Pharmacological Investigation of *Solanum incanum* against *P. falciparum*, *L. infantum*, *T. cruzi* and *T. brucei*: A Role of Antioxidant Effect and Clinical Overview

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The *in vitro* antiprotozoal and cytotoxic activity of the *Solanum incanum* leaves and fruit extract of Alba region was assessed against *Plasmodium falciparum* (chloroquine resistant K1 strain), *Leishmania infantum*, two Trypanosoma (*T. cruzi* and *T. brucei*) and MRC-5 cell-lines respectively. Additionally, ethnomedicinal studies by survey and interview, antioxidant potential by DPPH assay was studied for *Solanum incanum*. Results indicated that the *S. incanum* fruit was inactive (IC50: >64 µg/mL) against *P. falciparum* but leaves had shown low activity (IC50: 47 µg/mL) against *L. infantum* both fruit (IC50: 27.3 µg/mL) and leaves (IC50: 27.3 µg/mL) had good activity, against *T. brucei* both fruit (IC50: 34.1 µg/mL) and leaves (IC50: 32.7 µg/mL) had moderate activity. *S. incanum* fruits (IC50: 9.3 µg/mL) had pronounced activity against *T. cruzi* but leaves (IC50: 6.0 µg/mL) had pronounced activity against *T. cruzi* with selectivity index > 1. *Solanum incanum* fruits had stronger antioxidant activity (IC50: 98.7 µg/mL) than leaves (IC50: 293.2 µg/mL) but both fruit and leaves had lower antioxidant activity than standards (Ascorbic acid IC50: 19.1 µg/mL; Trolox IC50: 19.5 µg/mL). Our results demonstrate that *S. incanum* leaves has promising activity against *T. cruzi* possibly active constituents like flavonoid, solasonine and solamargine are contributing for this effect. Furthermore previous reports demonstrate that *T. cruzi* infection is inhibited by antioxidant effects through NRF2 upregulation, possibly our extracts inhibited *T. cruzi* through antioxidant pathway.

**Keywords:** *Solanum incanum*, antimalarial, antileishmaniasis, Antitrypanosomal, Alba, Saudi arabia.
agents caused concerns in usage in poor countries. Pentamidine, Suramin, Melarsoprol, Eflornithine and Nifurtimox-Eflornithine combination are currently used for trypanosomes infection. In stage II *T. brucei gambiense* and stages of *T. brucei rhodesiense*, Pentamidine, Suramin and Eflornithine proved to be ineffective. Melarsoprol have shown toxicity, around 5% of patients died due to post-treatment reactive encephalopathy (PTRE). Moreover 30% patients have shown resistance to these drugs, particularly combination of Nifurtimox–Eflornithine.

*Solanum incanum* commonly seen in Africa, Middle East and South Africa that belongs to subgenus Leptostemonum and Melongena section. It grows as a wild in Madagascar and Mauritius, fruits are used as edible. In Africa, treating for various diseases, entire plant of *S. incanum* is used as folk medicine. Roots consist of new spirostanol saponin, four known saponins, indoside D, dioscin, protodioscin, methyl-protodioscin and steroid glycoalcaloid solamargine. Aerial parts consist of two steroidal glycosidal alkaloids, solasone and solamargine and non-steroidal components like three phenylalkanoic acids, benzyl-O-b-D-xylopyranosyl(1®2)-b-D-glucopyranoside, flavonoids, chlorogenic acid, adenosine and new compound kaempferol 3-O-(6²¢-O-2,5-dihydroxycinnamoyl)-b-D-glucopyranosyl (1®2) b-D-glucopyranoside.

*S. incanum* have shown antibacterial effect against *E. coli, S. pyogenes, S. aureus,* and *P. aeruginosa*. Antipyretic, antinociceptive, hypoglycemic, anorexic effects, insect repellant properties and spasmylatic effects have been reported. Solamargine was found to induce apoptosis in HA549 lung adenocarcinoma cell and hepatoma cells. Solamargine was found to induce apoptosis in breast cancer by increasing the expression of external death receptors such as TNFR-1 related death domain (FADD), Fas, TNFR-1 and triggered death pathway mediated by mitochondria by augmenting the intrinsic proportion of Bax/Bcl-2. Solamargine was found to be effective against human K562 leukemia and squamous cell carcinoma by causing tumor cell bursting and injury by damaging the cell membrane.

*Solanum incanum* leaf extracts have shown antileishmanial activities against *Leishmania amazonensis*. In present research, our aim was to investigate antioxidant, antimalarial activity against *Plasmodium falciparum*, antileishmanial activity against *Leishmania infantum* and antitrypanosomal activity against *Trypanosoma cruzi* and *Trypanosoma brucei* of the methanolic extracts of *Solanum incanum* fruits and leaves from Alba region. To the best of our knowledge, these pharmacological activities have not been reported previously against the above parasites.

**METHODOLOGY**

**Plant Material**

The plant aerial parts were collected between the month of March – June from various sites in Alba town and suburbs of Alba region (Table 1). The plants were taxonomically studied, identified, numbered as voucher specimens and preserved at the pharmacology lab, College of Clinical Pharmacy, Alba University, Saudi Arabia.

**Preparation of Extracts**

Plant aerial parts were air dried and finely powdered, at room temperature 10 g powder was subjected to extraction with 100 mL methanol 4 times with continuous shaking. The crude extract was filtered and evaporated under vacuum at 40°C until dryness. The percent yield was calculated for each dried extract. The collected dried crude extracts were retained at 4°C.

**Antioxidant Activity: DPPH free radical scavenging assay**

The plant extract (500 µL) was added to 5 ml of DPPH solution (0.004% w/v in 80% methanol) in amber coloured bottle. The concentration of the plant extract tested was in the range between 1 µg/ml to 2.5 mg/ml. Ascorbic acid and trolox used as standard, 80% methanol as a blank and DPPH solution without plant extract was used as negative control. In the dark, the reaction complex was incubated at 37°C for 30 min and absorbance was read at 517 nm. The test was repeated 3 times and DPPH scavenging effect was calculated as per the following formula:

\[
\text{% DPPH scavenging effect} = \left( \frac{A_o - A_1}{A_o} \right) \times 100
\]

Where \( A_o \) is the measurement for the negative control, \( A_1 \) is the measurement for the DPPH in presence of plant extract - the measurement of plant extract in 80% methanol. An IC50 value was calculated from the dose inhibition.

curve and results were calculated as average ± SD.

**Antiprotozoal activity**

**Standard Drugs**

Following standard drugs were used for different assays as positive control: tamoxifen - cytotoxicity test against MRC-5, chloroquine - antiplasmodial test against *P. falciparum*, miltefosine - antileishmanial test against *L. infantum*, benznidazole - antityrpanosomal test against *T. cruzi* and suramin for *T. brucei*. All standard drugs were either purchased from Sigma-Aldrich (tamoxifen, suramin) or from WHO-TDR (chloroquine, miltefosine, benznidazole).

**Pharmacological Assays**

Standard screening methodologies and the integrated board for microbial screening was applied as described previously. All experimental tests were repeated thrice at the Microbiology, Parasitology and Hygiene laboratory, University of Antwerp, Belgium. To estimate IC50 (50% inhibitory concentration), the plant extract was studied at five different concentrations (64, 16, 4, 1 and 0.25 µg/mL). The DMSO concentration in final in-test was 0.5%. Simultaneously, fibroblast (MRC-5) cell line cytotoxicity evaluation was performed for estimating antiprotozoal selectivity. For positive activity the criteria was with an IC50 <10 µg/mL and selectivity index (SI) of >1.

**Antiplasmodial Activity**

*P. falciparum* (K 1-strain ; chloroquine resistant) was grown in human erythrocytes O+ in RPMI-1640 medium with human serum supplement (10%) at 37°C with atmosphere with low oxygen (3% - O2, 4% - CO2, and 93% - N2). In multiwall plate, infected human RBCs (200 µL, 1% - parasitaemia, 2% - hematocrit) were added to each well and followed by 72h incubation. Test plates were frozen at -20°C after incubation. The Malstat assay was used to measure parasite multiplication, a colorimetric test based on the 3-acetylpyridine adenine dinucleotide (APAD) reduction by parasite specific lactate dehydrogenase (pLDH).

**Antileishmanial Activity**

Primary peritoneal macrophages of mouse was infected with *L. infantum* (MHOM/MA(BE)/67) amastigotes procured from infected donor hamster spleen. In 96 multiwell plates, macrophages (3 × 10⁴) were seeded in each well to determine in vitro antileishmanial effect. After 48 hours of growth, amastigotes (5 × 10⁵ per well) were reseeded and kept for incubation for 2h at 37°C. Simultaneously pre-diluted plant extracts were added to each well and further kept for incubation for 5 days at 37°C with CO2 (5%). Giemsa staining was done on 500 cells; parasite burden (mean number of amastigotes/macrophase) was determined microscopically and expressed as percentage of the blank control without plant extract.

**Antityrpanosomal Activity**

*Tryptosoma brucei* (Squib-427 strain; suramin sensitive) was grown in hirumi 9 medium with fetal calf serum supplement (10 %) at 37°C with CO2 (5%). In 96-multiwell plate, around 1.5 × 10⁴ trypomastigotes were seeded in each well and after 72h at 37°C, parasite multiplication was determined by adding resazurin. To study effect of *S incanum* on Chagas disease, *T. cruzi* (Tulahuen CL2 ; benznidazole sensitive) was grown on MRC-5 cells in minimal essential medium with glutamine (20 mM) supplement, sodium hydrogen carbonate (16.5 mM) and fetal calf serum (5%). In 96-multiwell plate, MRC-5 cells (4 × 10⁴) and parasites (4 × 10⁴) were seeded in each well, incubated for 7 days at 37°C and parasite multiplication was determined by addition of chlorphenol red ²-D-galactopyranoside a ²-galactosidase substrate. After 4 hrs, the reaction mixture color was read at 540 nm and the readings were depicted as the percentage blank control.

**Cytotoxicity testing against MRC-5 Cells**

MRC-5 SV2 cells were grown in minimum essential medium with L-glutamine (20 mM) supplement, sodium hydrogen carbonate (16.5 mM) and fetal calf serum (5%). For the study, MRC-5 cells (10⁴ per well) were seeded onto multiwell plates containing pre-diluted sample and incubated at 37°C with CO2 (5%) for 72h. After 4 h resazurin was added and cell viability was determined by fluorometry. Fluorescence was determined at excitation 550 nm, emission 590 nm and the results were expressed as percent reduction in cell viability compared to control.

**RESULTS**

In table 1, the plants name, family, investigated parts, voucher specimen numbers, local names, percent yields, and their traditional uses are listed. The DPPH assay is directly
measures the ability of bioactive molecules to scavenge free radicals. The DPPH scavenging activity (IC50) of *Solanum incanum* fruits and leaves, ascorbic acid and trolox as standard are presented in Table 2. *Solanum incanum* fruits had stronger antioxidant activity (IC50: 98.7 µg/mL) than leaves (IC50: 293.2 µg/mL) but both fruit and leaves had lower antioxidant activity than standards (Ascorbic acid IC50: 19.1 µg/mL; Trolox IC50: 19.5 µg/mL).

In table 3, antiprotozoal and cytotoxic activity of standards and *S. incanum* (fruits and leaves) extracts of Al-baha region against tested parasites are presented. The listed IC50 values are the means of three determinations. The following criteria were applied to express their level of activity, when IC50 ≤ 5 µg/ml considered as pronounced activity, 5 < IC50 ≤ 20 considered as good activity, 20 < IC50 ≤ 30 considered as moderate activity, 30 < IC50 ≤ 60 considered as low activity, IC50 > 64 µg/ml considered as inactive. For cytotoxicity, test sample is considered as cytotoxic when CC50 < 32 µg/ml. For the samples tested, the cytotoxicity of MRC-5 cell

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant Family</th>
<th>Part testeda</th>
<th>Local name</th>
<th>Traditional uses</th>
</tr>
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<tbody>
<tr>
<td><em>Solanum incanum</em> L.</td>
<td>Solanaceae (CP-131)</td>
<td>F (7.6%)</td>
<td>Al-hadak</td>
<td>Antiseptic, Leishmaniasis*</td>
</tr>
<tr>
<td><em>Solanum incanum</em> L.</td>
<td>Solanaceae (CP-131)</td>
<td>L (3.9%)</td>
<td>Al-hadak</td>
<td>Leaves as dressing for healing wounds, paste of fruits for treating leishmaniasisa</td>
</tr>
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F: fruits; L: leaves. *most information obtained from interviewing with local people

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<th>Table 2. Antioxidant activity of extracts</th>
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<td>Plant</td>
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*AP, aerial parts; F: fruits; L: leaves.

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<tr>
<th>Table 3. Antiprotozoal effect of the <em>Solanum incanum</em> and its cytotoxicity against MRC-5 cell lines</th>
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<tbody>
<tr>
<td>Plant species (part tested)*</td>
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<tr>
<td>--------------------------------</td>
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<tr>
<td><em>S. incanum</em> (F)</td>
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<tr>
<td><em>S. incanum</em> (L)</td>
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<tr>
<td>Chloroquine</td>
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<td>Miltefosine</td>
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<td>Benznidazole</td>
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<td>Suramin</td>
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<td>Tamoxifen</td>
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*AP, aerial parts; F: fruits; L: leaves.

1IC50: Concentration of extract showing 50% growth inhibition.

1CC50: Concentration of extract showing 50% of mortality of MRC-5 cells

1SI: Selectivity index. —: Not done
activity (IC50: 47 µg/mL), against L. infantum both fruit (IC50: 27.3 µg/mL) and leaves (IC50: 27.3 µg/mL) had good activity, against T. brucei both fruit (IC50: 34.1 µg/mL) and leaves (IC50: 32.7 µg/mL) had moderate activity. S. incanum fruits (IC50: 9.3 µg/mL) had pronounced activity against T. cruzi but leaves (IC50: 6.0 µg/mL) had pronounced activity against T. cruzi with selectivity index > 1. Our results demonstrate that S. incanum leaves have shown promising activity against T. cruzi.

**DISCUSSION**

*Solanum Incanum* aerial parts consist of steroidal components such as two biologically active glycosidial alkaloids, solasonine and solamargine whereas non-steroidal constituents such as benzyl-O-b-D-xylpyranosyl(1®2)-b-D-glucopyranoside, chlorogenic acid, flavonoid, phenylalkanoic acids and adenine. Auta et al reported *Solanum incanum* contained 26.7% of flavonoid additionally they identified antioxidant elements such as riboflavin, ascorbic acid and tocopherol. Flavonoid compounds have been widely attributed for antioxidant properties. Previously reported antioxidant effect of *Solanum incanum* fruits varied from our findings may be due to geographical origin since we found that *Mytrus communis* L essential oil varied its pharmacological properties as per different origin. Steroidal alkaloids tomatidine and solatidine from *Solanum aculeastrum* has been reported for antioxidant properties. Flavonoid has been reported for wider role in counteracting free radicals, it is attributed to inhibit ros, reactive species scavenging, block free radical production and activate antioxidant protection signaling pathways. Furthermore, we previously demonstrated that phenolics compounds like flavonoid as cyanidin-3-O-glucoside are able to upregulate Nrf2 transcriptional factor. Until now there is no report for direct upregulation of Nrf2 by any active constituents of *solanum incanum* but Meybodi et al reported that steroidal alkaloids upregulate Nrf2 and have shown anticancer effect.

The position of oxidative insult in parasitic infection is ambiguous and widely debated, it is said that oxidative generation helps in combating infection, while it is also said that it is contributing in pathophysiology of infection. Furthermore, oxidative insult markers are found to be elevated in infected rats and humans rather in uninfected groups. Appreciation to upregulation of a redox-sensitive gene regulatory system mediated by the transcription factor (Nrf2) which is involved in response to oxidative insult and xenobiotics, communicated through Antioxidant Responsive Element (ARE). Nrf2 stimulates HO-1 (heme oxygenase -1) enzyme expression that counteracts pro-oxidant heme by balancing cellular redox status. Surprisingly, the stimulation of Nrf2 and upregulation of HO-1 expression significantly decreased parasite population in isolated macrophages and in infected animals. This process did not involve in eradicating of apoptotic infected cells and did not depend on immunity mediated by T-lymphocytes. Nrf2 regulated pathway induced infection inhibition did not depend on other effectors as IFN-1, TNF, NO. Particularly, it was proved in iNOS-deficient mice that Nrf2 is able to reduce high levels of *T. cruzi* burden independent of other pathways.

There are about sixteen species in Saudi Arabia profoundly seen in West and Southwest part of the country. Extracts of *S. incanum* were investigated phytochemically and biologically, but there is no reports regarding antiprotozoal activity so far. Another possibility for antitrypanosomal compounds in *S. incanum* extract is steroidal alkaloids or steroid derivatives such as cilistol-A because it was reported that steroidal alkaloids such as solasonine, solamargine and a-chaconine from *Solanum* species were found to have antitrypanosomal activity against *Trypanosoma cruzi*. Furthermore, Pavia et al reported that macrophage specific mechanism, since upregulation of antioxidant pathway decreased *T. cruzi* burden in only macrophages not in other type of cells. Particularly, this trend is important because macrophage is involved in in vivo iron storage. Iron is mobilized and controlled by regulated expression of a particular iron exporter protein-ferroportin. Antioxidant transcription factor Nrf2 transcribes ferroportin and ferritin proteins that are responsible for storage of iron in an inert redox form. So most of the iron is stored, and free iron is made unavailable to intracellular pathogens, predicting this may be a possible mechanism by which antioxidant pathway upregulation is inhibiting *T. cruzi*.  

**REFERENCES**

Developing and testing new drugs in humans that target parasitic protozoa is challenging. In humans, parasitic infections are in different clinical forms, drug resistance and genetic manipulation by parasites are observed and pharmacokinetic requirements of a new drug are complex. Around 1200 medicinal plants are used against malaria and fever throughout the world but most of them did not undergo clinical trials. Many medicinal plants and its isolated secondary metabolite like saponins, phenolics, alkaloids, cardiac glycosides, polyacetylenes and terpenoids screened for anti-trypanosomal activity. Among these metabolites, some have shown promising activity in sub-micromolar concentration but very few established in vivo studies, lack clinical data and not translated in clinical practices. The minimum requirement for herbal drug safety depends on duration of disease state. Pre-clinical and control clinical trial are required for acute diseases whereas for preclinical may be required and clinical trial may be or may not be required for chronic diseases. Challenges for clinical trial for herbal products are many, to name few of them, herbal drugs have improper standardization and quality control, dosage forms are not uniform, inadequate randomization studies, low patient numbers and lack of significant data and very long duration of treatment. USFDA since 1994, do not evaluate herbal medicines under “Food and Drug administration” regulation but evaluated under “Dietary Supplement Health and Education Act of 1994”, by this regulation herbal product as mere dietary supplement to improve and prevent diseases. All these parameters should be considered while testing *S. incanum* extracts in humans.

**CONCLUSION**

*Solanum incanum* antiprotozoal effect has been reported for the first time against *P. falciparum, L. infantum, T. brucei* and *T. cruzi*. *S. incanum* fruits had stronger antioxidant profile. Among tested protozoa, *S. incanum* have shown promising activity against *T. cruzi*, may be antioxidant properties is contributing molecular mechanism to inhibit *T. cruzi*. Further gene expression studies are needed to confirm and establish molecular mechanism of *T. cruzi* inhibition. Appropriate in vivo animal screening studies and clinical trial data should be established for human use.

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