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Investigating *Caralluma penicillata*'s Medicinal Properties: Effects on Haematological Parameters and Renal Functions in Cotton Pellet Induced Granuloma in Adult Guinea Pigs

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Abstract:

Background: *Caralluma penicillata* is a plant that possessing a great therapeutic potential in Yemeni folk medicines, such as antipyretic, antirheumatic, for the treatment of peptic ulcer and antihyperglycemic. **Aim:** The study was conducted to determine the impact of *C. penicillata* on a few haematological variables and tow aspects of renal functioning in cotton pellet-induced granuloma in adult male guinea pigs. **Method:** Thirty six guinea pigs were assigned randomly to six groups (I, II, III, IV, V and VI), each with six guinea pigs. Group (I) A normal control group got 5% Polysorbate 80 in dosages similar to those of the drugs. Group (II) Normal saline in an equivalent dosage was given to the untreated control group. Groups (III-VI) got tested drugs either singly or in combinations. **Results:** According to the findings, *C. penicillata* extract did not appear to reduce the increases in total leukocytic count, haematocrit value, or erythrocytic sedimentation rate. An increase in neutrophils, monocytes, and eosinophils was seen, along with a noticeably less number of lymphocytic cells. An increase in plasma urea and creatinine indicated a significant deterioration in renal functioning, according to the data. This nephrotoxic effect was unaffected by *C. penicillata* extract or indomethacin, either alone or in combination. **Conclusion:** The *C. penicillata* extract was unable to reverse the hematological or nephrotoxic consequences of cotton pellet-induced granuloma.

Keywords: *Caralluma penicillata*, Haematological parameters, Renal functions, Cotton Pellet, Granuloma

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Introduction

Due to the residents' poor economic situation, Yemen is one of the developing nations that relies on traditional medicine, particularly medicinal plants that provide basic medical needs. In Yemen, there are thought to be 3000 plants, 10% of which are indigenous. Yemen's customary usage of medicinal plants dates back to ancient Arabic medicine, which was in turn influenced by Greek and Indian practices¹. The genus *Caralluma* belongs to the family Asclepiadaceae (also known as the milk weed family) which comprises some 200 genera and 2500 species. There are approximately 100 variable species in the *Caralluma* genus which are distributed throughout the world, in Africa, Canary Islands, India, Arabia, Southern Europe, Ceylon and Afghanistan. Southern Arabia is an important center of diversity for the *Carallumas*, with about 35 species found mainly in the south west of the Arabian Peninsula². *Caralluma* species that are found all over the world are edible and are used in various nations' traditional medical practices. In Yemeni traditional medicine, *Caralluma* is a plant with significant therapeutic potential. Different illness conditions such as (peptic ulcer, diabetes mellitus, snake and scorpion bites rheumatic and inflammation) have been treated using the aerial sections of *Caralluma* species³⁻⁵. *C. penicillata*, which is widely utilized in folk medicine, is one of the species of *Caralluma* that is most prevalent in Yemen². The common vernacular names are (Ghulāf, Ghulāth, Kusmah...). The purpose of this study was to identify the effects of *C. penicillata* some haematological parameters and some aspects of renal functions of adult guinea pigs in chronic inflammation model was induced by cotton pellet implantation, which was described Sheth et al., 1972 and Albaser et al., 2014 with slight modification.

Materials and Methods

Reagents and Chemicals

Indomethacin was obtained from MSD (UK) in powder form. Diazepam of Hoffman La Roche (Switzerland) in injectable form was purchased. Polysorbate 80 USP was purchased (Sigma Chemical Co. Germany). Ethanol of Merck, Darmstadt (Germany), was purchased.

Plant collection and storage

The plant material was identified in Botany Division, Biology Department, Faculty of Sciences, Sana'a University. The aerial parts of the *C. penicillata*, were chopped and crushed in mixer and extracted with 10%

ethanol at room temperature. The extract was dried by using freeze dryer..

Animals

Thirty six adult male guinea pigs with uniform locally bred strain that weighing from 350 g to 405 g were used in this study with permission from the Faculty Research Board, Department of Pharmacology, Faculty of Medicine and Health Sciences, Sana'a University, Yemen. They were housed and cared for at the same facility, divided randomly into 6 groups (n = 6), marked clearly, housed under controlled temperature/humidity and 12 hours light/dark cycles with water and diet ad libitum. They were fed on grass and carrots. To acclimatize to the new environment, the guinea pigs were placed in a separate room for one week prior to the experiment and were used according to Sana'a University guidelines. The animals were divided into six groups:

- Group I: Normal control received 5% Polysorbate 80 in amounts comparable to those of the medications.
- Group II: Non-treated control received equivalent amounts of normal saline.
- Group III: *C. penicillata*; (400 mg/kg)
- Group IV: Indomethacin (10 mg/kg) daily as aqueous suspensions using 5% Polysorbate 80
- Group V: *C. penicillata*-indomethacin combination (200-5 mg/kg).
- Group VI: *C. penicillata*-indomethacin combination (400-10 mg/kg).

All animals groups were given oral dose once daily of the tested drugs at 8 PM for 7 consecutive days from the day of cotton pellet implantation. The combination doses of *C. penicillata* and dose of indomethacin were administered to animals groups separately with 30 minutes between them.

Experimental design

The inflammation model was induced by cotton pellet implantation, which was described by Sheth et al., 1972⁶ and Albaser et al., 2014 with slight modification.

Blood sample collection

After 7 days of drugs administration for all animals. All animals were sacrificed by decapitation. A blood sample was taken in EDTA tube for clinical hematological study. For renal functions test, the blood samples were left at room temperature for 30 min for coagulation and then centrifuged at 3000 rpm for 15 min at 4°C (Centurion Scientific Ltd K240R, UK) to separate serum. The serum

was stored at -20°C for 48 h (Aftron AFF 545, Denmark) until subjected for analysis of creatinine and BUN levels.

Hematological analysis

Hematocrit (HCT), erythrocyte sedimentation rate (ESR), total white blood cells count (WBC), neutrophil, monocyte, and eosinophil counts. were determined using the Automated Hematological Analyzer (Sysmex, SE 9500, Kobe, Japan).

Serum urea and creatinine analysis

The concentration of serum creatinine and BUN were measured by Cobas integra 400 (Roche, Switzerland) using commercial kits according to the manufacturer's protocol.

Statistical analysis

All data obtained were statistically analyzed using students' t-test. Descriptive statistics are shown as the mean \pm standard error (s.e.) and values of $P < 0.05$ were considered statistically significant.

Results

Effect on haematological parameters

The hematological parameters such as total white blood cells count, hematocrit, erythrocyte sedimentation rate (ESR), and percentages of neutrophils, lymphocyte, monocytes, and eosinophils were examined across the comparative groups. The results of the effect of *C. penicillata* on hematological parameters were summarized in Tables 1& 2. The hematocrit was significantly increased (p value = <0.05) in *C. penicillata*, full and half doses combination groups compared with normal control. Similarly, the ESR was significantly enhanced in various groups compared with normal control. However, it was significantly decreased in all treated groups compared with non-treated control. The

total WBC's counts were significantly increased in cases of non-treated control, *C. penicillata* and both full and half doses combination compared with normal control. The percentages of neutrophils, monocytes, and eosinophils when inflammation was induced by the implantation of cotton pellets were all considerably higher than they were in the normal control. The total leukocytic count, neutrophil, and eosinophil counts from indomethacin alone were all considerably higher than in the control group. The neutrophils, monocytes, and eosinophils levels were all significantly higher when *C. penicillata* was used alone. There was a reduction of 30%, 40%, 22%, and 43% in cases of indomethacin, *C. penicillata*, half, and full doses combinations, respectively.

Compared with indomethacin eosinophils count there was significantly increase by 27% in case of *C. penicillata* and half dose combination. The monocytic count was significantly increased by 16% in *C. penicillata*, 66% in half dose combination and 18% in full dose combination compared with non-treated control. However, in indomethacin treated group there was reduction amount to 35%. Compared with Indomethacin treated group monocyte count was significantly higher in *C. penicillata*, half dose combination, and in full dose combination treated group by +80%, +158% and +83% respectively.

The lymphocytic count was significantly decreased by 27% in model control, 29 % in Indomethacin 25% in case of *C. penicillata*, -32% in half dose combination and 36% in full dose combination groups compared with normal control. These values were insignificant compared with non-treated control table.

Table 1. Effect of *C. penicillata* on haematocrit, erythrocyte sedimentation rate and total W.B.C count in cotton pellet induced granuloma in adult male guinea pigs

Groups Parameters	Normal control (NC)	Non-treated control (NTC)	Indomethacin (10 mg/kg)	<i>C. penicillata</i> (400 mg/kg)	<i>C. penicillata</i> -indomethacin combination (200 - 5mg/kg)	<i>C. penicillata</i> -indomethacin combination (400 - 10 mg/kg)
Haematocrit (ml %)	36.2 ± 0.69	41.8 ± 1.2	43.0 ± 1.4	45.3 ± 1.3	46.0 ± 1.07	46.5 ± 1.12
% change compared with NC		+19	+ 22	+ 29*	+ 31*	+ 32*
% change compared with NTC			+ 3	+ 8	+10	+ 11
ESR (mm /h)	1.8 ± 0.13	3.8 ± 0.13	2.7 ± 0.20	2.3 ± 0.08	3.0 ± 0.12	7.3 ± 0.33
% change compared with NC		+ 111*	+ 50*	+ 28*	+ 67*	+ 22*
% change compared with NTC			- 30**	- 40**	- 22**	- 43**
Total W.B.C (Cell x10³/mm³)	3.6 ± 0.06	6.2 ± 0.78	5.4 ± 0.6	7.0 ± 0.6	6.4 ± 0.6	6.1 ± 0.26
% change compared with NC		+ 82*	+ 59	+ 106*	+ 88*	+ 79*
% change compared with NTC			- 13	+ 13	+ 4	- 0.5

* Significant compared with Normal control value at p < 0.05 ** Significant compared with non-treated control value at p < 0.05.

Table 2. The effect of *C. penicillata* differential white blood cells count (%) in cotton pellet induced granuloma in adult male guinea pigs.

Parameters	Normal control (NC)	Non-treated control (NTC)	Indomethacin (10 mg/kg)	<i>C. penicillata</i> (400 mg/kg)	<i>C. penicillata</i> -indomethacin combination (200 - 5mg/kg)	<i>C. penicillata</i> -indomethacin combination (400 - 10 mg/kg)
Neutrophils (%)	43.2 ± 0.7	51.8 ± 1.2	56.2 ± 3.0	50.5 ± 1.6	50.7 ± 0.8	56.5 ± 1.5
% change compared with NC		+20*	+30*	+17*	+17*	+31*
% change compared with NTC			+8	-3	-2	+9
% change compared with Indomethacin				-10	-10	+0.5
Lymphocytes (%)	51.8 ± 0.65	38 ± 1.34	36.8 ± 2.1	38.8 ± 1.5	35.2 ± 1.1	33.3 ± 1.23
% change compared with NC		-27*	-29*	-25*	-32*	-36*
% change compared with NTC			-3	+2	-7	-12
% change compared with Indomethacin				+5	-4	-10
Monocytes (%)	3.7 ± 0.2	6.2 ± 0.6	4 ± 0.4	7.2 ± 0.6	10.3 ± 0.6	7.3 ± 0.33
% change compared with NC		+68*	+8	+95*	+178*	+97*
% change compared with NTC			-35**	+16**	+66**	+18**
% change compared with Indomethacin				+80***	+158***	+83***
Eosinophils (%)	1.3 ± 0.1	3.2 ± 0.3	3 ± 0.3	3.8 ± 0.3	3.8 ± 0.3	3.3 ± 0.33
% change compared with NC		+146*	+131*	+192*	+192*	+154*
% change compared with NTC			-6	+18	+18	+3
% change compared with Indomethacin				+27***	+27***	+10

* Significant compared with Normal control value at p < 0.05 ** Significant compared with non-treated control value at p < 0.05. *** Significant compared with Indomethacin value at p < 0.05.

Effects on renal functions

The average urea and creatinine concentration in normal adult guinea pigs were 26.5 ± 0.5 mg /dl and 0.5 ± 0.05 mg/dl respectively. The cotton pellet induced granuloma increased creatinine concentration by + 80%. It is increased by +40% in Indomethacin and *C. penicillata* treated groups compared with normal control. Compared

with non-treated control the average serum creatinine concentration was significantly decreased by 20%.

The combination of Indomethacin and tested plant extract either in half or full doses significantly increased creatinine concentration by 60% and +80% respectively table-3

Table 3. Effect of *C. penicillata* on serum urea and creatinine in cotton pellet induced granuloma in adult male guinea pigs.

Parameters	Normal control (NC)	Non-treated control (NTC)	Indomethacin (10mg/kg)	<i>C. penicillata</i> (400 mg/kg)	<i>C. penicillata</i> -indomethacin combination (200 - 5mg/kg)	<i>C. penicillata</i> -indomethacin combination (400 - 10 mg/kg)
Urea (mg/dl)	26.5 ± 0.5	31.5±1.9	29.3±1.2	31.8±1.11	34±1.6	30.3±0.7
% change compared with NC		+19	+11	+20	+28	+14
% change compared with NTC			-7	+1	+8	+4
Creatinine (mg/dl)	0.5±0.03	0.9±0.04	0.7±0.05	0.7±0.07	0.8±0.05	0.9±0.05
% change compared with NC		+80*	+40*	+40*	+60*	+80*
% change compared with NTC			-22**	-22**	-11	0

* Significant compared with Normal control value at $p < 0.05$. ** Significant compared with non-treated control value at $p < 0.05$

Discussion

As regards the tested haematological parameters, the values of normal control group were within the same range of previous studies⁷. Induction of inflammation by cotton pellet implantation significantly increased white blood cell count. This may be due to the stimulating effect of the inflammatory mediators namely interleukins on synthesis of leukocytes in bone marrow⁸. Such effect was not reversed after administration of either drugs or their combinations. The haematocrit value and erythrocytic sedimentation rate (ESR) were significantly increased by the effect of either drug. This is in good correlation with the observed increase in white blood cells and fibroblastic activity respectively found in previous study⁹. The differential cell counts revealed that the non-treated cotton pellet model of inflammation had significant increase in neutrophils, monocytes and eosinophils with marked decrease lymphocytes counts. This may reflect inflammatory mediators induced stimulation of bone marrow. The significant decrease in lymphocyte may be due to its migration from blood stream to tissues.

Indomethacin administration normalized cotton pellet induced in macrophage count probably due to its role in promotion of macrophage migration¹⁰. Indomethacin alone produced significantly increased in total leukocytic count, neutrophil, and eosinophil counts by 59%, 30% and 131% respectively compared with normal control tables. *C. penicillata* alone, significantly increased haematocrit, total leukocytic count, neutrophil, monocyte, and eosinophil counts by 29%, 106%, 17%, 95% and 192% compared with normal control table -2. The half dose combination of above mentioned drugs showed significant increase in haematocrit, total leukocytic count, neutrophil, monocyte, and eosinophil counts by 31%, 88%, 17%, 178%, and 192% respectively compared with normal control, tables. Regarding the full dose combination there was significant increase in haematocrit, total leukocytic count, neutrophil, monocyte, eosinophil counts by 32%, 79%, 31%, 97%, and 154% compared with normal control, tables. In addition, either the adopted model of chronic inflammation or tested drugs increased the erythrocytic sedimentation rate (ESR) from a normal control value of 1.8 ± 0.13 ml in the first

hour by 111% in case of non-treated model control, 50% in case of indomethacin, 28% in case of *C. penicillata*, 67% in case of half dose combination and 22% for full dose combination compared with normal control. *C. penicillata* either singly or in combination with Indomethacin significantly increased all tested white blood cell types probably due to emigration inhibition of leukocytes. This effect is correlated with the anti-inflammatory effect observed in this work. The above mentioned result is in agreement with the study of Hong, 2005¹¹ who showed that ketorolac which is a non-steroidal anti-inflammatory agent significantly increase leukocytic and neutrophil and decreasing lymphocytic count. Nevertheless, it is contradictory with the results of this work in decreasing monocytes and eosinophils. The discrepancy between the results of the two studies may be explained by the difference in animal species and pathological conditions. This work was conducted in guinea pigs in which inflammation was induced by cotton pallet implantation. The study of Hong, 2005¹¹ was conducted on postoperative patients with no apparent signs of inflammation. Compared with non-treated control, there was a reduction amount to 30%, 40%, 22% and 43% in cases of indomethacin alone, *C. penicillata* alone, half and full dose combination respectively. Compared with indomethacin eosinophils count there was significantly increase by 27% in case of *C. penicillata* and half dose combination. Moreover, the bone marrow stimulant action of *C. penicillata* was confirmed by the study conducted Al-Bekairi, et al.1992¹² who showed that *C. tuberculata* antagonized the DNA inhibitory effect of cytotoxic drug – cyclophosphamide.

The combination of indomethacin and tested plant extract either in half or full doses significantly increased creatinine concentration by 60% and + 80% respectively, table-3. Induction of inflammation with cotton pellet implantation deteriorated renal function as evidenced by significant increase in plasma creatinine. This may be attributed to foreign body induced glomerulonephritis. Appel, 2004¹³ showed that glomerular lesion similar to human glomerulonephritis may be induced by injection of foreign materials. The mechanism may be due to formation of antigen –antibody complexes which were precipitated in the glomeruli. The latter activated complement system that resulted in cytotoxic reaction. This nephrotoxic action persisted after administration of indomethacin or *C. penicillata* either singly or in-combination. This means that the tested drugs failed to modify foreign body induced

nephrotoxicity i.e. the tested drugs had no effect on complement induced inflammatory reaction.

Conclusion

The *C. penicillata* extract failed to reverse either the hematological or nephrotoxic effects of cotton pellet induced granuloma in adult male guinea pigs. More studies are needed for explanation the mechanism and the effect of *C. penicillata* on other hematological parameters and renal functions.

Conflict of interest

No conflict of interest is associated with this work.

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دراسة الخواص الطبية لنبات الكاريولوما بنيسلاتا: التأثيرات على بعض مؤشرات الدم ووظائف الكلى في الالتهاب المحدث بزراعة قطعة قطن في خنازير غينيا البالغة

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الملخص

نبات الكارالوما بنيسلاتا هو نبات يمتلك إمكانات علاجية كبيرة في الأدوية الشعبية اليمنية، مثل خافض الحرارة، ومضاد للروماتيزم، لعلاج القرحة الهضمية وخافض سكر الدم. أجريت هذه الدراسة لتحديد تأثير مستخلص نبات الكارالوما بنيسلاتا على بعض مؤشرات الدم ووظائف الكلى في الالتهاب المحدث بزراعة قطعة قطن في ذكور خنازير غينيا البالغة. تم تقسيم ستة وثلاثين خنزير غينيا بشكل عشوائي إلى ست مجموعات (الأولى والثانية والثالثة والرابعة والخامسة والسادسة)، وتضم كل مجموعة ستة خنازير غينيا. المجموعة (الأولى) مجموعة المراقبة الطبيعية على 5% بوليسوربات 80 بجرعات مشابهة لجرعات الأدوية. المجموعة (الثانية) مجموعة المراقبة الغير معالجة أعطيت محلول ملحي عادي بجرعة مكافئة و حصلت المجموعات (من الثالثة الى السادسة) على الأدوية التي تم اختبارها إما منفردة أو معا. اظهرت النتائج أن مستخلص نبات الكارالوما بنيسلاتا لم يقلل من الزيادات في إجمالي عدد كريات الدم البيضاء، أو قيمة الهيماتوكريت، أو معدل ترسيب كرات الدم الحمراء. وقد لوحظت زيادة في كرات الدم البيضاء ذات الصيغة المتعادلة وذات الصيغة الحمضية والخلايا الاحادية. وعلى العكس من ذلك فان عدد الخلايا الليمفاوية قد قل بشكل ملحوظ. تشير الزيادة في اليوريا والكرياتينين في البلازما إلى تدهور كبير في وظائف الكلى، ووفقا للبيانات. لم يتأثر هذا التأثير الكلوي مستخلص نبات الكارالوما بنيسلاتا أو الإندوميثاسين، سواء بمفرده أو مجتمعاً. وعليه لم يتمكن المستخلص النباتي موضع الدراسة من معاكسة تأثير الالتهاب المحدث بزراعة قطعة القطن على الكلى وكريات الدم المختلفة .

الكلمات المفتاحية: الكارالوما بنيسلاتا. مؤشرات الدم، وظائف الكلى، حبيبات القطن، الورم الحبيبي