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# Study the Effects of Some Herbs on Rats Suffering from Diabetes

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**Abstract:**

This investigation aimed to evaluate the effect of colocynth, teucrium and their combination on male diabetic rats. Thirty (30) adult male Sprague Dawley rats were divided into five groups. Group (1): Normal rats fed on basal diet as control negative (C-), Group (2): Control positive (C+) (fed on basal diet). Group (3): Diabetic rats fed on basal diet containing 5% colocynth .Group (4): Diabetic rats fed on basal diet containing 5% teucrium. Group (5): Diabetic rats fed on basal diet containing mixture of them (5%). At the end of experiment, 28 days of feeding, all serum samples were analyzed for biochemical parameters. Injection with alloxan caused a significant decrease in BWG and high density lipoprotein cholesterol (HDL-c) while a significant increase was recorded in Total cholesterol (TC), triglyceride (TG), very low density lipoprotein cholesterol (VLDL-c), low density lipoprotein cholesterol (LDL-c), uric acid (U.A), Creatinine, Urea, Aspartate amino transaminase (AST), alanine amino transferase (ALT), Alkaline phosphatase (ALP) and Glucose. Diabetic rats treated with various diets, showed the improvement in all previous parameters.

**Key words:** Diabetes, Colocynth, Teucruim and Mixture of them.

**Introduction:**

*Citrullus colocynthis (L.)* Schrad. is a Cucurbitaceae family plant **(Riaz *et al*., 2015)**. The plant is generally accessible in the Sahara and Arabian deserts, Sudan and a Southern piece of Asia including Pakistan, India and Southern Islands. The fruit is intense and globular with a smooth surface. It is hard and has a skin around it and contains 200–300 seeds/gourd. Seeds are small (6mm in length), ovoid, compressed, smooth and brownish when ripe. Seed constitute about 75% of the weight of fruit of *Citrullus colocynthis* **(Hussain *et al*., 2014)**.

A few dynamic synthetic constituents of *C. colocynthis* plant were surveyed. They are grouped as saponins, carbohydrates, tannins, glycosides, alkaloids, flavonoids and essential oils. Plant-based characteristic constituents can be obtained from any part of the plant like leaves, roots, flowers, stems, fruits, and seeds. Various plant secondary metabolites including flavonoids and cucurbitacins have already been accounted for from *C. colocynthis* **(Salama, 2012)**.

*Citrullus colocynthis (L.)* Schrad. has a wide range of therapeutic and nutritional uses. It possessed antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflamatory, analgesic, gastrointestinal, reproductive, protective and many other pharmacological effects **(Al-Snafi, 2016)**. Traditionally this plant is used in the treatment of diseases like cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases **(Dhakad *et al*., 2017)**.

*Teucrium polium* is perennial, herbaceous, with almost woody plants, to a height of 30 cm and has a white appearance and cotton. Flowers can be seen in white, yellow and white to yellow. This variability is seen not only in color, but also in flower stems that are branched or lying **(Ricci *et al*., 2005)**.

Some compounds of *T. polium* have been intro-duced in different investigations including tannin, terpenoid, saponin, flavonoid, sterol, β-caryophyllene, diterpenoids, caryophyllene oxide, asparagine, ditryne and resinous sub-stances **(Niazmand *et al*., 2008)**.

The effects of *T. polium* on the liver, kidney, stomach, brainhas investigated and antidiabetic, antioxidant, antimicrobial, and anticancer effects of this agent, have introduced. Several studies revealedthat *T. polium* has a hypoglycemic effect and can help to control blood sugar. In addition, due to the undeniable effects of this plant againstcancer cells, it

can be considered as a natural resource, for the treatment of cancer **(Khazaei *et al*., 2018)**.

Therefore this study aimed to evaluate the effect of colocynth, teucrium and their combination on some nutritional and biochemical parameters of male diabetic rats.

**Materials and Methods**

**Materials:**

Colocynth and teucrium were obtained dry from herb shop in Cairo, Egypt.

**Chemicals:**

Alloxan obtained from El-Gomhoria Company, Cairo .Egypt.

**Animals:**

Thirty (30) adult male Sprague Dawley rats, average body weight (150± 10 g) were used in this study. Rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

**Methods:**

**Basal diet composition**:

The basal diet in the experiment consisted of casein (12%), corn oil (4%), mineral mixture (3.5%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (74.o%) according to **AIN (1993)**.

**Preparation of materials**:

All materials were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to **Russo (2001)**.

**Induced diabetic for rats:**

Rats were injected by Alloxan in 0.9% Nacl intraperitoneally at150 mg /kg body weight to induce male diabetic for rats. After 4 days feeding on basal diet, blood sugar was determined for healthy and injected rats to make sure the induction of diabetes.

**Experimental design and animal groups:**

Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week for adaptation period. The rats were divided into 5 groups each n=6 rats. All groups of rats were housed in wire cages at room temperature 25 $C^0$, and kept under normal healthy condition. Rats were divided into the following groups:

**Group (1):** Control negative group (-), normal rats were fed on basal diet.

**Group (2):** Control positive group (+), diabetic rats were fed on basal diet.

**Group (3):** Diabetic rats fed on diet containing 5% colocynth.

**Group (4):** Diabetic rats fed on diet containing 5% teucrium.

**Group (5):** Diabetic rats fed on diet containing 5% mixture of both herbs.

**Determination of Biochemical Blood Parameters:**

Blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. The rats were scarified under ether anaesthesia. Blood samples were received into in clean dry centrifuge tubes, in which blood was left to clot at room temperature, and then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean cuvette tubes and stored frozen at-20$^o$C for biochemical analysis as described by **Schermer, (1967)**. All serum samples were analyzed for determination the following parameters:

Urea was determined according to the enzymatic method of **Patton and Crouch (1977)**, creatinine was determined according to kinetic method of **Henry (1974)** and uric acid was according to the enzymatic colorimetric test of **Fossatti and Prencipe (1980)**. Aspartate amino transaminase (AST) and alanine amino transferase (ALT) were carried out according to the method of **Yound (1975) and Tietz (1976)**. Alkaline phsphatase (ALP) was determined according to **Belfield and Goldberg (1971)**. Total cholesterol (TC) was determined according to **Allain (1974),** and high density lipoprotein cholesterol (HDL-c) according to **Lopez (1997)**. The calculation of low density lipoprotein cholesterol (LDL-c) was carried out according to the method of **Lee and Nieman (1996),** atherogenic index (AI) was calculated according to **Kikuchi *et al.*, (1998)** and triglycerides as **Fossati and Prencipe (1982)**.Serum glucose determined according to **Kaplan (1984)**.

**Statistical Analysis:**

The data were statistically analyzed using a computerized Costat Program by one way ANOVA using a Completely Randomized Factorial Design **(SAS, 1988)** when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant. The results are presented as mean ± SD.

**Results and Discussion:**

Data presented in table (1) illustrate the effect of colocynth, teucruim and their combination on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats. It could be observed that the mean value of (BWG) of control (-) group was higher than control (+) group, being 1.10±0.009 and 0.70±0.005 g⁄ day ⁄each rat respectively. The best (BWG) level was showed for groups 5 (rats fed on basal diet containing 5% mixture of both herbs) when compared to the other treated groups and control (+) group.

It could be noticed that the mean value of FI of control (-) group was higher than control (+) group, being 22.40±0.004 and 18.06±0.003 g⁄ day ⁄each rat respectively. The highest mean value of (FI) level was showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to the other treated groups and control (+) group.

Also, data of table (1) observed that the mean value of (FER) of control (-) group was higher than control (+) group, being 0.049±0.0006 and 0.039±0.0008 respectively. The best FER was shown for group 5 (rats fed on basal diet + 5% mixture of both) this group showed significant increase in FER, as compared to the other treated groups and the control (+) group.

It seems possible that the combination of both Colocynth and Teucruim revealed asynngistic action.

**Table (1): Effect of colocynth, teucruim and mixture of them on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats**

| Parameters / Groups | BWG (g) Mean ± SD | FI (g) Mean ± SD | FER (%) Mean ± SD |
|---|---|---|---|
| **G1:** Control –ve | $1.10^{a}$±0.009 | $22.40^{a}$±0.004 | $0.049^{a}$±0.0006 |
| **G2:** Control +ve | $0.70^{e}$±0.005 | $18.06^{c}$±0.003 | $0.039^{e}$±0.0008 |
| **G3:** Colocynth (5%) | $0.72^{d}$±0.001 | $17.75^{e}$±0.002 | $0.041^{d}$±0.0003 |
| **G4:** Teucruim (5%) | $0.75^{c}$±0.008 | $17.84^{d}$±0.008 | $0.042^{c}$±0.0004 |
| **G5:** Mixture of both (5%) | $0.84^{b}$±0.002 | $18.40^{b}$±0.004 | $0.046^{b}$±0.0005 |
| **LSD** | **0.11** | **0.0085** | **9.96** |

Values in each coloumn with different letters are significantly different (P<0.05).

Data presented in table (2) illustrate the effect of colocynth, teucruim and mixture of both on total cholesterol and triglycerides of diabetic rats. It could be observed that the mean value of total cholesterol (TC) of control (+) group was higher than control (-) group, being 236±0.54 and 102±0.57 mg/dl respectively.

Feeding diabetic group on diet containing 5% teucruim showed significant decrease in serum total cholesterol, as compared to the group which was fed on diet containing 5% colocynth. On the other hand, treating diabetic group with 5% combination of both herbs recorded the best result in TC, this group showed significant decrease in the parameter, as compared to the other treated groups and the control (+).

It could be noticed that the mean value of triglycerides TG of control (+) group was higher than control (-) group, being 250±0.80 and 125±0.75 mg/dl respectively. All treated groups showed significant decrease in serum TG, as compared to the positive control group on the other hand results of serum (TG) level was showed for group 5 (rats fed on basal diet + 5%mixture of both) when compared to control (+) group.

**Ayoubi *et al*., (2013)** reported that Teucrium (*Teucrium polium*) was accompanied by significantly lower total cholesterol and triglycerides in streptozotocin-induced diabetic Wistar rats.

**Ebrahimi *et al*., (2016)** indicted that hydro-alcoholic leaf extract of *C. colocynthis* decreased total cholesterol and triglycerides in streptozotocin-induced diabetic rats.

**Table (2): Effect of colocynth, teucruim and mixture of them on total cholesterol (TC) and triglycerides (TG) of diabetic rats**

| Parameters / Groups | TC (mg/dl) Mean ± SD | (TG mg/dl) Mean ± SD |
|---|---|---|
| **G1:** Control –ve | $102^{e}$±0.57 | $125^{d}$±0.75 |
| **G2:** Control +ve | $236^{a}$±0.54 | $250^{a}$±0.80 |
| **G3:** Colocynth (5%) | $220^{b}$±0.62 | $212^{b}$±0.73 |
| **G4:** Teucruim (5%) | $215^{c}$±0.65 | $210^{c}$±0.79 |
| **G5:** Mixture of both (5%) | $201^{d}$±0.70 | $209^{c}$±0.71 |
| **LSD** | **1.12** | **1.38** |

Values in each coloumn with different letters are significantly different (P<0.05).

Data presented in table (3) show the effect of colocynth, teucruim and mixture of both on serum lipoprotein cholesterol HDLc, LDLc, VLDLc & atherogenic index AI of diabetic rats.

It could be observed that the mean value of ($VLDL_C$) of control (+) group was higher than control (-) group, being 50±4.0 and 25±2.0 mg/dl respectively.

All treated diabetic groups with the two types of herbs and their combination induced significant decrease in VLDL-c as compared to the control (+) group. On the other hand, non- significant change in serum VLDL-c was observed between all treated groups.

It could be showed that the mean value of (HDLc) of control (-) group was higher than control (+) group, being 60±1.7 and 39±0.4 mg/dl respectively. The best serum HDLc was shown for group 5 (rats fed on basal diet containing 5% mixture of both) the two types of herbs which used in this study and their combination increased the mean value of serum HDL-c, as compared to the positive control group.

The same table indicated that the mean value of (LDLc) of control (-) group was lower than control (+) group, being 17±2.0 and 147±1.5 mg/dl respectively. The best serum LDLc was shown for group 5 (rats fed on basal diet +5% mixture of both) and the group which treated with 5% teucruim,

Also, data of table (3) observed that the mean value of (AI) of control (+) group was higher than control (-) group, being 5.05±0.001 and 0.70±0.007 respectively. Followed by the groups which treated with 5% Teucruim and 5% Colocynth, respectively results in AI was shown

for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

**Ebrahimi *et al*., (2016)** indicted that hydro-alcoholic leaf extract of *C. colocynthis* decreased low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) in streptozotocin-induced diabetic rats.

**Safaeian *et al*., (2018)** found that hydroalcoholic extract (72.9%, $P < 0.001$) of *T. polium* reduced low-density lipoprotein and butanol fraction of hydroalcoholic extract of *T. polium* increased increased HDL (33%, $P < 0.05$) in rats.

**Table (3): Effect of colocynth, teucruim and mixture of them on serum lipoprotein cholesterol (VLDLc), (HDLc), (LDLc) (mg/dl) and Atherogenic index (AI) of diabetic rats**

| Parameters / Groups | VLDL (mg/dl) Mean ± SD | HDL (mg/dl) Mean ± SD | LDL (mg/dl) Mean ± SD | AI Mean ± SD |
|---|---|---|---|---|
| **G1:** Control –ve | $25.0^{c}$±2.0 | $60^{a}$±1.7 | $17^{e}$±2.0 | $0.70^{e}$±0.007 |
| **G2:** Control +ve | $50.0^{a}$±4.0 | $39^{d}$±0.4 | $147.0^{a}$±1.5 | $5.05^{a}$±0.001 |
| **G3:** Colocynth (5%) | $42.4^{b}$±2.3 | $42^{c}$±0.5 | $135.6^{b}$±2.4 | $4.24^{b}$±0.004 |
| **G4:** Teucruim (5%) | $42.0^{b}$±1.67 | $45^{b}$±1.6 | $128.0^{c}$±3.0 | $3.78^{c}$±0.005 |
| **G5:** Mixture of both (5%) | $41.8^{b}$±4.8 | $47^{b}$±1.8 | $112.2^{d}$±3.0 | $3.28^{d}$±0.006 |
| **LSD** | **5.82** | **2.45** | **4.46** | **0.009** |

Values in each coloumn with different letters are significantly different ($P<0.05$).

Data of table (4) illustrate the effect of colocynth, teucruim and mixture of them on serum levels of AST, ALT, ALP enzymes & (AST/ALT) ratio of diabetic rats.

It could be observed that the mean value of AST enzyme of control (+) group was higher than control (-) group, being 158±1.73 and 126±0.78 (U/L) respectively. Treating diabetic rats with 5% Teucruim improved the mean value of serum AST enzyme, than that the group which treated with 5% Colocynth. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to other treated groups and the control (+) group.

It could be noticed that the mean value of ALT enzyme of control (+) group was higher than control (-) group, being 130±0.83 and 36±0.87 (U/L) respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

Data of the same table (4) show the mean value of ALP enzyme

of control (+) group was higher than control (-) group, being 220±0.68 and 55±0.78 (U/L) respectively. Treating diabetic rats with 5% Teucruim improved the mean value of serum ALP, than that the group which treated with 5% Colocynth. Group 5 showed the lowest mean value of ALP enzyme level as compared to control (+) group which and recorded the best result**.**

It could be noticed that the mean value of (AST/ALT) of control (-) group was higher than control (+) group, being 3.50±0.62 and 1.22±0.21 respectively.

Non-significant changes in the ratio between AST/ALT were observed between the groups which treated with the two types of herbs and the combination of them.

**Amini *et al*., (2009)** reported that ethyl acetate (EtOAc) extract of *Teucrium polium* reduced alkaline phosphatase, aspartate aminotransferase and alanine aminoteransferase activities in rats.

**Ebrahimi *et al*., (2016)** indicted that hydro-alcoholic leaf extract of *C. colocynthis* decreased aspartate amino-transferase and alanine aminotransferase in streptozotocin-induced diabetic rats.

**Table (4): Effect of colocynth, teucruim and mixture of them on GOT, GPT, GOT/GPT and ALP (U/L) of diabetic rats**

| Parameters / Groups | AST (U/L) Mean ± SD | ALT (U/L) Mean ± SD | AST/ALT Mean ± SD | ALP (U/L) Mean ± SD |
|---|---|---|---|---|
| **G1:** Control –ve | $126^{c}$±0.87 | $36^{d}$±0.87 | $3.50^{a}$±0.62 | $55^{e}$±0.78 |
| **G2:** Control +ve | $158^{a}$±1.73 | $130^{a}$±0.83 | $1.22^{c}$±0.21 | $220^{a}$±0.68 |
| **G3:** Colocynth (5%) | $130^{b}$±2.68 | $61^{b}$±1.82 | $2.13^{b}$±0.07 | $85^{b}$±0.73 |
| **G4:** Teucruim (5%) | $126^{c}$±0.81 | $59^{b}$±0.85 | $2.14^{b}$±0.14 | $82^{c}$±0.59 |
| **G5:** Mixture of both (5%) | $120^{d}$±1.75 | $56^{c}$±0.89 | $2.14^{b}$±0.19 | $74^{d}$±0.62 |
| **LSD** | **3.11** | **2.038** | **0.57** | **1.24** |

Values in each coloumn with different letters are significantly different (P<0.05).

Data presented in table (5) show the effect of colocynth, teucruim and mixture of both on serum glucose of diabetic rats. It could be noticed that the mean value of glucose of control (+) group was higher than control (-) group, being 275±3 and 95±1 (mg/dl) respectively. The best serum glucose was observed for group 5 (basal diet containing 5% mixture of both) when compared the other treated groups and the control positive group.

**Oryan *et al*., (2014)** found that *C. colocynthis* was able to reduce blood glucose significantly compared with the control diabetic group (P<0.05).

**Barghamdi *et al*., (2016)** reported that 125 mg *C. colocynthis* once per day for 2 months can lead to considerable decrease in the mean levels of HbA1c and fasting blood glucose (FBS) among the patients with type II diabetes without any side effects.

**Zabihi *et al*., (2018)** indicated that chronic administration of *Teucrium polium* TP in STZ-induced diabetic rats could decrease blood glucose in streptozotocin (STZ)-induced diabetic rats.

**Table (5): Effect of colocynth, teucruim and mixture of them on serum glucose (mg/dl) of diabetic rats**

| Parameters / Groups | Glucose (mg/dl) Mean ± SD |
|---|---|
| **G1:** Control –ve | $95^{e}$±1 |
| **G2:** Control +ve | $275^{a}$±3 |
| **G3:** Colocynth (5%) | $132^{b}$±2 |
| **G4:** Teucruim (5%) | $125^{c}$±2 |
| **G5:** Mixture of both (5%) | $120^{d}$±3 |
| **LSD** | **4.23** |

Values in each coloumn with different letters are significantly different (P<0.05).

Results of table (6) show the mean value of serum creatinine, urea and uric acid (mg/dl) on diabetic rats fed on various diets.

It could be observed that the mean value of uric acid of control (+) group was higher than control (-) group, being 6.75±0.006 and 2.80±0.007 mg/dl respectively. All treated groups showed significant decrease in serum uric acid, as compared to the positive control group. The best results in this parameter recorded for the group which treated with the combination of the two types of herbs, this treatment showed significant decrease in serum uric acid, as compared to the other treated groups and the positive control group.

The same table (6) results illustrate that mean value of creatinine of control (+) group was higher than control (-) group, being 1.60±0.001 and 0.65±0.006 mg/dl respectively. In concern to creatinine the best treatment was recorded for the group 5 (rats fed on basal diet +5%

mixture of both) when compared to control (+) group.

It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being 85±2 and 20±2 mg/dl respectively. Group 5 (rats fed on basal diet +5% mixture of both) recorded the best result as compared to control (+) group.

**Abdel-Muein (2005)** found that petroleum ether and ethanolic extract of *Teucrium polium L* decreased uric acid, total urea and total creatinine in diabetic rats.

**Bagherizadeh *et al*., (2015)** investigated that *Citrullus colocynthis* pulp decreased serum levels of urea, uric acid, and creatinine in diabetic rats.

**Table (6): Effect of colocynth, teucruim and mixture of them on uric acid (U.A), creatinine and urea (mg/dl) of diabetic rats**

| Parameters / Groups | U.A (mg/dl) Mean ± SD | Creatinine (mg/dl) Mean ± SD | Urea (mg/dl) Mean ± SD |
|---|---|---|---|
| **G1:** Control –ve | $2.80^{e}$±0.007 | $0.65^{e}$±0.006 | $20^{d}$±2 |
| **G2:** Control +ve | $6.75^{a}$±0.006 | $1.60^{a}$±0.001 | $85^{a}$±2 |
| **G3:** Colocynth (5%) | $5.00^{b}$±0.1 | $0.74^{b}$±0.007 | $44^{b}$±1 |
| **G4:** Teucruim (5%) | $4.71^{c}$±0.004 | $0.69^{c}$±0.002 | $40^{b}$±3 |
| **G5:** Mixture of both (5%) | $4.22^{d}$±0.005 | $0.67^{d}$±0.005 | $25^{c}$±4 |
| **LSD** | **0.082** | **0.0087** | **4.74** |

Values in each coloumn with different letters are significantly different (P<0.05).

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# دراسة تأثيربعض الأعشاب على الفئران المصابة بمرض السكر

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**الملخص العربى**

يهدف هذا البحث إلى تقييم تأثير أعشاب الحنظل والجعدة والخليط منهما علي ذكور الفئران المصابة بالسكر. تم تقسيم ثلاثون فأر من الذكور البالغين سبراغ داولي إلى خمس مجموعات. مجموعة (1): وهي المجموعة الضابطة السالبة (-) تغذت على الوجبة الأساسية ، المجموعة (2): وهي المجموعة الضابطة الموجبة (+) وهي الفئران المصابة بالسكر وتغذت على الوجبة الأساسية. المجموعة (3): الفئران المصابة بالسكر التي تغذت علي نبات الحنظل بنسبة 5%. المجموعة (4): الفئران المصابة بالسكر التي تغذت على نبات الجعدة بنسبة 5 %. المجموعة (5): الفئران المصابة بالسكر التي تغذت على الاثنين معا بتركيز 5%.. في نهاية التجربة ، بعد 28 يومًا من التغذية ، تم تقدير الاختبارات البيوكيميائية للدم. الحقن بالألوكسان سبب ارتفاع في مستويات الجلوكوز واليوريا والكرياتينين واليوريك اسيد وALTوAST وALP ومستوى الكوليسترول الكلي وجلسريدات ثلاثية والليبوبروتين منخفض الكثافة والليبوبروتين منخفض الكثافة جدا وانخفاض مستويات الليبروتين مرتفع الكثافة والوزن المكتسب في الفئران المصابة بالسكر وتحسنت النتائج باستخدام الأغذية المعالجة.

**الكلمات المفتاحية:** مرض السكر, عشبة الحنظل, عشبة الجعدة والخليط من الاثنين معا.