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Antileishmanial Activity of *Cassia fistula* L., *Morus nigra* L. and *Ziziphus jujuba* Mill., Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To investigate the different antileishmanial activities of extracts of *Cassia Fistula* L.*, Morus Nigra* L. and *Ziziphus Jujuba* Mill.
Methodology: In this method, three (03) plants having concentrations between 500 – 2000 µg/mL were subjected to KWH23 strains of *L. tropica* in which Standard drug was Amphotericin B and have negative control for 24 – 48 hours. To check the *in-vivo* studies, plant extract was tested on BALB/c mice (Iqbal et al., 2016).
Results: It showed that inhibition (mean) of KWH23 strains at 500, 1000, 1500, 2000 µg/mL after 48 hours were 92.1 ± 0.02, 95.00 ± 0.05, 97.09 ± 0.07 and 98.05 ± 0.05 % respectively. It decreased the lesion size (mean) from 0.8 ± 0.1 mm to 0.40 ± 0.2 mm having significance value $p < 0.01$ after 8th week, and cure at 200 mg/Kg against intracellular amastigotes in BALB/c mice was 90.00% (95% Cl = 80.05 – 97.00).
Conclusion: The result shows that *Ziziphus jujuba* Mill. leaves possess significant antileishmanial activity.

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Keywords: Ziziphus jujuba Mill.; Cassia fistula L.; Morus nigra L.; Leishmania tropica; promastigotes; amastigotes.

1. INTRODUCTION

Leishmaniasis is an infectious disease, which is prevalent all over the world. It belongs to genus *Leishmania*. It affects millions of people annually around the globe [1]. Existing remedies have high cost, drug resistance, toxicity and high dose regimen. Natural entities exhibit safety and efficacy in terms of drug potential against infectious diseases [2].

Ziziphus jujuba Mill. is commonly known as "Ber" belong to family Rhamnaceae. It is native to china and is cultivated in Australia, southern and eastern Asia and Europe. As traditional medicine, different parts of the plant, especially the fruits, leaves, and stem bark are used for thousands of years. In this plant, various pharmacological activities observed including antifungal, antioxidant, anti-inflammatory, anticancer, immunostimulating, hepatoprotective, antiobesity and antiulcer due to presence of flavonoids, phenolics, triterpenic acids etc [3].

Cassia fistula L. locally known as "Amaltas" (family: Caesalpinioidease) is cultivated in Asia, China, West Indies and South Africa. Its fruit is used to treat fever, leprosy, abdominal pain and various skin diseases, contain aspartic acid, Fe and Mn, Glutamic acid, lysine, Isoflavone, biochanin A, Kaempherol, tannic matter etc [4].

Morus Nigra L. is locally termed as "Shahtoot" (family: Moraceae), which is cultivated in Africa, Asia, America and Europe. Different parts of plant including root, fruits, bark and twigs contain flavonoids, triterpenes and saponins exhibit pharmacological effects like antioxidant, anticancer, antibacterial [5] and antileishmanial [1].

In this work, Anti-leishmanial effects (*in-vitro* and *in-vivo*) of Leaves of *Ziziphus jujuba* Mill*.,* Fruit of *Morus Nigra* L. and Fruit of *Cassia Fistula* L. were checked against *L. tropica* parasites.

2. MATERIALS AND METHODS

2.1 Chemicals

Amphotericin B, dimethyl sulfoxide (DMSO), RPMI-1640 medium, penicillin, streptomycin, formic acid, Fetal bovine serum (FBS), and analytical grade methanol as solvent were purchased from Merck Pakistan.

2.2 Plant Material Collection and Crude Extract Preparation

Leaves of *Ziziphus jujuba* Mill. (ZJL)*,* Fruit of *Morus Nigra* L. (MNF) and Fruit of *Cassia Fistula* L. (CFF) having identification number (Bot, 200104 (pup), Bot, 200103 (pup) and Bot, 200105 (pup)) were collected from the area of D. I. Khan, KPK, Pakistan in March 2016. Identification was done by Prof. Dr. Habib, Faculty of Agriculture, and was deposited at Department of Botany, Gomal University, DIKhan. This method was followed by Iqbal et al. [1].

2.3 Antileishmanial Activity *(In-vitro)*

In this study, KWH23 strains of *L. tropica* were used for antileishmanial analysis of MNF, ZJL and CFF where as method was adopted from Iqbal *et al.*, 2017b & Iqbal *et al.*, 2016.

2.4 Anti-leishmanial Activity *(In-vivo)*

In this process, BALB/c mice (Male) having weight of 20-32 gm and aged of 6-8 weeks were used which were supplied by National Institute of Health, Pakistan. This method was adopted from Iqbal *et al.*, 2017a.

2.5 Statistical Analysis

In-vivo experimental work having IC_{50} value, percentage cure rate and mean lesion size (mm) was determined by Non-linear regression and was measured by Graph Pad Prism 6 software, whereas level of significance was $p < 0.05$. Percentage inhibition of cell was Analysed in triplicate and measurements of data was analyzed in Mean±SD.

3. RESULTS

3.1 Antileishmanial *(In-vitro)* Test

At 24^{th} h the percentage inhibition of plant extracts at 500 µg/mL and 2000 µg/mL ranging between 29.02 % and 51.21 % and 40.05 % and 65.82 % respectively. The percentage inhibition at 48^{th} h ranged between 68 % and 92.10 % at

500 µg/mL and between 80.23 and 98.05 % at 2000 µg/mL (Table 1). All extracts showed promising parasite inhibition against *L. tropica* that is 98.05 % inhibition at 2000 µg/mL exhibited by *Ziziphus Jujuba* Mill. and 24th h analysis of MNF and CFF shows 40.05 % and 42.22 % at 2000 µg/mL in contrast to the negative control.

The significant results against anti-leishmanial activity were shown by ZJL after 24th h at 2000 µg/mL ranging between 51.21% and 65.62%.

3.2 Antileishmanial (*In-vivo)* Test

In the albino mice that are infected with 0.02 mL KWH23 strains (1.5 × 10^6 promastigotes/mL) effective *in-vivo* anti-leishmanial results of ZJL, MNF, and CFF after 36 – 120 days has been shown (Table 2). After treatment with the plant extracts of 3 sample groups at the end of the 8th week, the mean lesion size of mice decreased significantly from 0.80 ± 0.1 mm to 0.40 ± 0.2 mm, but in comparison with the negative control group mean lesion size reached 1.51 ± 0.2 mm ($p > 0.05$), and of Amphotericin-B group it decreased from 0.85 ± 0.4 mm to 0.36 ± 0.4 mm. After 8 weeks of treatment, the mean lesion size and % cure in mice that received MNF and CFF methanolic extracts are 0.50 ± 0.3 mm and 0.45 ± 0.1 mm, respectively; with % cure of 72.15 % and 85.10 % respectively.

The anti-leishmanial effects of ZJL against KWH23 strains shows a decreased average mean lesion size to 0.40 ± 0.2 mm correlate to 90.00% cure and the result possessed significant activity.

4. DISCUSSION

In current work, ZJL at different concentrations showed inhibition of KWH23 strains ($p<0.0)1$ after 48 hours which is an agreement with previously work reported [1].

Secondary metabolites such as triterpenic acid, saponins, and phenolics are confirmed in ZJL as per previous reportings by *Yoshikawa* et al. [6-7]. Antileishmanial activity in ZJL is due to the presence of these secondary metabolites. This antileishmanial activity in ZJL is more than other plants (CFF and MNF) as studied in this work. Mean lesion size was decreased after 8th week and cure after 120 days in BALB/c mice when ZJL extract was administered at regular interval of time. In this case, significant inhibition of Metabolic pathways of KWH23 strains are due to administration of ZJL extract [1].

It is first time to report the antileishmanial activities of ZJL, CFF and MNF against KWH23 strains which showed significant results.

Table 1. Plant extracts *in-vitro* anti-leishmanial effect

Sample	Sample Concentration (µg/mL)	Inhibition (%)	
		24 h	48 h
ZJL	500	51.21 ± 0.05	92.1 ± 0.02
	1000	56.11 ± 0.01	95.00 ± 0.05
	1500	60.00 ± 0.03	97.09 ± 0.07
	2000	65.82 ± 0.01	98.05 ± 0.05
MNF	500	29.02 ± 0.02	68.00 ± 0.03
	1000	35.01 ± 0.01	75.05 ± 0.05
	1500	38.02 ± 0.05	78.09 ± 0.09
	2000	40.05 ± 0.08	80.23 ± 0.08
CFF	500	34.20 ± 0.01	72.00 ± 0.09
	1000	36.15 ± 0.05	75.20 ± 0.06
	1500	40.18 ± 0.03	80.31 ± 0.08
	2000	42.22 ± 0.09	84.50 ± 0.02
Amp	10	60.02 ± 0.01	95.43 ± 0.02
	25	65.05 ± 0.03	97.52 ± 0.04
	50	70.06 ± 0.00	98.05 ± 0.10
	100	72.55 ± 0.09	99.01 ± 0.09
NC	500	00.00 ± 0.00	00.00 ± 0.00
	1000	00.00 ± 0.00	00.00 ± 0.00
	1500	00.00 ± 0.00	00.00 ± 0.00
	2000	00.00 ± 0.00	00.00 ± 0.00

NC= Negative Control; p< 0.05; Amp= Amphotericin B (Standard Drug)

Table 2. Plant extracts *in-vivo* anti-leishmanial effect

Sample	Dosage (for 5 Days)	Mean lesion size (mm) before treatment	Mean lesion (mm) size after treatment (8 weeks later) ($p < 0.01$)	% Cure rate (with 95 % level of confidence intervals, CI)	No: of BALB/c mice cured/ No: of mice infected	Mean survival time (Days)
ZJL	40 mg/Kg	0.80 ± 0.1	0.40 ± 0.2	90.00 (80.05 – 97.00)	5/6	≥ 60
MNF	40 mg/Kg	0.80 ± 0.2	0.50 ± 0.3	72.15 (60.00 – 85.13)	3/6	≥ 60
CFF	40 mg/Kg	0.82 ± 0.1	0.45 ± 0.1	85.10 (71.02 – 90.15)	4/6	≥ 60
Amp	15 mg/Kg	0.85 ± 0.4	0.36 ± 0.4	96.16 (80.91 – 98.25)	6/6	≥ 60
NC	40 mg/Kg	0.90 ± 0.2	1.51 ± 0.2	0.00	0/6	≥ 0

NC= Negative Control; $p< 0.05$; Amp= Amphotericin B (Standard Drug)
Table shows the mean lesion size (mm) ± SD

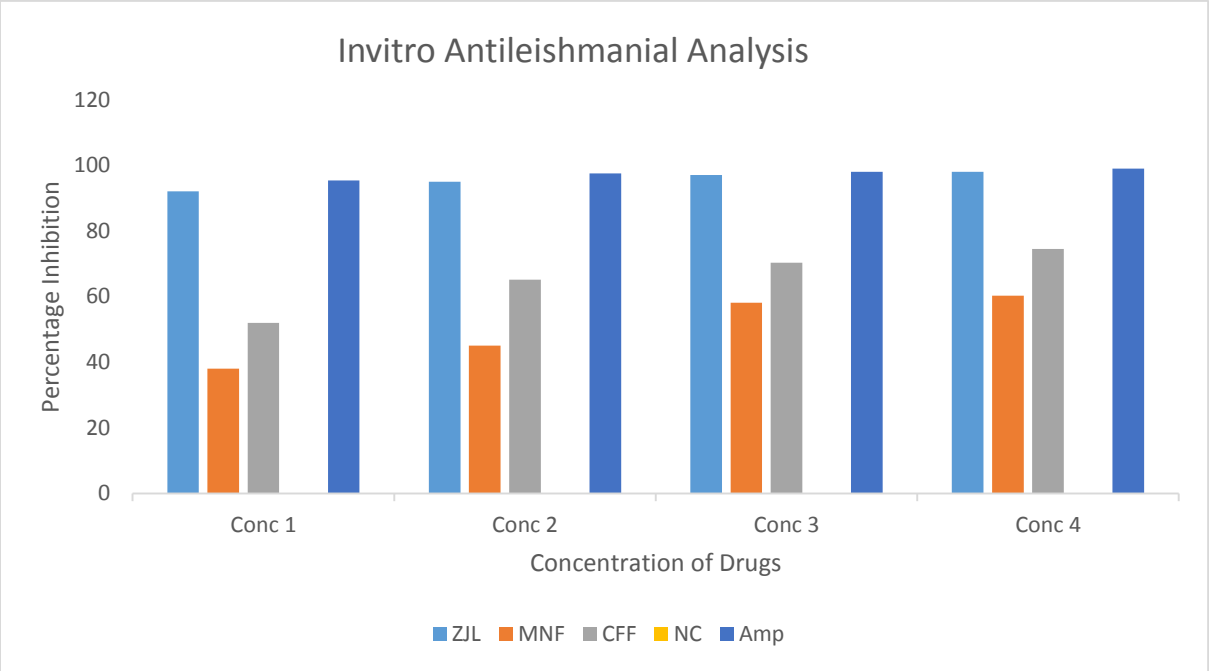

Fig. 1. Plant extracts *in-vitro* anti-leishmanial effect

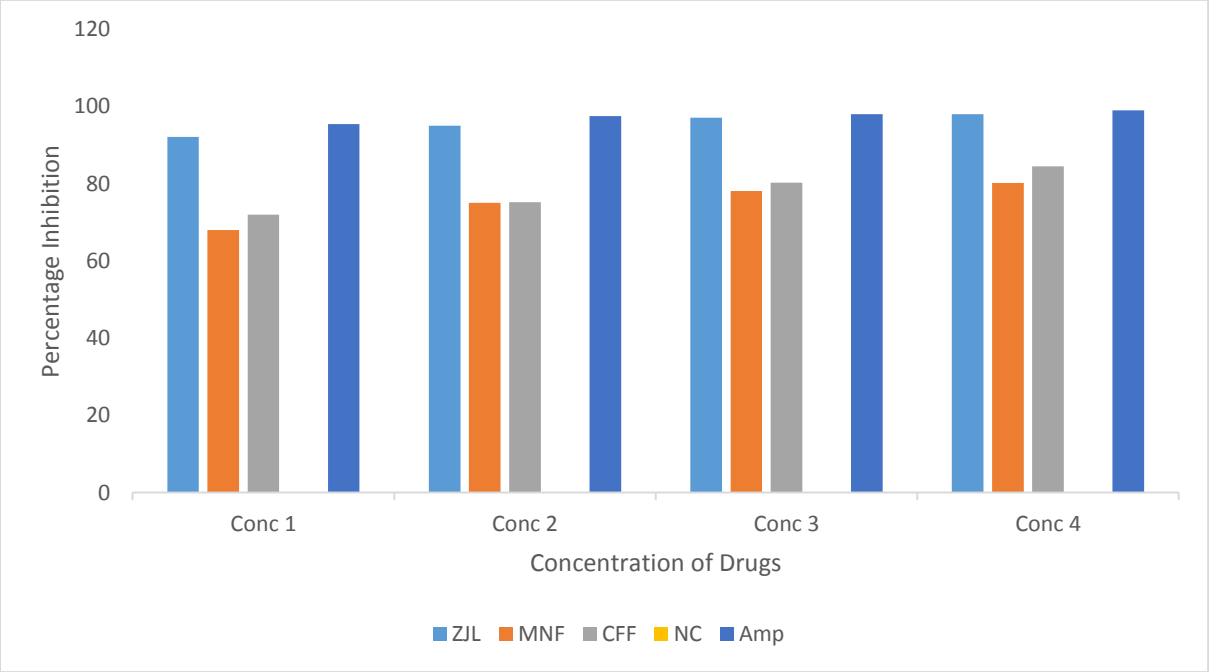

Fig. 2. Plant extracts *in-vivo* anti-leishmanial effect

5. CONCLUSION

The result highlighted that due to presence of phenol, flavonoids, and triterpenes in *Ziziphus jujuba* Mill. It showed remarkable antileishmanial effects against *L. tropica* and this effective part of the plant can be a significant source for the latest anti-leishmanial agents.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for

any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Institutional Animal Ethical committee of Department of Pharmacology, The University of Lahore - Islamabad campus having approval ref. no. DOP/UOL/039, approved this study. [The National Academies Press; 2010]. During the experiments, the animals were given the standard diet and water.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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