

ASSESSMENT OF THE EFFECT OF *ALLIUM SATIVUM* (GARLIC) AND *CURCUMA LONGA* (CURCUMIN) IN COMBINATION WITH DRUGS ON CULTURED *TRICHOMONAS VAGINALIS*

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Abstract

Trichomoniasis is an infectious disease caused by *Trichomonas vaginalis*. It inflicts severe complications to human genitourinary system. The devastating negative effects and resistance emerged to known medication impose the search for effective and safer alternatives. This study evaluated the effect of curcumin and garlic as well as combination between their effective doses with Metronidazole® & Tinidazole®. So, vaginal swabs were obtained from the symptomatic patients, and cultured on modified Diamond's medium. Assessment of the herbs various concentrations at different follow-up periods was done by counting dead *T. vaginalis* trophozoites by using hemocytometer and trypan blue staining. The results showed that curcumin 400mg/ml gave a promising anti-*Trichomonas* especially when combined with garlic 90mg/ml that showed a high synergistic effect.

Keywords: Curcumin, Garlic, *Trichomonas vaginalis*, Culture, Metronidazole, Tinidazole

Introduction

Trichomoniasis is a sexually transmitted worldwide disease caused by protozoan *T. vaginalis* (Poole and McClellan, 2013). It was considered as a re-emerging infectious disease (Setzer *et al*, 2017). It mainly affects male & female urogenital tract particularly childbearing ones (Paz-Bailey *et al*, 2010). Trichomoniasis prevalence between 2012 & 2016 was 110.4million cases (Rowley *et al*, 2019). Clinically, it varies between asymptomatic and carriers, to symptomatic; cervicitis, urethritis and serious pelvic inflammations (Poole and Mc Clellan, 2013), to infertility, cervical cancer and HIV (Tsevat *et al*, 2017). Trichomoniasis in man causes urethritis, epididymitis, prostatitis and infertility or prostatic cancer (Sutcliffe *et al*, 2012). There were many diagnostic methods, as staining, immunochromatography and nucleic acid amplification (Chalamilla *et al*, 2006), and serology as ELISA (Sharma *et al*, 1991) or PCR (Walsh *et al*, 1991). But, Diamond's medium (modified) gave a more prolific growth over a shorter time as the golden standard for *T. vaginalis* diagnosis and research studies (Gelbart *et al*, 1990).

Trichomoniasis ongoing drugs are metronidazole (MTZ) and tinidazole. Metronidazole is an antibiotic member of 5-nitroimidazole family (Muzny and Schwebke, 2013). It has many side effects (Ali and Nozaki, 2007) particularly when higher doses were indicated in steadily increased resistant cases (Howe and Kissinger, 2017). MTZ causes nausea, dizziness, hypersensitivity, dermatitis (Schwebke and Barrientes, 2006), and with teratogenic & carcinogenic effects on fetus (Calzada *et al*, 2007). But, no alternative safe therapy to overcome these serious side effects or treat the resistant trichomoniasis cases (Unemo *et al*, 2017). This emphasized the urgent need for safe effective treatment of medicinal plants or herbs origin (Abdel Hady *et al*, 2008).

Allium sativum (Garlic) is a popular vegetable which is used as a spice and food additive (Singh *et al*, 2009) and in modern medicine (Martins *et al*, 2016). Thiosulfates, sulphur-containing amino acids, and allicin are responsible for the therapeutic benefit of garlic (Hornickova *et al*, 2010).

Sulphur compounds have antimicrobial, anticancer, antioxidant, anti-inflammatory,

cardio-protective, antidiabetic and immunomodulatory activities (Lanzotti *et al*, 2014), and the produce glutathione has antioxidant activity (Banerjee *et al*, 2003). Garlic active the immune cells via allicin bioactive properties (Alorainy, 2011), anti-protozoal effect (Ayrle *et al*, 2016) and anti-cryptosporidiosis (Abouel-Nour *et al*, 2016). *Nigella sativa* (*N. sativa*) is an ancient annual plant, with therapeutic activities against *Trichomonas vaginalis* (Al-Am-mash, 2017), hepatitis C virus, and *Helicobacter pylori* (Tavakkoli *et al*, 2017).

Curcumin is a polyphenol extract from the common spice turmeric (*Curcuma longa*), a rhizomatous perennial plant use in Indian Ayurvedic Medicine for thousands of years (Sharma *et al*, 2005). It is also used for food coloring (Buescher and Yang, 2000) and studied for biological effects. An impressive array of potent pharmacological effects were reported; as inhibitory effects on *Giardia lamblia* (Perez-Arriaga *et al*, 2006), *Eimeria tenella* sporozoites (Khalafalla *et al*, 2011), *Plasmodium* spp. (Mimche *et al*, 2011), *Cryptosporidium parvum* (Shahiduzzaman *et al*, 2009), *Schistosoma mansoni* (Allam, 2009), *Leishmania amazonensis*, *L. mexicana* & *Trypanosoma brucei* (Changtam *et al*, 2010), and HIV (Barthelemy *et al*, 1998), Also, it is anti-oxidant (Wei *et al*, 2003) anti-inflammatory (Chainani-Wu, 2003), anti-carcinogenic (Devasena *et al*, 2003), immuno-modulatory (Yadav *et al*, 2005), and supported curcumin and its derivatives as broad-spectrum antiviral agents (Jennings and Parks, 2020) Also, it has protective effect on β -pancreatic cells (Srivivasan *et al*, 2003). So, curcumin fulfils the basic requirements of a potential effective & safe drug, cheap, available and well tolerated.

This study aimed to evaluate the anti-*Trichomonas* effect of curcumin & garlic alone or combined versus metronidazole and tinidazole.

Material and Methods

This study was done in the Research Laboratory, Department of Medical Parasit-

ology, Faculty of Medicine, Aswan University from December 2019 to November 2020.

Culture: Vaginal swabs were taken from 30 females with suspected trichomoniasis. Wet mounted samples were immediately examined (Patil *et al*, 2012) or preserved in amines transport medium for 24-48hrs. From 30 positive samples, parasites were grown on pH6.2 modified Diamond's medium (Diamond, 1957) and Broth medium (Rayner, 1968), supplemented with fetal bovine serum and mixture of penicillin and streptomycin at 37°C. *T. vaginalis* maintenance was subculture (Diamond and Bartgis, 1962).

Drugs and herbs: MTZ (250mg) tablets were crushed, dissolved in distilled water, and diluted in culture medium (100mg/ml). Garlic or Tomex (90mg/ml) were prepared. Curcumin was dissolved in polyethylene glycol (PEG) to have 10% stock solution that was diluted to gain an appropriate working solution of 1.600mg curcumin/ml. This solution was diluted into wells as 800 & 400mg/ml, respectively (Ibrahim, 2013).

Experimental design: *T. vaginalis* trophozoites were cultured on Broth and modified Diamond's media. Cultures treated with different metronidazole, tinidazole, curcumin and garlic. Different MTZ doses were tested to highest effective dose or as a positive control.

T. vaginalis trophozoites were incubated (3×10^4 cells/tube) with each herb as duplicate for 24, 48 & 72hrs. Both MTZ treated culture and control cultures (parasite only), were procedure for extracts. Combined groups included MTZ and the highest effective herbal dose.

Effect of different agents' on *T. vaginalis* was done by: a- Inverted microscopic examination after 24hrs, 48hrs & 72hrs. b- Hemocytometer and trypan blue stained to count the dead trophozoites after 24hrs, 48hrs & 72hrs (Arbabi *et al*, 2016).

Statistical analysis: Data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 25.0 and the ANOVA.

Ethical approval: The study was approved by the Ethics Committee and Review Board, Faculty of Medicine, Aswan University, and signed consents were taken from patients.

Results

Dead *T. vaginalis* trophozoites on modified Diamond's medium were less than Broth medium with significant difference at follow up. Combination between curcumin 400 mg/ml. & tomex 90mg/ml. gave the highest mean of trophozoites death at different periods with significant ($p < 0.001$). Curcumin 400mg/ml. & MTZ100 mg/ml. gave a good

trophozoites death at different periods. Combination between curcumin 400mg/mL and tomex 90mg/ml gave highest dead *T. vaginalis* trophozoites at different follow up periods with significant ($p < 0.001$). Combination between tomex 90mg/ml. and tinidazole 100mg/ml. gave better mean of trophozoites death than tomex 90mg/ml and metronidazole 100mg/ml. at different periods. Combination between curcumin 400mg/ml. and tinidazole100mg/ml. gave good trophozoites death at different follow ups.

Details were given in tables (1, 2 & 3).

Table 1: Comparison between modified Diamond & Broth medium as to dead *T. vaginalis* trophozoites.

Culture	After 24hrs	After 48hrs	After 72hrs
Modified Diamond	15±5	18±3	35±2
Broth medium	100±4	220±7	270±9

Modified Diamond's medium gave better survival of *T. vaginalis* trophozoites.

Table 2: Effect of metronidazole, tinidazole, tomex & curcumin on mean dead *T. vaginalis* trophozoites at different periods.

Agent	After 24hrs	After 48hrs	After 72hrs	P value
Modified Diamond's medium (negative control)	15±5	18±3	35±2	0.01
Metronidazole100mg/ml (positive control)	6±2	24±8	70±20	0.001
Tinidazole 100mg/ml	10±4	30±7	100±20	0.001
Tomex 90mg/ml	15±2	40±4	50±9	0.001
Curcumin 400mg/ml	18±2	45±3	120±8	0.001
P value	<0.001	<0.001	<0.001	

Curcumin 400mg/ml gave highest death after 72hrs with significant ($p < 0.001$).

Table 3: Combined effect with metronidazole, tinidazole, tomex & curcumin on trophozoites death at different periods.

Agent	After 24hrs	After 48hrs	After 72hrs	P value
Modified Diamond's medium (negative control)	15±5	18±3	35±2	0.01
Metronidazole100mg/ml(positive control)	6±2	24±8	70±20	0.001
Metronidazole100mg & tomex 90 mg/ml	40±5	60±9	110±15	0.001
Tinidazole 100mg/ml & tomex 90mg/ml	60±4	90±7	120±11	0.001
Metronidazole100mg/ml & curcumin400mg/ml	30±3	50±6	70±8	0.001
Tinidazole 100mg/ml & curcumin 400mg/ml	60±9	95±8	130±5	0.001
Tomex 90mg/ml & curcumin 400mg/ml	70±3	100±3	135±4	0.001
P value	<0.001	<0.001	<0.001	

Discussion

The present study investigated the efficacy of different concentrations of commercially available garlic tablet (Tomex) and curcumin 400mg/ml, their combination between their highest effective doses with metronidazole and Tinidazole against *T. vaginalis* infection at 24, 48, 72hrs follow up periods.

In the present study, the mean number of dead trophozoites on modified Diamond's medium was less than Broth medium with significant difference at all follow up periods. This agreed with Gelbart *et al.* (1990) who recommended modified Diamond's medium as the medium of choice for *T. vaginalis*. gave the highest dead *T. vaginalis* trophozo-

In the present study, Tomex 90mg/ml caused the highest dead trophozoites after 72hrs (50.00±9.00), but MTZ 100mg/ml gave higher effect (70.67±20.50). This agreed with both Ahmed (2010), and Ibrahim (2013) reported that garlic was as efficient as metronidazole to prevent trophozoites multiplication and motility. Alyasari *et al.* (2018) reported that aqueous garlic extract completely inhibited *T. vaginalis* growth, viability and motility. No doubt, allicin production of tomex (Ross *et al.*, 2001) disrupted the normal *Trichomonas* physiological functions (Masamha *et al.*, 2010).

In the present study, curcumin 400mg/ml ites (120.00±8.01) after 72hrs compared to

was used positive control. This agreed with Tonkal (2009) who found that curcumin 400 mg/ml markedly inhibited *T. vaginalis* trophozoites' growth. Mahmoud *et al.* (2011) and Al-Ammash (2017) reported that curcumin 400mg/ml had an *in vitro* anti-*Trichomonas* effect. Curcumin proved to have an anti-adhesion effect for *T. vaginalis* to human epithelial cells (Shaaban *et al.*, 2011).

In the present study, metronidazole 100mg/ml alone or combined with the most effective Tomex concentration (90mg/ml) and curcumin (400mg/ml) on *T. vaginalis* trophozoites gave the best anti-trichomonas effect (70±8.00) as a new treatment era.

No doubt, the herbs showed a synergistic effect when combined with drugs by modifying the intestinal and hepatic metabolizing enzymes (Boullata, 2005). Wachter *et al.* (2014) in Austria found that curcumin proved to be a promising candidate for trichomonosis topical treatment. Mady *et al.* (2016) in Egypt showed that combination between Daraprim® and NSO against murine toxoplasmosis was better than the herb alone. Nassef *et al.* (2018) in Egypt combined between cisplatin, a cytotoxic drug, and NSO for *Trypanosome evansi* treatment. Mallo *et al.* (2020) in Spain demonstrated the potential usefulness of curcumin as an anti-parasitic and anti-inflammatory treatment for trichomoniasis, and added that could be used to control the disease and mitigate the associated immunopathogenic effects.

In the present study, significantly higher metronidazole was needed to kill trichomonads, but, curcumin the not toxic and can be applied in very high concentrations. Besides, curcumin would preferentially be applied topically, so that effective drug concentrations can be reached easily as topical curcumin, as a commercial solution (Curcummall®) as well as a gel containing 2% curcumin used successfully for trichomoniasis treatment. Kotha and Luthria (2019) in USA reported that turmeric is a curry spice that originated from India, attracted great interest in recent decades due to bioactive curcuminoids (curcum-

in, demethoxycurcumin, and bisdemethoxycurcumin) it contains. They added that the curcumin, a lipophilic polyphenol may work as an anticancer, antibiotic, anti-inflammatory, and anti-aging agent as reported by several *in vitro*, *in vivo* and clinical trials.

Conclusion

The outcome results proved that curcumin 400mg/ml in combination with tomex 90mg/ml are promising and safe anti-*Trichomonas vaginalis*. But, when combined with MTZ showed an active synergistic effect. Besides, Modified Diamond's medium was better than Broth medium for *T. vaginalis* culture.

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