .coli* to *Datura innoxia* extract (mg/ml).**

Determination of Minimum Inhibitory Concentration (MIC) of *Datura* Extract Against Bacterial Growths :- The results of MIC showed that *Datura innoxia* extract had lower MIC (5 mg/ml) against *E. coli* in comparison with 10 mg/ml against *Staph. aureus*. Sensitivity of *E. coli* to *Datura* extract is in agreement with(29) who indicated that, microorganisms may need a higher concentration of extracts to

inhibit their growths or kill them and it depends on their cell wall components, antimicrobial substances may affect the synthesis of peptidoglycan in the bacterial cell wall, leads to osmotic shock and death of bacterial cell. (36) reported that the minimum inhibitory concentration (MIC) of the plant extract ranged between 10% (w/v) and 25% (w/v), It can be concluded that the bacterial spp., its virulence, cell wall structure and resistance to antibacterial agent may play a critical role in determining the MIC of each bacteria spp Ciprofloxacin was used as a reference antibiotic because it is consider as a broad spectrum fluoroquinolone antibiotic that is beneficial in the treatment of a variety of infections (37). It acts by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase type IV, these enzymes are necessary to separate replicated DNA, thereby inhibiting cell division (38). Both kinds of bacterial spp. growth were sensitive to ciprofloxacin (table 5) . *E .coli* was sensitive significantly (P<0.05) more than *Staph. aureus* in a dose dependent concentration 2.5, 5, 10 and 20 mcg /ml respectively.

Table (5): Antibacterial activity of ciprofloxacin against *Staph. aureus* and *E.coli*

Concentration mcg/ml	***Staph. aureus* MIC(zone-mm)**	***E. coli* MIC(zone-mm)**
	Mean±SE	**Mean±SE**
2.5	9.66 ±1.20 **Db**	20.33±0.33 **Da**
5	15.33±0.33 **Cb**	24.66 ±0.66 **Ca**
10	21.33±1.45 **Bb**	28.33 ±0.33 **Ba**
20	28.66±0.88 **Ab**	33.00 ±0.67 **Aa**
D.W	0.00±0.00	0.00±0.00

Values represent mean ±S.E

Different capital letters mean significant (P<0.05) results between different concentrations .-

Different small letters mean significant (P< 0.05) results between bacterial spp. Growths-

The minimum inhibition concentration test results (MIC) for ciprofloxacin was the concentration 2.5 mcg /ml against both *E. coli* and *Staph. aureus* bacterial spp. growth.

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