AMERICAN JOURNAL OF PHARMTECH RESEARCH

**Journal home page:** http://www.ajptr.com/

# In-Vitro Anthelmintic Activity of Ditoxifie Capsule

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## ABSTRACT

Present generation is a fast moving generation and no doubt about the potency of allopathic medicine, they provide fast result, but the darkest side of this medicine is their several side effects and contraindications. On the other hand the Ayurvedic medicines are good substitution for those medicines because of their less or no side effect and their ability to cure the problem from their root. The present study is based on the pharmacological activity of Dhanwantari's Ditoxifie capsule. This drug is having great potential to deal with ache of muscles or joints, frequent headaches, low energy or frequent being tired, frequent cold or sickness, coughs or a sore throat, runny nose or sinus congestion, dense or yellow urine, painful bowel movements, acne or oily skin, lack of taste and many more. In the present work, experiments were conducted to evaluate the possible anthelmintic effects of Ditoxifie capsule on Indian earthworm (*Pheretima posthuma*) at 400mg/ml, 600mg/ml, and 800mg/ml concentration. Results were expressed in terms of time for paralysis and time for death of worms.

**Keywords:** Anthelmintic, Ditoxifie capsule.

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Received 12 June 2018, Accepted 23 June 2018

Please cite this article as: Gandhi A *et al.*, In-Vitro Anthelmintic Activity of Ditoxifie Capsule. American Journal of PharmTech Research 2018.

# INTRODUCTION

Helminthes parasite infections are global problems with serious social and economic repercussions in the third world countries. Parasitic helminths affect animals and man and animals that any single group of parasites, causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans. The diseases affect the health status of a large fraction of the human population as well as animals. Herbal drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance. (Glibert A.Castro.et.al, 1996) Literature survey revealed that Detoxifie capsule has yet not been screened for anthelmentic activity. Therefore, the objective of this work was to assess the anthelmentic activity of Detoxifie capsule. (www.dhanwantari.com)

**Introduction to Helminths (Metazoa)**

The term helminth has been derived from a Greek word meaning worn. It was originally meant to refer to only intestinal worms, but now includes tissue parasites as well as many free living species. These are metazoa.(Jeffery H.C.et.al, 1968 )

**Classification of helminths**

The metazoa are classified into two phyla: Platyhelminthes and Nemathelminthes. Platyhelminthesis divided into two classes: Cestodea (tapeworms) and Trematodea (flukes) while Nemathelminthes has only one class Nematodea (roundworms). (Glibert A. Castro, 1996)

**Adult worm of cestode (tapeworm)**

**Figure 1: Adult worm of cestode**

**Figure 2: Adult worm of trematode (fluke)**

**Figure 3: Adult worm of nematode(roundworm)**

**General characteristics of helminths**

1. They do not possess organs of locomotion, so locomotion is by muscular contraction & relaxation.
2. The outer covering, known as cuticle or integument. It is situated on its outer surface & may be armed with spines or hooks. It is resistant to intestinal digestion.
3. Nervous system and excretory system are primitive.
4. Digestive system is complete, partially lost (rudimentary) or absent. The alimentary tract has entirely disappeared from all stages of the tapeworms (cestodes); it is greatly or nearly absent in many of the trematodes, but its present and complete in most nematodes. The digestive system is Partially lost (rudimentary) or absent in certain parasitic helminths because of their location in the hosts (intestine or tissue), where predigested nutrient are abundant.
5. Reproductive system is very well developed.
6. They may be monocious or diecious. Both self-fertilization and cross-fertilization may take place.
7. Reproduction to increase the parasite population within the same host (internal autoinfection) does not occur among certain helminths; more over under usual conditions of host & environment, the number of worms that reach maturity in any given host is limited levels that are Tolerable to both host & parasite. Thus most of the people who are infected with helminths are asymptomatic carriers, & the diseased individuals among the infected group are those with the heaviest worm burdens. The terms, light moderate, and heavy as applied to worm burdens are relative and differ for the various species of helminths & in people of different ages & physical status.
8. When worms are crowded the collective egg output is great, but the output per worm is relatively low, depending on the degree of crowding.

9. The factors that determine helminth population, are those associated with the host-parasite relationship (i.e. the immune factors derived from the host responses & the complex role of co-existing infection).

   Massive infection depends initially on massive inoculation of infective larvae & eggs.
10. The co-existence of several species of helminths in the same individual (poly-helminthism) is widely prevalent. (en.m.wikipedia.org)
11. In some helminths, the life cycle is direct & relatively simple; involving only one host species and a brief period of development of an infective stage, an example is the pin worm (*Enterobius vermicularis*).

    In a group referred to as soil transmitted helminths, the life cycle involves only one host (man) but the infective stage (larvae) remaining in the egg, as in *Ascaris lumbricords* & *Trichuris trichiura*; or free in soil as in hookworm species which requires a period of development in soil, i.e. the soil functions as an intermediate host.

    In other, the man-to-man cycle involves essential development in one intermediate host as in the filarial worms & most tapeworms, or two intermediate hosts, as in most trematodes; the first being a snail or other mollusk, the 2nd is an animal or plant that is eaten by people. Intermediate hosts provide the parasite with sustenance for essential development, protection & availability to its final host.
12. Worms & larvae that migrate through or reside in tissue generally produce eosinophilia, focally in tissue, in the blood or in both.

    Persistent hyper-eosinophilia is the most recognized general sign of helminthic infection.

    Helminthic infections frequently are occult or cryptic because certain helminths of animal develop in man, but do not produce eggs or larvae & therefore the infection are not patent. Such infections are referred to as non patent
13. In addition to eosinophilia, common signals to occult helminthic infections, somewhat in order of their significance or frequency, are hepatomegaly, pneumonitis, bronchial asthma, urticaria, subcutaneous cyst or swelling, neurologic disturbance, and deviations in behaviour.(Jeffery H.C.et.al, 1968)

**Life cycle of Helminthics**

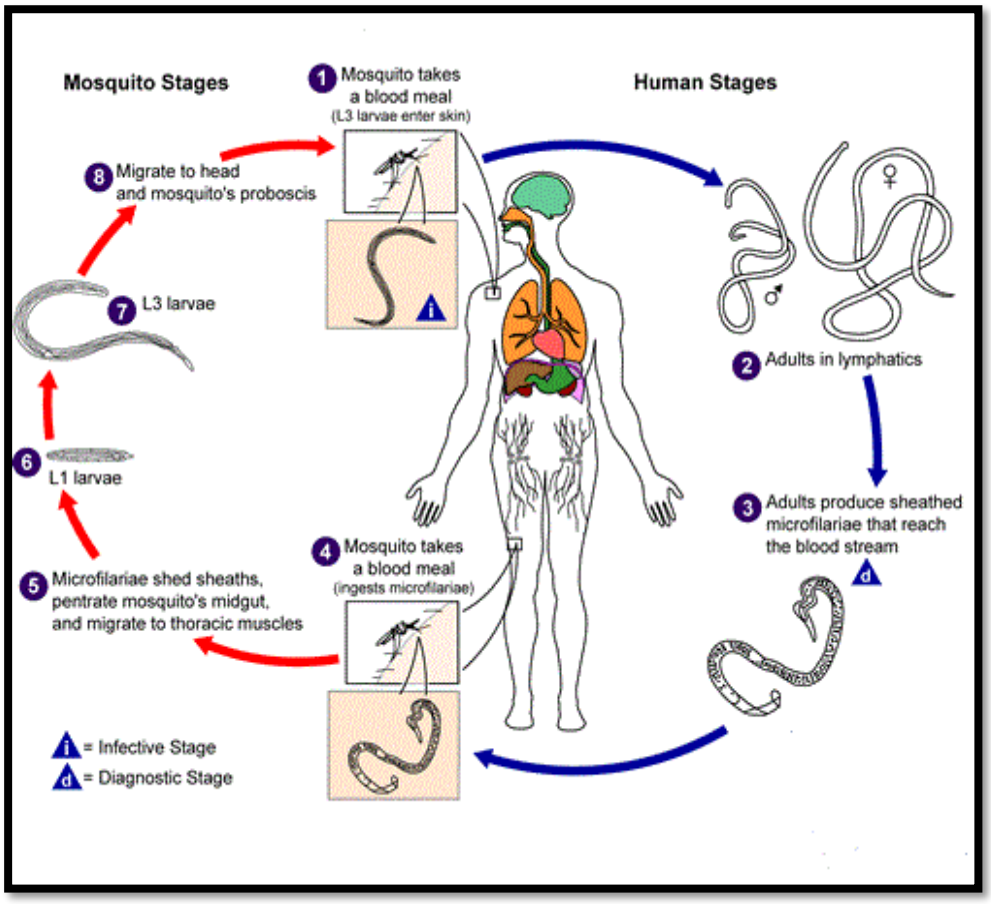


**Figure 4: Life cycle of Helminthics** (Geoff A. parker.et.al, 2003)

**Pathogenicity and clinical features**

1. The invasion stage extending from the period of exposure to cercariae till formation of adult and beginning of egg laying generalized and localized reaction occurs due to metabolites of growing and mature worms.
2. The stage of egg deposition and extrusion. The period between egg deposition and their appearances in the urine or faeces. Trauma and hemorrhage as eggs pass from the venules is the main pathogenic feature.
3. The stages of tissue proliferation and repeat it is a late stages initiated after egg deposition in tissue and their extrusion is progressing .the present of eggs in tissue then tissue reaction producing formation of local tubercles or granulomatus lesion with deposition of histocytes, plasma cells and fibroblastic proliferation and development of scar tissue which may lead to strictures chronic ulcers and other pathological changes. (Sciencedirect.com)

**DIAGNOSIS**

**Direct method**

The most important diagnosis method to find living eggs:

1. Urine for *s.hamatobium*, eggs with characteristic terminal spines can be demonstrated by microscopic examination

2. Specimens collected between midday and 2 pm are most likely to contain eggs sedimentation:

- Filtration
- Stool for mansoni ,lateral spin
- Best egg. concentration technique formal - ether TIF (Thiomersal, iodine formal)

3. Glycerol sedimentation:

B-rectal biopsy for all schistosomes a small piece of rectal mucosa is removed by biopsy forceps .it is placed on slide under a cover slip and examined with x10 power *S.hamemotobium* eggs are often trapped in the rectal mucosa *S.mansoni* more often look.

**Indirect method**

- As intradermal test(Fairley's test)
- Compliment fixation test
- ELISA

Although the better ones correlate well with the results of direct diagnostic methods. (Sciencedirect.com)

**Treatment**

- Metriphonate is the drug of choice in schistosomiasis to haematobum.
- Prazignatel is effective against all schistosomes prevention and control.
- Eradication of the intermediate hosts (copper sulphate 10 ppm).
- Prevention of environmental pollution with urine and faeces.
- Effective treatment of infected persons.

**Mechanism of Action**

The site of action of drug appears to be the microtubular protein ‘β-tubulin’ of the parasite. It binds to β-tubulin of susceptible worms with high affinity and inhibits its polymerization. Intracellular microtubules in the cell of the worms are gradually lost. In addition, it probably blocks glucose uptake in the parasite and deplets its glycogen stores. Hatching of nematode eggs and their larvae are also inhibited. Ascaris ova are killed. (K.D.Tripathi, 2013)

**Ditoxifie**

Scientists estimate that everyone alive today carries within her or his body at least 700 contaminants, most of which have not been well studied. This is true whether we live in a rural or

isolated area, in the middle of a large city, or near an industrial area. Because many chemicals have the ability to attach to dust particles and/or catch air and water current sand travel far from where they are produced or used, the globe is bathed in a chemical soup. Our bodies have no alternative but to absorb these chemicals and sometimes store them for long periods of time. There are more than 84 chemicals found in human body. This capsule helps our body to remove these toxic chemicals. (www.dhanwantari.com)


Dhanwantari's **Ditoxifie capsule** is having great potential to deal with ache of muscles or joints, frequent headaches, low energy or frequent being tired, frequent cold or sickness, coughs or a sore throat, runny nose or sinus congestion, dense or yellow urine, painful bowel movements, acne or oily skin, lack of taste and many more. (www.dhanwantari.com)

**Each capsule contains extract of:**

**Table 1: Each capsule contains 20mg drug**

| Sr. No. | Name of drug | Figure | Chemical constituents | Uses |
|---|---|---|---|---|
| 1 | Haridra |  | Curcumene, Curcumenone, curcone | Diabetes, Urinary tract infection, wound healing, skin diseases |

| | | | | |
|---|---|---|---|---|
| 2 | Guduchi |  | Terpenoids, Alkaloids, steroids | Anticancer, antitumor, antidiabetic, antistress |
| 3 | Rasna |  | Resin , Galangol | Stimulant, carminative, antibacterial |
| 4 | Yastimadhu |  | Glycyrrhizin, Glucose | Anti-inflammatory ,treatment on respiratory system |
| 5 | Shirisha |  | Tannin, Sitosterol | Anti-inflammatory, Toothache |
| 6 | Anantmool |  | Lupeol, | Purify blood, improve skin |
| 7 | Nirgudi |  | Alkaloids, Sitosterol | Anti-inflammatory, Treatment of ulcer |

| | | | | |
|---|---|---|---|---|
| 8 | Bhumy |  | Glycosides, flavanoids, alkaloids | Anti-inflammatory, Treat skin infection |
| 9 | Bhringraj |  | Ecliptic wedelolactane | Hair fall treatment, liver disorder |
| 10 | Kamala |  | Resins, Flemingin | Treatment of GI tract,skin infection |

**Table 2 :Each capsule contains 10mg drug**

| **Sr. No.** | **Name of drug** | **Figure** | **Chemical constituents** | **Uses** |
|---|---|---|---|---|
| 1 | Kumari |  | Emodin, barbaloin | Useful in constipation, antitoxic |
| 2 | Nimbpatra |  | Nimbidin, nimbin, resin | Fever, antimicrobial, wound healing |
| 3 | Kushtha |  | Essential oil, costol | Headache, shoulder and backpain |

| | | | | |
|---|---|---|---|---|
| 4 | Arjuna |  | Calcium, Magnesium | Antioxidant , treatment on respiratory system |
| 5 | Babbul |  | Tannin, magnesium, calcium | Wound healing, treat diarrhea |
| 6 | Priyangu |  | Propionic acid, Apigenin | Treat headache, skin treatment, peptic ulcer |
| 7 | Jatamansi |  | Carotin, virolin | Purify blood, useful in depression |
| 8 | Daruhalad |   | Quaternary ammonium salt | Antifungal, antiviral, Antidiabetic |

| | | | | |
|---|---|---|---|---|
| 9 | Kalevala |  | Alkaloid | Antibacterial, Antidiabetic |
| 10 | Ashoka bark |  | Tannin, flavanoids | Used in bleeding disorder,dizziness, jaundice |

**Table 3: Each capsule contains 30mg drug**

| Sr. No. | Name of drug | Figure | Chemical constituents | Uses |
|---|---|---|---|---|
| 1 | Draksha |  | 70 to 80% water, sugar, organic acid | Act as cardiac tonic, treatment of respiratory system |
| 2 | Dadim |  | Vit- B, minerals | Imbalance in body, use for healthy immunity |

| | | | | |
|---|---|---|---|---|
| 3 | Spirulina |  | Carbohydrate, fat, protein, vitamin | Antidiabetic |

**Table 4: Each capsule contains 5mg drug**

| Sr. No. | Name of drug | Figure | Chemical constituents | Uses |
|---|---|---|---|---|
| 1 | Vidanga |  | Embelin, vilangin | Used to cure worm infection |
| 2 | Punarnava |  | Alkaloid, lunamarine | Anticancer activity, antiestrogenic |
| 3 | Tulasi |  | Aleanolic acid, ursolic acid, eugenol | Dysentery, diarrhea, malaria |
| 4 | Sabja |  | Linalool, estragole | Antifungal, insect-reppeling property |
| 5 | Lodhra |  | Alkaloid loturine, colloturine | Improve the womens heath |

| | | | | |
|---|---|---|---|---|
| 6 | Ashwagandha |  | Alkaloid, steroid-withanolides | Arthritis, anxiety, bipolar disorder |

(WHO monographs, 2009)

**Need of Work**

Synthetic drugs are having high cost and having major side effects. Hence there is need to use Ayurvedic drugs for anthelmintic purpose. Ayurveda, the science of life, prevention and longevity is believed to be oldest and most holistic or comprehensive medical system available. Ayurveda is one of the most ancient systems of life, health and cure. Ayurveda is highly evolved and codified system of life and health science based on its own unique and original concepts and fundamental principles. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in developing countries. The medicinal values of these plants are found in some chemical active substances that produce a definite physiological action on human body.

The active principles responsible for the Anthelmintic activity of Ditoxifie capsule need to be explored and mechanism of action need to be studied in detail. Hence, further detailed study needs to be conducted to evaluate the clinical efficacy of Ditoxifie capsule in treatment of helmintic mediated diseased patients.

**Objective**

1. To evaluate anthelmintic activity of Ditoxifie capsule by using Indian earthworms (*Pheretima posthuma*).
2. To compare the results of anthelmintic activity of Allopathic medicine (Albendazole) with Herbal product (Ditoxifie).

**Plan of Work**

| Sr. No. | Activity | Period of work |
|---|---|---|
| 1 | Selection of marketed herbal product | 10 Days |
| 2 | Collection of information | 15 Days |
| 3 | Selection of method for Anthelmintic activity | 15 Days |
| 4 | Collection of worms (*Pheretima posthuma*) | 10 Days |
| 5 | Selection of dose | 5 Days |
| 6 | Study the Anthelmintic activity | 20 Days |
| 7 | Comparative study of both Drugs | 10 Days |

# MATERIALS AND METHOD

**Collection of Marketed Product:**

The Ditoxifie capsules were collected from Dhanwantari Distributors, Satara. Collected capsules were emptied and powder was weighed accurately.

**Experimental worms:**

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to Its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all faecal matters.

**Selection of Dose:**

The dose for experiment was decided on the basis that the patient is advised to administer 1-2 capsules each of 400mg twice a day as directed by physician. (www.dhanwantari.com)

***In-vitro* Anthelminitic activity:**

1. The Anthelminitic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.
2. The earthworms are collected and washed with normal saline with removal of fecal matter.
3. The earthworms are 5 to 6 cm length and 0.2- 0.3 cm widths were used for experiment protocol.
4. Different concentrations of Ditoxifie were prepared and examined systematically for their *in-vitro* Anthelminitic activity against *Pheretima posthuma.*
5. The *in-vitro* Anthelminitic assay procedures were carried out with slight modifications.
6. Five groups of equal size Indian earthworm consisting of six earthworms in each groups were released into 400 mg/mL, 600 mg/mL , 800 mg/mL l, of desired formulation.
7. Each group was treated with one of the following: Vehicle, Albendazole (400mg/mL), and different concentrations of ditoxifie and in normal saline.
8. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded (each reading taken for 6 times). The time taken by the worms to become motionless, consider as paralysis was recorded and the lethal time was recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin. Albendazole (400mg/mL) was taken as reference drug. (Satish B. Kosalge.et.al, 2009) (V. Murugamani, 2009)

# RESULTS AND OBSERVATION

**Anthelmintic screening:**

Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

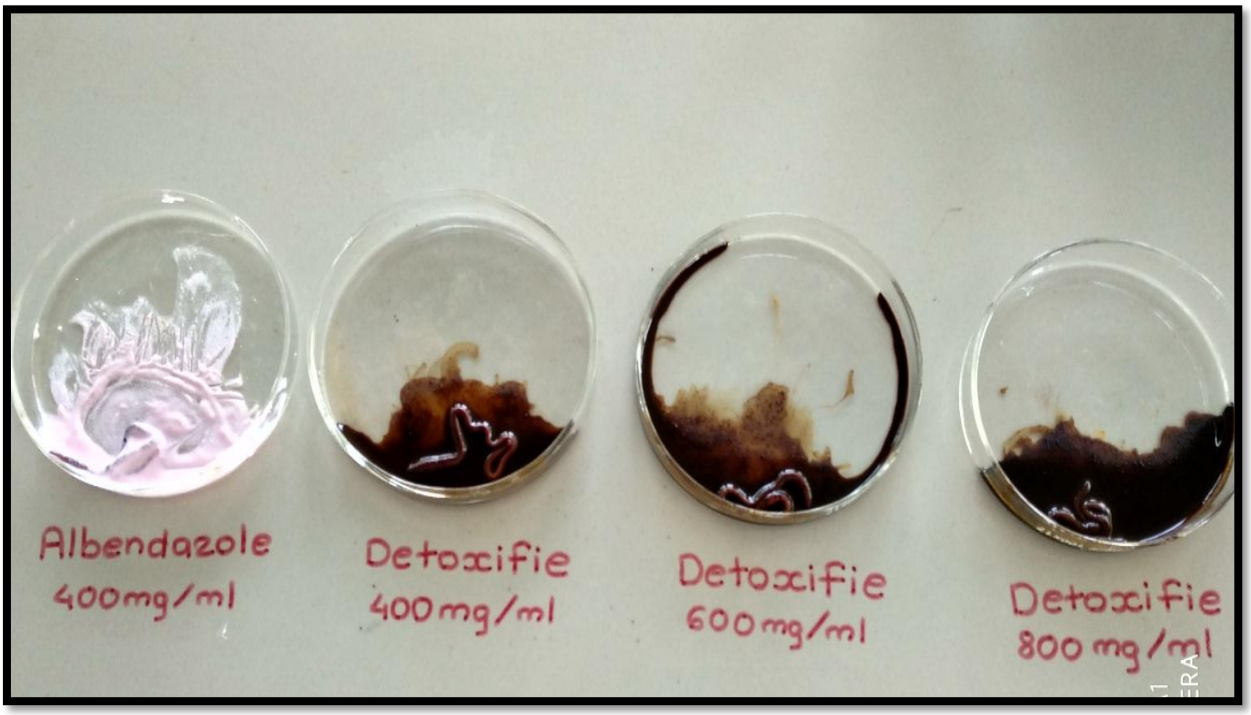


**Figure 6: Observation of anthelmintic activity**

**Table 4: Characteristic of different conc. of Ditoxifie**

| Characteristics | Ditoxifie solution |
|---|---|
| Colour | Brown |
| Odour | Pungent |
| Taste | Bitter |

**Observation table:**

**Table 6: Anthelmintic Activity of Ditoxifie capsule against Earthworm.**

| Name of group | Treatment | Concentration (mg/ml) | Time taken to paralysis and death of worms | |
|---|---|---|---|---|
| | | | Paralysis time (Min.) | Death time (Min.) |
| Control | Saline solution | - | - | - |
| Standard | Albendazole | 400mg/ml | 10.40 | 17.20 |
| Test sample | Ditoxifie | 400mg/ml | 6.42 | 12.31 |
| | | 600mg/ml | 5.51 | 10.30 |
| | | 800mg/ml | 4.21 | 9.31 |

In the present work, Ditoxifie capsule was used to evaluate in- vitro anthelmintic activity against Indian earthworm.

The perusal of the anthelmintic activity data reveals Ditoxifie capsule at the concentration of 400 mg/ml showed paralysis and death in 6.42 min and 12.31 min. Respectively. 600 mg/ml showed paralysis and death in 5.51 min and 10.30 min. And 800mg/ml showed paralysis and death in 4.21 min and 9.31 min. Different concentrations of Ditoxifie capsule showed less time of death of earthworms (*Pheretima posthuma*) than the standard drug Albendazole.

## CONCLUSION

It is concluded that the Dhanwantari's Ditoxifie capsule (Ayurvedic drug) had significant activity than Alendazole (Allopathic drug). And it is concluded that herbal medicines are having higher resistance and less side effects and contraindications as compared to allopathic medicines. There are less chances of development of resistance for Ditoxifie capsule as it contains combination of various herbs. Further in future researches will also being carried out in our department to establish the prescribed mechanism of action responsible for this activity.

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