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# Effect of Some Synthesized Pyrrolidines in Growth of *L. infantum* Promastigotes

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**Abstract**

In this research, three pyrrolidine compounds (P1-P3) were synthesized and then tested for efficacy against *L. infantum* promastigotes in vitro. The study included preparation of some chalcones and schiff bases then the condensation of both to get the pyrrolidines and studying the effect of pyrrolidine compounds in growth, generation number and time of parasites. They determined using concentrations between (5-30) µg/ml. The effect of the compounds was found to be important to the parasite growth as the more the concentration the less the growth, generation number and increased the generation time. At the logarithmic phase, the $LD_{50}$ were (10), (20) and (30)µg/ml for (P1-P3) compounds, respectively.

## Introduction

Leishmaniasis is a poverty-associated disease with several different forms, of which the two visceral and cutaneous leishmaniasis are most common. Visceral form is fatal without treatment while the cutaneous infections has a spectrum of presentations, typically with self-healing or chronic lesions on the skin.

Leishmaniasis results from infection by various species of Leishmania, approximately 30 species have been described and at least 20 of these organisms are pathogenic for mammals. Human visceral leishmaniasis is primarily caused by *L. donovani, L. archibaldi*, *L. infantum,* and *L. chagasi*. *L. infantum* that cause cutaneous and visceral infections it, which mainly causes visceral leishmaniasis in people, can cause both visceral and cutaneous disease in dogs, and primarily causes skin lesions in cats and horses [1].

*Leishmania spp*. is digenetic organisms shuttling between a flagellated-promastigote in the mid-gut of the sand fly vector and an intracellular-amastigote in the mammalian host. Sand flies are blood feeders and the infectious promastigotes are transmitted during a blood feeding meal. Promastigotes attach to mononuclear phagocytes and are taken up by phagocytosis into a phagosome, which fuse with lysosomes to form the phagolysosome. Once inside the macrophage, promastigotes undergoes biochemical and metabolic changes and differentiate to the obligatory intracellular form of the parasite. Amastigotes are released from macrophages and can re-invade dendritic and fibroblasts cells as well as new macrophages [2].

Leishmania parasites are spread in Iraq, observed in several provinces and considered one of the areas affected by visceral and cutaneous leishmaniasis [3].

From decades ago, there is no effective drug to treat leishmaniasis, there are 25 compounds or chemical formula showed effectiveness against leishmania but a few of these compounds proved its worth. None of the available drugs can be considered ideal due to their high toxicity, higher prices, long duration of treatment, and severe adverse reactions, which often lead to treatment abandonment.

Pentavalent antimony compounds have been the first-line drugs for treatment of leishmaniasis, which are available in two formulations (Pentostam, $C_{12}H_{38}O_{26}Sb$) and (Glucantime, $C_{14}H_{29}O_{10}N_2Sb$). The second line drug for the treatment is Amphotericin B ($C_{47}H_{73}NO_{17}$) as well Miltefosine, Paramomycine and Pentamidine but none of them are effectiveness [4].

Therefore, there is a need to develop or use new compounds. The compounds that have attracted the attention of many researchers are heterocyclic compounds, which are among the most important types of organic chemistry. They are characterized by containing one or several atoms in their molecular structure, at least one atom other than carbon. Heterocyclic compounds build up by 3 to 6 rings, the

most common consist of 3 or 6 rings which include heteroatoms such as nitrogen, oxygen or sulfur. They synthesize pyrrolidine compounds with formula $C_5H_9N$. These compounds are cyclic secondary amine containing five heterogeneous rings, four carbon atoms and one nitrogen. The five-heterogeneous system is considered the most important part of these compounds, many of them have been shown biological activity as antibacterial [5], antifungal [6], anticancer [7], antitumor [8] and antiviral [9].

Thus, the current research drives at the synthesis of some five rings compounds (pyrrolidines) and study their effects in the growth of *L. infantum* promastigote.

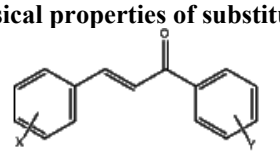
## Material and methods

### 1. Preparation of Chalcones (1,3-Diphenyl prop-2-ene-1-one)

Chalcones were prepared according to [10]. A mixture of 2.2 gm (0.05 mole) of sodium hydroxide pellets, (20) ml of water and (12.5) ml of ethanol was magnetically stirred in a (100) ml round-bottomed flask which immersed in an ice-bath. A (0.043 mole) of freshly distilled of appropriate acetophenone was poured on the stirred mixture followed by (0.043 mole) of substituted benzaldehyde.

The temperature of the mixture was kept at (20-25) C° with a vigorous stirring for three hrs. until they become thick, and then kept in a refrigerator overnight. The product was filtered and washed until the neutralization of filtrates, then washed with (20) ml of cold-ethanol. After drying the crude chalcone in air, it was recrystallized from ethanol. Some physical properties data were illustrated in Table (1).

**Table (1): Physical properties of substituted chalcones**

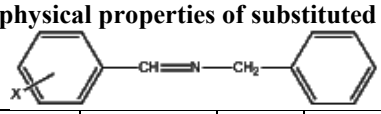


| Comp No. | X | Y | Melting point (C°) | Yield (%) | Color |
|---|---|---|---|---|---|
| C1 | H | $4NO_2$ | 134-136 | 95 | Yellow |
| C2 | 4-$N(CH_3)_2$ | H | 97-99 | 88 | Cumin |
| C3 | 3,4-$CH_2O_2$ | H | 114-116 | 64 | Earthy |

### 2. Preparation of Schiff bases

In a (100) ml beaker, (0.01) mole of benzyl amine was heated with (0.01) mole of substituted benzaldehyde for (10) min. at (100) C° after adding (10) ml of ethanol. The reaction mixture was then cooled and filtered, the precipitate was dried and recrystallized from ethanol, [11]. Table (2) illustrates some physical properties.

**Table (2): physical properties of substituted schiff bases**

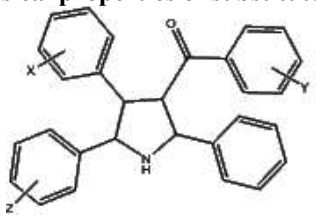


| Comp No. | X | Melting point (C°) | Yield (%) | Color |
|---|---|---|---|---|
| S1 | 4-$NO_2$ | 57-59 | 54 | Crystal yellow-orang |
| S2 | 3,4-$CH_2O_2$ | 74-76 | 72 | Crystal earthy |

### 3. Synthesis of pyrrolidines

These compounds were prepared from a mixture of (0.01) mole schiff-base with (0.01) mole of chalcone. The mixture was magnetically stirred then, (10) ml of ethanol, (3) ml 50% sodium hydroxide added `and the stirring continues for one hour at room temperature. The mixture was kept overnight, and then add (100) ml of water. The product filtered and washed, after drying it was recrystallized by methanol-ethyl acetate [12] as shown in Table (3).

**Table (3): physical properties of substituted pyrrolidines**



| Comp No. | X | Y | Z | Melting point (C°) | Yield (%) | Color |
|---|---|---|---|---|---|---|
| P1 | H | 4-$NO_2$ | 4-$NO_2$ | 115-118 | 53 | Pale brown |
| P2 | 4-$N(CH_3)_2$ | H | 3,4-$CH_2O_2$ | 65-58 | 88 | Distinct yellow |
| P3 | 3,4-$CH_2O_2$ | H | 3,4-$CH_2O_2$ | 63-65 | 88 | Earthy |

**Biological activity of synthesis Pyrrolidines**

**A. *Leishmania* used:**

*MONI / EP 126 Leishmania infantum* standard strain was used. They obtained from the Bioengineering Department, Faculty of Chemistry-Metallurgical, Yildiz Technical University. The culture was cultivated in Tobie medium [13].

**B. Cultivation of parasites:**

1.9 ml of liquid phase was added to McCantry vials that contained 5 ml solid phase slants, then 0.1 ml of promastigotes inoculums was taken from stock culture during log phase, so that the initial density of the organism was 2 x $10^5$ / ml, then the organisms of new culture incubated at 25 $C^o$ for 5 days, was counted using a haemocytometer.

**C. Effect of the pyrrolidines on growth, number of generation and generation time of *L. infantum* promastigotes:**

Effect of the pyrrolidines on growth was studied in vitro in comparison with untreated groups. The compound was dissolved in 2% DMSO. Five concentrations (5, 10, 15, 20, 30) µg / ml were used to determine the ($LD_{50}$) of the cultivated organisms. Numbers of promastigotes were determined at (72,120) hrs. time interval, then generation number and time were estimated according to [14].

**Apparatus used:**

1. Electrothermal Melting Point Apparatus 9300.
2. Shimadzu U.V-Vis Recording U.V 160 Spectrophotometer.
3. F.T.I.R-600 Biotech Engineering magement Co. LTD. (UK).
4. Nuclear magnetic resonance $^1$HNMR.

**Statistical Analysis**

The statistical program (SPSS) version (21) was used to analyze the data [15].

## Results and discussion

**Chemical:**

Pyrrolidines are a five-membered heterocycle containing carbon atoms and nitrogen. Schiff bases (N-Arylidene benzylamine) were added to chalcones (α,β- unsaturated ketone) via 1,3 - anionic cycloaddition under strong basic conditions to afford the substituted pyrrolidines[16]. For the purpose first prepared substituted chalcones, from benzaldehyde and acetophenone in the presence of NaOH and ethanol as solvent using Claisen – Schmidt condensation [17].

Spectroscopic measurements were used in the characterization, UV spectra showed wavelengths at the highest absorption (λ max) for two types of transitions: (n → π*) at range (334-354) nm and (π → π*) a range of (266-324) nm.

On the other hand IR spectra manifested absorption in different locations, the strong range (1649-1662) $cm^{-1}$ referring to stretching vibration of carbonyl group (υ C=O), While the range of the double bond (υ C=C) was (1589-1597) $cm^{-1}$, and the medium package in range (1502-1574) $cm^{-1}$ related to stretching vibration of aromatic ring bonds (υ C—C).

The compounds 1-(p-Nitro phenyl)-3- phenyl prop-2-ene-l-one **(C1)** showed stretching vibrations, symmetric and asymmetric referred nitro group (υ N—O) at (1334) $cm^{-1}$ and (1516) $cm^{-1}$ respectively. Whereas 1-phenyl-3- (p-N,N-dimethyl amino phenyl) prop-2-ene-l-one **(C2)** only show an absorption package at (1167) $cm^{-1}$ which belongs to single bond (υ C-N). Finally, 1-phenyl-3-(3,4-methylene dioxy phenyl) prop-2-ene-l-one **(C3)** manifested the stretching vibrations, symmetric and asymmetric of ether bond (υ C-O-C) at (1167) $cm^{-1}$ and (1254) $cm^{-1}$ respectively. These values support the synthesized possibility of compounds C1, C2 and C3 as shown in the Table (4).

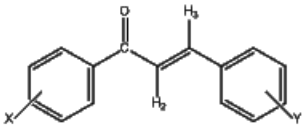
**Table (4): Spectral data of substituted chalcones C1-C3**

| Comp. No. | U.V.( λ max) nm $CHCl_3$ | IR (KBr disc), υ $cm^{-1}$ | | | |
|---|---|---|---|---|---|
| | | C = O | C = C | C — C | Others |
| C1 | 324 , 354 | 1662 | 1591 | 1574 | (N—O) 1516 asym. 1334 sym. |
| C2 | 306 , 336 | 1649 | 1597 | 1529 | (C-N) 1167 |
| C3 | 266 , 334 | 1658 | 1589 | 1502 | (C-O-C) 1254 asym. 1107 sym. |

The Nuclear Magnetic Resonance $^1$HNMR has reversed values for chemical shifts of the protons of C1and C2, as mentioned in the Table (5). We will discuss only NMR spectrum data of chalcones C1, the Olefinic protons H2 and H3 showed doublet of doublet signal (dd) at δ (7.75) and δ (7.97) ppm respectively, whereas the para-substituted ring showed a doublet of doublet (dd) signal for 4H (four protons) at δ (7.6-8.4) ppm and the mono-substituted ring showed a multiplet signal (m) for 5H (five protons) at δ (7.7-8.0) ppm, almost this is evidence lead to correct results. On the other hand, the two methyl ($2CH_3$) group bonded to the nitrogen atom revealed a singlet signal (s) for 6H (six protons) at δ (3.1) ppm this results is in agreement with [5,18,19].

The previous discussion of UV, IR and NMR data was applied on the chains (schiff bases and pyrrolidines) as shows in Tables (6), (7), (8) and (9). Spectral data was mentioned to the compatible with the suggested structures of chalcones, schiff bases and Pyrrolidines.

**Table (5): $^1$HNMR of substituted chalcones C1and C2**



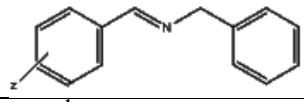
| Comp. No. | $^1$H-NMR (DMSO) , δ (ppm) | | | | |
|---|---|---|---|---|---|
| | Substituents | Olefinic protons | | Aromatic protons | |
| | | H2 | H3 | Para disubstituted ring | Mono substituted ring |
| C1 | $-NO_2$ | 7.75<br>1H, dd | 7.97<br>1H, dd | 7.60-8.40<br>4H, dd | 7.70-8.00<br>5H, m |
| C2 | $-N(CH_3)_2$ 3.1<br>6H, s | 7.54<br>1H, dd | 7.63<br>1H, dd | 7.70-8.09<br>4H, dd | 7.56-7.70<br>5H, m |

In the second stage, they prepare schiff bases: N-(P-nitrobenzylidene) benzyl amine **(S1)** and N-(3',4'-methylene dioxybenzylidene) benzyl amine **(S2)** shows in Tables (6) and (7) by nucleophilic addition of primary amine on aldehyde or ketone carbonyl atom to form carbinol amine with loses of $H_2O$ molecule [20].

**Table (6): Spectral data of substituted schiff bases S1 and S2**

| Comp. No. | U.V.( λ max) nm $CHCl_3$ | IR (KBr disc), υ $cm^{-1}$ | | | |
|---|---|---|---|---|---|
| | | C = N | C — C | C — N | Others |
| S1 | 264 , 336 | 1637 | 1448 | 1107 | (N–Θ) 1518 asym. 1342 sym. |
| S2 | 244 , 316 | 1641 | 1603 | 1207 | (C-O-C) 1255 asym. 1109 sym. |

**Table (7): $^1$HNMR of substituted schiff bases S1 and S2**



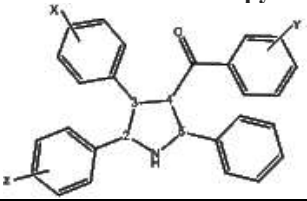
| Comp. No. | $^1$H-NMR (DMSO) , δ (ppm) | | | | |
|---|---|---|---|---|---|
| | Z | H2 | H3 | Aromatic protons | |
| | | | | Disubstituted ring | Mono substituted ring |
| S1 | 4-$NO_2$ | 4.80<br>2H, s | 8.60<br>1H, s | 8.04-8.31<br>4H, dd | 7.28-7.36<br>5H, m |
| S2 | $-OCH_2O$ 6.10<br>2H, s | 4.70<br>2H, s | 8.40<br>1H, s | 7.00-7.25<br>3H, m | 7.30-7.38<br>5H, m |

Finally, schiff bases were condense with chalcones to give the pyrrolidines, 2,4-Diphenyl -3-(p-nitrbenzoyl) -5- (p-nitrophenyl) pyrrolidine **(P1)**, 2-phenyl-3-benzoyl-4-(p-N,N-dimethyl amino phenyl)-5-(3',4'-methylene dioxy phenyl) pyrrolidine **(P2)** and 2-phenyl-3-benzoyl-4,5-[Bis (3',4'-methylene dioxy phenyl)] pyrrolidine **(P3)**. Table (8) and (9).

**Table (8): Spectral data of substituted pyrrolidine P1-P3**

| Comp. No. | U.V.( λ max)nm $CHCl_3$ | IR (KBr disc), υ $cm^{-1}$ | | | |
|---|---|---|---|---|---|
| | | NH | C = O | C — C | ··· Others |
| P1 | 258 , 332 | 3442 | 1641 | 1585 | (N–Θ) 1523 asym. 1344 sym. |
| P2 | 260 , 312 | 3437 | 1641 | 1579 | (C-O-C) 1255 asym. 1097 sym. |
| P3 | 256 , 340 | 3454 | 1658 | 1574 | (C-O-C) 1255 asym. 1105 sym. |

**Table (9): $^1$HNMR of substituted pyrrolidine P1-P3**



| Comp No. | $^1$H-NMR (DMSO) , δ (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | X | Y | Z | NH | H3 | H4 | H2 | H5 | Aromatic protons | |
| | | | | | | | | | Para disubstituted ring | Other Ar-H |
| P1 | H | 4-$NO_2$ | 4-$NO_2$ | 3.15<br>1H, d | 4.10<br>1H, t | 4.30<br>1H, t | 4.75<br>1H, d | 4.95<br>1H, d | 7.62-8.11<br>8H, dd | 7.03-8.50<br>9H, m |
| P2 | $-N(CH_3)_2$<br>3.00<br>6H, s | H | $-OCH_2O-$<br>6.75<br>2H, d | 3.00<br>1H, d | 4.20<br>1H, t | 4.35<br>1H, t | 4.77<br>1H, d | 4.90<br>1H, d | 7.00-8.11<br>4H, dd | 7.24-8.40<br>13H, m |
| P3 | $-OCH_2O-$<br>5.80<br>2H, s | H | $-OCH_2O-$<br>6.12<br>2H, s | 3.10<br>1H, d | 4.15<br>1H, t | 4.37<br>1H, t | 4.70<br>1H, d | 4.89<br>1H, d | ---- | 7.00-8.40<br>12H, m<br>(all rings) |

## Biological

### Effect of synthesized pyrrolidines on parasite growth

Table (10), (11) and (12) show the inhibitory effect of different concentrations of the synthesized pyrrolidines on *L. infantum* growth in comparison with control group during different (72) and (120) intervals. (10), (20) and (30) µg / ml seemed to be the 50% inhibitory-concentration of the promastigotes ($LD_{50}$) at the log phase (120) hrs. of the compounds P1, P2 and P3 respectively. On the other hand, the highest concentrations inhibit more than 80% of growth.

**Table (10): Effect of different concentrations of P1 on *L. infantum* promastigote numbers**

| Exposure time (hrs.) / Treatments (µg / ml) | 72 Mean* ± SE | Percentage inhibition | 120 Mean* ± SE | Percentage inhibition |
|---|---|---|---|---|
| Control | 3.50 ± 0.17 [e] | -- | 16.5 ± 0.27 [f] | -- |
| 5 | 2.89 ± 0.00 [d] | 17 | 12.2 ± 0.40 [e] | 26 |
| 10 | 2.60 ± 0.07 [c] | 26 | 7.56 ± 0.34 [d] | 54 |
| 15 | 2.41 ± 0.06 [c] | 31 | 4.90 ± 0.14 [c] | 70 |
| 20 | 1.81 ± 0.05 [b] | 48 | 2.80 ± 0.02 [b] | 83 |
| 30 | 0.92 ± 0.00 [a] | 74 | 1.21 ± 0.03 [a] | 92 |

* 3-replicates were used for each treatment. Mean and Standard Error were multiplied x $10^6$.
** According to Duncan-test, different letter refers to presence of significant differences between treatments at $P \leq 0.05$.

**Table (11): Effect of different concentrations of P2 on *L. infantum* promastigote numbers**

| Exposure time (hrs.) / Treatments (µg / ml) | 72 Mean* ± SE | Percentage inhibition | 120 Mean* ± SE | Percentage inhibition |
|---|---|---|---|---|
| Control | 12.6 ± 0.31 [f] | -- | 47.4 ± 0.17 [f] | -- |
| 5 | 10.5 ± 0.17 [e] | 17 | 37.2 ± 0.62 [e] | 21 |
| 10 | 9.60 ± 0.57 [d] | 24 | 32.7 ± 0.87 [d] | 31 |
| 15 | 8.70 ± 0.08 [c] | 31 | 29.8 ± 0.03 [c] | 37 |
| 20 | 7.60 ± 0.08 [b] | 40 | 23.7 ± 0.82 [b] | 50 |
| 30 | 4.60 ± 0.05 [a] | 64 | 12.7 ± 0.15 [a] | 73 |

* 3-replicates were used for each treatment. Mean and Standard Error were multiplied x $10^6$.
** According to Duncan-test, different letter refers to presence of significant differences between treatments at $P \leq 0.05$.

**Table (12): Effect of different concentrations of P3 on *L. infantum* promastigote numbers**

| Exposure time (hrs.) / Treatments (µg / ml) | 72 Mean* ± SE | Percentage inhibition | 120 Mean* ± SE | Percentage inhibition |
|---|---|---|---|---|
| Control | 11.8 ± 0.15 [f] | -- | 45.4 ± 1.49 [e] | -- |
| 5 | 10.5 ± 0.12 [e] | 11 | 38.9 ± 0.17 [d] | 14 |
| 10 | 9.60 ± 0.05 [d] | 19 | 36.4 ± 0.79 [c] | 20 |
| 15 | 8.40 ± 0.21 [c] | 29 | 33.1 ± 0.55 [b] | 27 |
| 20 | 7.50 ± 0.05 [b] | 36 | 29.2 ± 0.64 [b] | 36 |
| 30 | 5.50 ± 0.12 [a] | 53 | 22.8 ± 0.60 [a] | 50 |

* 3-replicates were used for each treatment. Mean and Standard Error were multiplied x $10^6$.
** According to Duncan-test, different letter refers to presence of significant differences between treatments at $P \leq 0.05$.

These results which is agreement with [21] used the pyridine derivatives at the concentration that kills 50% of *L. tropica* parasites, they demonstrated that some of these compounds had an inhibitory effect in concentration lower than used with Glucantime which was used to treatment these parasites. Other researchers used low molecular weight compounds as antileishmanial agents. For example, [22] which indicated an inhibitory effect of amide compounds, that extracted from *Paper amalago* and their synthetic analogs on *L. amazonensis* in $LD_{50}$ at logarithmic phase.

Also [23] used both piperzine and pyrrolidine compounds and their derivatives, they explained that five of these compounds at (10) mM concentration had given an effective inhibitory in reducing growth of *Plasmodium falciparum* parasites by killing 50% of these parasites, as well as they mentioned that these compounds are easily intestinal absorbed. In addition, other researchers tried to use the five ring thiohydantions derivatives as antitrypanosomal, [24] indicated that these compounds gave a disincentive effect on growth and gave 100% cure rate in mice infected with parasites after 4 days of oral treatment at 50 mg/kg twice per day.

It is worthy to mention [25,5] confirmed that some pyrrolidines synthesis and their derivatives are effective as antibacterial.

**Effect of synthesized pyrrolidines on parasite generation number and time**

On the light of the previously growth indices, effect of the pyrrolidines on generation number and generation time of *L. infantum* promastigotes were estimated. As for the effect of different concentrations of the pyrrolidines on generation number Table (13) inverse correlation between generation number and concentration were observed, generation number at log phase ranged from (5.92) at 5.0 µg to (2.59) at 30 µg, when compared with control (6.36 generations) of P1, also ranged from (7.54) at 5.0 µg to (5.98) at 30 µg, when compared with control (7.89 generations) of P2 and ranged from (7.60) at 5.0 µg to (6.83) at 30 µg, when compared with control (7.82 generations) of P3.

**Table (13): Effect of different concentrations of P1-P3 on generation numbers of *L. infantum* promastigotes**

| Exposure time (hrs.) / Treatments (µg / ml) | P1 | | P2 | | P3 | |
|---|---|---|---|---|---|---|
| | 72 | 120 | 72 | 120 | 72 | 120 |
| | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE |
| Control | 4.12 ± 0.06 $^{e}$ | 6.36 ± 0.02 $^{f}$ | 5.97 ± 0.06 $^{f}$ | 7.89 ± 0.00 $^{f}$ | 5.88 ± 0.06 $^{f}$ | 7.82 ± 0.00 $^{e}$ |
| 5 | 3.85 ± 0.00 $^{d}$ | 5.92 ± 0.04 $^{e}$ | 5.71 ± 0.04 $^{e}$ | 7.54 ± 0.04 $^{e}$ | 5.71 ± 0.04 $^{e}$ | 7.60 ± 0.04 $^{d}$ |
| 10 | 3.65 ± 0.04 $^{c}$ | 5.23 ± 0.06 $^{d}$ | 5.58 ± 0.15 $^{d}$ | 7.35 ± 0.06 $^{d}$ | 5.58 ± 0.15 $^{d}$ | 7.50 ± 0.06 $^{c}$ |
| 15 | 3.58 ± 0.03 $^{c}$ | 4.62 ± 0.04 $^{c}$ | 5.44 ± 0.55 $^{c}$ | 7.21 ± 0.00 $^{c}$ | 5.39 ± 0.55 $^{c}$ | 7.37 ± 0.00 $^{b}$ |
| 20 | 3.17 ± 0.04 $^{b}$ | 3.80 ± 0.01 $^{b}$ | 5.25 ± 0.03 $^{b}$ | 6.88 ± 0.09 $^{b}$ | 5.22 ± 0.03 $^{b}$ | 7.19 ± 0.09 $^{b}$ |
| 30 | 2.21 ± 0.01 $^{a}$ | 2.59 ± 0.04 $^{a}$ | 4.52 ± 0.02 $^{a}$ | 5.98 ± 0.03 $^{a}$ | 4.78 ± 0.02 $^{a}$ | 6.83 ± 0.03 $^{a}$ |

* 3-replicates were used for each treatment. Mean and Standard Error were multiplied x $10^{6}$.
** According to Duncan-test, different letter refers to presence of significant differences between treatments at $P \leq 0.05$.

However, generation time appeared to depend upon the concentration of the pyrrolidines Table (14) which is increased when concentration increased (direct correlation). At log phase, generation time values ranged from (20.0) at 5 µg to (46.3) at 30 µg when compared with (18.8 hrs.) of control group of P1, also ranged from (15.9) at 5 µg to (20.0) at 30 µg when compared with (15.2 hrs.) of control group of P2, and ranged from 15.7 at 5 µg to 17.5 at 30 µg when compared with (15.3 hrs.) of control group of P3.

**Table (14): Effect of different concentrations of P1-P3 on generation time of *L. infantum* promastigotes**

| Exposure time (hrs.) / Treatments (µg / ml) | P1 | | P2 | | P3 | |
|---|---|---|---|---|---|---|
| | 72 | 120 | 72 | 120 | 72 | 120 |
| | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE | Mean* ± SE |
| Control | 17.4 ± 0.06 $^{d}$ | 18.8 ± 0.02 $^{f}$ | 12.0 ± 0.05 $^{f}$ | 15.2 ± 0.00 $^{f}$ | 12.2 ± 0.05 $^{f}$ | 15.3 ± 0.08 $^{e}$ |
| 5 | 19.1 ± 0.00 $^{c}$ | 20.0 ± 0.04 $^{e}$ | 12.6 ± 0.03 $^{e}$ | 15.9 ± 0.06 $^{e}$ | 12.6 ± 0.05 $^{e}$ | 15.7 ± 0.03 $^{d}$ |
| 10 | 19.5 ± 0.04 $^{c}$ | 22.9 ± 0.06 $^{d}$ | 12.9 ± 0.03 $^{d}$ | 16.3 ± 0.10 $^{d}$ | 12.9 ± 0.03 $^{d}$ | 16.0 ± 0.08 $^{c}$ |
| 15 | 20.0 ± 0.03 $^{c}$ | 25.9 ± 0.04 $^{c}$ | 13.2 ± 0.03 $^{c}$ | 16.6 ± 0.00 $^{c}$ | 13.3 ± 0.10 $^{c}$ | 16.3 ± 0.05 $^{b}$ |
| 20 | 22.7 ± 0.04 $^{b}$ | 31.5 ± 0.01 $^{b}$ | 13.7 ± 0.03 $^{b}$ | 17.4 ± 0.15 $^{b}$ | 13.8 ± 0.03 $^{b}$ | 16.6 ± 0.12 $^{b}$ |
| 30 | 32.5 ± 0.01 $^{a}$ | 46.3 ± 0.04 $^{a}$ | 15.9 ± 0.05 $^{a}$ | 20.0 ± 0.05 $^{a}$ | 15.0 ± 0.08 $^{a}$ | 17.5 ± 0.17 $^{a}$ |

* 3-replicates were used for each treatment. Mean and Standard Error were multiplied x $10^{6}$.
** According to Duncan-test, different letter refers to presence of significant differences between treatments at $P \leq 0.05$.

This results agree with [26] they proved that *L. tropica* parasites when treated with 2.5 µg / ml of 3,4-dihydro-4-(p-anisyl)-6-phenyl pyrimidine -2(1H)-one at the lethal concentration 50% reduced one generation number and increased generation time by 2.5 hrs., and the use of the lethal concentration 90% led to reduction 5 generations and increased the generation time to about 40 hrs. [27] used some synthesized pyrazoline and pyrimidine at $IC_{50}$ concentration, which resulted in a reduction of 0.5-1.5 generation and increasing generation time by about 1-5 hrs.

The synthesized pyrrolidines exhibited relatively high antileishmanial activity. However, the mechanism by which the pyrrolidines killed the parasite is not known. The mechanism of kills may be due to the affected of compounds on cell membrane through effects on osmosis, this will alter the permeability of the cell and may be effect on protein and nucleic acids metabolism.

The difference in effect between the compounds might be due to substituted group, for example: nitro group, dimethyl and methylene as well the aromatic ring effect. Thus there is a need for test the toxicity of the compounds and subsequent biochemical studies to determinate the molecular location that may targeted by this antileishmanial agent.

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# تأثير عدد من مركبات البايروليدين Pyrrolidines المشيدة في نمو بروماستكوت اللشمانيا الأحشائية *L. infantum*

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**الملخص**

في بحثنا هذا تم تصنيع ثلاث مركبات من البايروليدين (P3–P1) ومن ثم اختبارها وملاحظة فعاليتها ضد بروماستكوت اللشمانيا الأحشائية *L. infantum* خارج الجسم الحي.

تضمنت الدراسة تحضير بعض الجالكونات مع قواعد شيف وشيدت مركبات البايروليدين من تكثيف الجالكونات مع قواعد شيف ثم اختبرت فعالية المركبات الناتجة على نمو وعدد وزمن الجيل إذ استخدمت تراكيز تراوحت ما بين (5–30) مايكرو غرام / مل وأظهرت النتائج بأن هنالك تأثير واضح على نمو الطفيليات إذ كلما زاد التركيز قل النمو وكذلك عدد الجيل فضلاً عن زيادة زمن الجيل. وتبين النتائج بان التركيز الذي يقتل 50% من الطفيليات $LD_{50}$ هو (10) و (20) و (30) مايكرو غرام / مل للمركبات (P3–P1) على التوالي عند الطور اللوغاريتمي من النمو.