PRELIMINARY STUDY ON THE BROAD SPECTRUM ANTIPARASITIC EFFECTS OF NITAZOXANIDE ON *ECHINOCOCCUS GRANULOSUS* AND *BLASTOCYSTIS SPP.*, *IN-VITRO*.

Thesis
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ABSTRACT

Objective: The main objective of this work is to study the broad spectrum effects of Nitazoxanide on *Echinococcus granulosus* protoscoleces as an example of Helminthes and *Blastocystis* forms as an example of protozoa *in vitro* compared to the standard drugs for both parasites.

Methods: Hydatid protoscoleces and *Blastocysis* were cultivated each on its specific medium. The Culture tubes were divided into three groups to which; Nitazoxanide, Albendazole and combined both drugs were added in case of hydatid. While for *Blastocystis*, the culture tubes were divided into three groups to which; Nitazoxanide, Metronidazole and combined both drugs were added. These culture tubes were compared with that of the control culture with no added drugs. Viability of both parasites was assessed before and after adding the drugs by monitoring movement of suckers and rostellum and the use of Trypan blue exclusion test in case of hydatid, while for *Blastocystis*, viability assessment was done by Neutral red stain. Further confirmation of viability was done by subjecting samples to electron microscopic study for both parasites.

Conclusion: For hydatid, there was direct proportional relationship between number of dead protoscoleces and tested Nitazoxanide concentration together with the exposure time. The lethal dose of Nitazoxanide which killed 50% of protoscoleces was 10µg/ml at 24 hours i.e. LD50 of Nitazoxanide was10µg/ml at 24 hours while its Lethal dose which killed 90% of protoscoleces was15µg/ml at 24 hours i.e.LD90 was 15µg/ml at 24 hours. LD50 of Albendazole was 30µg/ml at 6 hours while its LD90 was 30µg/ml at 48 hours. Combined both drugs demonstrated variable effect against hydatid. 80% of control culture protoscoleces remained viable till the end of exposure time. For ***Blastocystis***, LD50 of Nitazoxanide was 2µg/ml at 48 hours in some cultures. LD90 of the drug was 10µg/ml at 48 hours in one of the cultures. Metronidazole produced variable effect against *Blastocystis* (40-76% deaths) at a concentration of 200µg/ml after 48 hours in all cultures. Combined both drugs showed variable effect against *Blastocystis*.
Electron microscopic study showed structural and morphological changes in drug treated cultures of both parasites.

Key words: *In vitro*, Nitazoxanide, Albendazole, Hydatid, Metronidazole, *Blastocystis*, electron microscope.

LIST OF ABBREVIATIONS

AE:	Alveolar echinococcosis.
AIDS:	Acquired immunodeficiency syndrome.
ATP:	Adenosine triphosphate.
B.hominis:	*Blastocystis hominis.*
***C.parvum*:**	*Cryptosporidium parvum.*
CE:	Cystic echinococcosis.
CT scan:	Computed tomography scan.
DMSO:	Dimethylsulphoxide.
DNA:	Deoxyribonucleic acid.
E.dispar:	Entamoeba dispar.
***E.granulosus*:**	*Ecchinococcus granulosus*.
***E.histolytica*:**	*Entamoeba histolytica*.
E.I.P.I.C.O.:	Egyptian International Pharmaceutical Industries Company.
ELISA:	Enzyme-linked immunosorbent assay.
F. hepatica:	*Fasciola hepatica.*
FDA:	The US Food and Drug Administration.
FECT:	Formol ethyl acetate concentration technique.
G.intestinalis:	*Giardia intestinalis*.
GIT :	Gastrointestinal tract.
GM-CSF:	Granulocyte-macrophage colony-stimulating factor.
***H. nana*:**	*Hymenolepis nana*.
HEPES:	Hydoxyethylpiperazine-N-2-Ethanesulfonic Acid.
HIV:	Human immunodeficiency virus.
IBS :	Irritable bowel syndrome.
IFA :	Indirect fluorescent antibody.

IgG:	Immunoglobulin G.
IL:	Interleukin.
INF-γ:	Interferon -γ.
LD:	Median lethal dose.
mM:	Millimol.
MRI:	Magnetic resonance imaging.
Mz:	Metronidazole.
Mzr:	Metronidazole resistant.
OIE:	Office of International Education.
PAIR:	Puncture, Aspiration, Injection and Reaspiration.
PBS :	Phosphate buffered saline.
PCR:	Polymerase chain reaction.
PFOR:	Pyruvate-ferredoxin oxidoreductase.
Psi:	Pressure/square inch.
Rpm	Rotor speed/ minute.
RPMl:	Roswell Park Memorial Institute medium.
SEM:	Scanning electron microscope.
Spp.:	Species.
***T. saginata*:**	*Taenia saginata*.
TEM:	Transmission electron microscope.
Th1&2:	T- helper 1&2.
WHO:	World health organization.

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INTRODUCTION

Parasitic infections rank among the most significant causes of morbidity and mortality worldwide, yet economic and other factors have contributed to lack of innovation of new drugs to treat these diseases (Gilles and Hoffman, 2002). Despite the development of cases refractory to treatment and the withdrawal of several antiparasitic drugs from the market due to their adverse effects, there has been very little effort to develop new agents to treat such human parasitic infections being more prevalent in poor communities (White, 2004).

In this context, the development of Nitazoxanide is quite remarkable (White, 2003). Nitazoxanide was originally discovered in the 1980s by Jean François Rossignol *et at* the Pasteur Institute. Initial studies demonstrated activity versus tapeworms. Further *in vitro* studies demonstrated much broader activity (Rossignol and Maisonneuve, 1984). Clinically, Nitazoxanide, was found to be effective in the treatment of a broad range of helminthic and protozoal infections (Abaza *et al*, 1998 and Cedillo-Rivera *et al*. 2002). Such a new broad-spectrum antiprotozoal and anthelmintic drug offers interesting possibilities for managing parasitic infections (Gilles and Hoffman, 2002). Cystic echinococcosis (CE) and blastocystosis were selected in the present study to investigate the *in vitro* effect of Nitazoxanide on both infections.

The preferred treatment strategy for CE is radical resection of the parasitic mass. Dissemination of protoscoleces during surgery can be considered a new source of infection. Thus, as a protoscolicidal agent such as Praziquantel can be included in surgical drainage approaches (Naguleswaran *et al*., 2006). For inoperable cases, chemotherapy is the only option.

Benzimidazole carbamate derivatives, such as Albendazole and Mebendazole, are currently the drugs of choice (Walker *et al*., 2004). However, benzimidazoles were shown to have several adverse side effects including

affection of liver function, leucopenia and alopecia. Albendazole has been shown to be teratogenic in rats and rabbits; it should therefore be avoided during pregnancy and lactation (Kern, 2003). As a consequence, the development of alternative means of treatment of CE is necessary. Amphotericin B is an alternative drug for patients who develop hepatic complications with Benzimidazoles (Walker *et al*., 2004).

Recently, Stettler *et al*., 2003 and walker *et al.*, 2004 have reported that Nitazoxanide has a lethal effect on *Echinococcus multilocularis* and *Echinococcus granulosus* protoscoleces and metacestodes *in-vitro*. The drug effects were comparable to the drug of choice, Albendazole, and when combined with Albendazole, chemotherapy exhibits profound anti-parasitic activity (Stettler *et al*., 2004). In 2009, Winning and his collegues have reported a case with long history of progressive bony hydatid disease treated with Nitazoxanide that showed marked clinical and radiologic response.

As regards *Blastocystis* infection the treatment of choice is Metronidazole, also emetine, Trimethoprim-Sulfamethoxazole, and Pentamidine are effective for refractory infections. However, recurrence of symptoms after treatment and cases refractory to treatment have been reported in some cases. (Boorom *et al*., 2008).

In a clinical study among Egyptian children done by Rossignol *et al*, 2005, persistant diarrhea in children caused by *Blastocystis spp* was treated effectively with Nitazoxanide. A combination therapy of drugs including Nitazoxanide has been proven to be more successful for the treatment of resistant forms of *Blastocystis* infection (Borody, 2007). Therefore Nitazoxanide with its promising results appears to be the clue for many parasitic diseases, giving the chance for further researches to be done.

AIM OF THE WORK

The aim of the present work is to study the broad spectrum effects of Nitazoxanide on *Echinococcus granulosus* as an example of helminthes and *Blastocystis* as an example of porotozoa *in vitro* as compared to the standard drugs for both parasites.

REVIEW OF LITERATURE

CYSTIC ECCHINOCOCCOSIS (CE)

Human echinococcosis is a zoonotic infection caused by the tapeworm of the genus *Echinococcus*. Of the four known species of *Echinococcus*, three are of medical importance in humans. These are *Echinococcus granulosus*, causing cystic echinococcosis (CE); *Echinococcus multilocularis*, causing alveolar echinococcosis (AE); and *Echinococcus vogeli* causing Polycystic echinococcis. *E. granulosus* is the most common of the three (Eckert and Deplazes, 2004) and the one of our concern in this literature.

Scientific classification (Lymbery *et. al*., 1990):

Kingdom: *Animalia.*

Phylum: *Platyhelminthes*.

Order: *Cyclophyllidea*.

Family: *Taeniidae*.

Genus*: Ecchinococcus*.

Species: *granulosus*.

Epidemiology

Hydatid disease is prevalent throughout most of the world. CE occurs on every continent except Antarctica and may be transmitted in arctic, temperate and tropical regions. Human infection with CE is caused by the larval stage of *E.granulosus* and is cosmopolitan in distribution in north and northwestern China, parts of South America, East Africa, Australia, Central Asia, the Mediterranean littoral (including North Africa) and Russia. CE is also endemic in parts of Western Europe and southern USA (Gemmell *al et*, 1987)(Fig 1). By 2002, two countries, Iceland and New Zealand, and