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## Formulation and Clinical Efficacy of Myrrh Extract in Hard Gelatin Capsules

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**Abstract:** The development of myrrh extract from natural origin capable of eradication of schistosomal infections has gained the interest of many scientists. Recently; myrrh extract is considered as the drug of choice from natural origin used to treat Schistosomiasis. This work includes the preparation of myrrh extract by maceration and the formulation of these extracts in hard gelatin capsules as a pharmaceutical dosage form followed by clinical investigation of the effectiveness of these capsules. As a result, myrrh extract capsules with different doses showed a variable effectiveness against *Schistosoma mansoni* (*S. mansoni*) with different severity but n-hexane extract capsules showed better effectiveness in comparison to alcohol extract capsules. The percent cure increased by increasing the treatment time. In additions the number of positive cases decreased by increasing the treatment time. On the contrary, increasing the severity of the infection from mild to heavy cases led to decrease the cure percent and increased the number of the positive cases. There was a significant reduction of infection intensity in cases which were incompletely cured. It could be concluded from these results that hard gelatin capsules of myrrh extract are highly effective as a pharmaceutical dosage form against Schistosomiasis.

**Key words:** *Commiphora molmol*, *Schistosoma*, Clinical investigation.

### Introduction

Schistosomiasis is endemic in 74 countries and widespread infection in the tropics caused by blood flukes belonging to *Schistosoma* species. It is the second most prevalent parasitic disease after malaria in the developing world with a huge impact on public health. In Egypt, it is considered the first health problem, because it has high morbidity and mortality (due to its complications). It affect active group with peaks at age of 10-20 years. It is associated with low

productivity and its fatal complications such as cancer liver and urinary bladder occur at young age causing social and medical burden and premature deaths.

For many of the *Schistosoma* species that infect humans, isoquinolin-4-one, or praziquantel (PZQ), is the only effective drug for treatment <sup>1</sup>. However, praziquantel has been in use for more than 20 years <sup>2</sup>, and concern is increasing the resistance has emerged in human parasites <sup>3-10</sup>. The presence of a gene or genes that confer

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resistance to PZQ cannot be excluded<sup>11-12</sup>. Low efficacy of praziquantel was reported by many authors in several clinical trials in many countries including Egypt<sup>3, 13-15</sup>. Laboratory isolation of schistosome strains resistant to standard and high doses of the drug was reported<sup>7, 11-12, 16</sup>. The role of praziquantel cannot be ignored as a possible etiological factor implicated in the process of carcinogenesis associated with schistosomiasis<sup>17</sup>.

Myrrh is the air-dried oleo-gum resin obtained from the stems and branches of *Commiphora molmol*. Myrrh is a safe natural substance approved by FDA for food use<sup>18</sup>. The Council of Europe (1981) included Myrrh in the list of plants and parts, which are acceptable for use in foods<sup>18</sup>. Essential oil of myrrh is commercially available from numerous sources. It is said to be beneficial when used as a chest rub to treat bronchitis, and externally in diluted form on ulcers and wounds. It is also showed a significant improvement in the childhood atopic eczema<sup>19-20</sup>. Myrrh is burned as incense and used to repel mosquitoes. Histological examination of myrrh treated mosquito larvae showed great pathological effect on their fat, muscles, and nervous tissues<sup>21</sup>. Myrrh acts as a broad-spectrum antiseptic and can be applied directly to sores and wounds. It is antifungal, and has been used to treat athlete's foot and *Candida*. It stimulates the production of white blood corpuscles, and their pathogenic actions.

Myrrh is widely used in Somalia for the treatment of diarrhea and stomach complains. Beside its anti-inflammatory activity<sup>22</sup>, it is successfully used in chronic bronchitis, bronchial asthma, and pulmonary tuberculosis.

*Commiphora molmol* (Myrrh) showed no clastogenic effect, no mutagenicity and was found to have anti-carcinogenic effect<sup>23</sup>. Experimental studies on albino rats revealed no chromosomal aberrations, no fetal abnormalities; safety for liver, kidney<sup>23</sup>, haemopoietic system and chromosomes<sup>21, 24</sup>. Recently myrrh extract formulated in soft gelatin capsules proved its effectiveness in treatment of Schistosomiasis. The development of myrrh extract from natural origin capable of eradication of schistosomal infections has gained the interest of many scientists. Myrrh

extract is considered as the drug of choice from natural origin used to treat Schistosomiasis. Experimental and clinical studies proved that myrrh extract obtained from oleo-gum resin of *Commiphora molmol* is a safe, potent, economic and anti-bilharzial agent. In the present work, the clinical evaluation of different doses of formulated myrrh extract capsules against different grades of *S. mansoni* infections was carried out.

## Materials and methods

### Materials

Myrrh purchased from France (Ethiopian origin), n-Hexane and ethyl acetate were from (Lab-Scan limited Ireland), ethyl Alcohol 96 %, sulphuric acid, methanol and diethyl ether were from (Scharlau Chemie S.A.-Spain), chloroform and toluene were from (El-Nasr Pharmaceutical Chemicals Co.-Egypt), Vanillin (Sigma-Aldrich), lactose anhydrous, starch, and calcium carbonate were supplied by Egyptian Company for Pharmaceutical and Chemical Industries (EPCI), Hard gelatin capsules shell No. 0 was supplied by Jedco International Pharmaceuticals Company.

### Methods

#### Preparation of Myrrh extract

Myrrh extract was prepared by maceration method. 5 Kg of myrrh soaked in glass container with n-hexane solvent at room temperature. Yellow extract developed evaporated under vacuum at about 40°C (Buchi EL 131 Rota vapor, Germany), and n-hexane returned to the vessel. This process is continued until the n-hexane solvent being colorless. N-Hexane extract was concentrated under vacuum at about 40°C until it gives thick brown sticky extract (Fraction 1=624.6 gm). Residue after n-hexane extraction was soaked again with ethyl alcohol (96 %). Yellow extract evaporated under vacuum at about 50°C and evaporated. Alcohol was returned to the vessel and the process was continued until ethyl alcohol in the vessel was colorless. Ethyl alcohol extract was concentrated under vacuum at about 50°C and air dried until it gives yellowish brown fine powder (Fraction 2=896.6 gm).

Part of alcohol extract (Fraction 2) is fractionated by diethyl ether into ether-soluble

extract (Fraction 3) and ether-insoluble extract (Fraction 4).

Myrrh extract was also prepared by Percolation using Soxhlet apparatus where 10 gm myrrh was extracted with ethyl alcohol (96 %) in continuous extract apparatus (soxhlet). Extract was evaporated and concentrated until it gives thick sticky extract (Fraction 5=3.65 gm). In addition, 30 gm myrrh was extracted using continuous extract apparatus (soxhlet) also, but successively with different solvents n-hexane, chloroform, and ethyl alcohol (96 %) which gives respectively:

n-Hexane sticky extract (Fraction 6=4.1719 gm)

Chloroform powder extract (Fraction 7=1.3410 gm)

Alcohol fine powders extract (Fraction 8=.5750 gm)

### **Chromatographic analysis of myrrh extract**

#### ***Thin layer chromatographic analysis of myrrh extract***

To prepare test samples, 5 ml of ethyl alcohol 96 % was added to 0.5 gm of powdered myrrh. The mixture warmed on a water-bath for 2-3 minutes, then cooled and filtered. On the other hand, 0.1 gm of extract (n-hexane and alcohol) was dissolved in 5 ml ethyl alcohol 96 %, and filtered. Also samples of volatile oil of myrrh were prepared by adding 2 drops of volatile oil of myrrh obtained by steam distillation in 2 ml of toluene, and filtered. Vanillin-sulphuric acid reagent was prepared by dissolving 1 gm of vanillin powder in 100 ml methanol (solution 1) and 25 % v/v solution of sulphuric acid was added to methanol (solution 2).

Pre-coated silica gel TLC plates (Silica gel 60 F254 (MERCK HX 614286) were used. Aliquots of 15 µl from each test sample were applied as a circular spot 2-cm above the edge of the plate. The plate was putted in a glass chromatographic jar containing a mixture of toluene-ethyl acetate (93:7) as mobile phase. When the mobile phase migrates to about 15 cm, the plate was removed and air-dried, and examined under UV-365 nm lamp. The plate sprayed by vanillin-sulphuric acid reagent, and visualized after drying at 105°C for 10 minutes.

### **Gas chromatographic/mass spectrometric analysis of myrrh**

Volatile oils obtained from hydro-distillation were subjected to GC-MS analysis (Finnigan U.S.A.) in Nutrition Institute-Ministry of health-A.R.E. under the following conditions:

Acquisition time: GC runs time, Seconds per scan: 1.00 seconds, Mass defect: 0.0 mmu/amu, Acquire cal Gas: no, Source temp: 200°C, Transfer line: 275°C

Tune File: Lens 1: -25 volts, Lens 2: -100 volts, Lens 3: -25 volts, Trap offset: -10 volts, ElecLens On: 15 volts, ElecLens Off: 65 volts, ElecEnergy On: -70volts, ElecEnergy Off: -20 volts, Emission current: 250 micro amps, High mass adjust: 50 %, AGC Target: 50

Injection waveform: Off, Environmental tuning factors: None

Oven temperature program: Initial value: 80°C, Initial time: 1.00 minutes, Maximum oven temperature: 280°C, Injection temperature: 250°C, Injector used: Right, Injector subambient: Off, Splitless injection, Split close time: 0.10 minute, Split open time: 1.00 minute, Constant velocity: 40.00 cm/sec, Surge pressure: Off

### **Screening of Anti-bilharzial Activity of the eight Fractions**

A stock of *Biomphalaria Alexandria* was purchased from Schistosoma Biological Supply Program (SBSP) in Theodor Bilharz Research Institute (TBRI), which were maintained for many years. The techniques used for maintaining snails were similar to technique of Frandsen F<sup>25</sup>. Eggs of *Schistosoma mansoni* were purchased in physiological saline (0.85 %) from SBSP, extracted from hamsters infected with Egyptian strain.

Each fraction diluted up to (1 part per million) prepared on basis of weight/volume using dechlorinated tap water (DTW). For each stock solution (8 stock solutions) three replicates were used, each of 10 snails (4-6 mm diameter) being immersed in a liter of mallusicide concentration (1 ppm). The exposure period was 24 hrs at room temperature. For each test, 3 replicates of control snails were maintained under the same experimental conditions in DTW.

After exposure (24 hrs), the snails were washed thoroughly with clean DTW, and transferred to clean plastic aquaria filled with DTW with lettuce for 24 hrs for recovery.

Snails were individually exposed to miracidia within 30 minutes after hatching. The eggs in suspension were washed with cold DTW, put in a Petri dish, hatched by adding warm water, and exposed to artificial light (60 w lamps, 40 cm above) for an hour.

Snails were put individually in cavities of tissue culture plates with 2 ml DTW and 8-10 miracidia. The hatched miracidia were collected by a Pasteur pipette under a binocular microscope.

Snails were left exposed to miracidia for 24 hrs under normal laboratory light. After exposure, snails were placed in plastic trays containing 1.5 L of DTW supplied with lettuce, blue green algae (*Nostic miscoram*) and mud and kept at a density of 50 snails/tray.

Snails were maintained under standard conditions; water was changed twice weekly, and kept under close observations to remove dead ones. Starting from the 21st day post -exposure, survived snails were examined individually for cercarial shedding twice weekly by placing individual snail in the cavities of tissue culture plate containing 2 ml of DTW for an hour under bench lamp. The water was examined by binocular microscope. The cercarial suspension was poured in a graduated Petri-dish and cercariae were counted after adding few drops of Bowin's solution. Infected snails were isolated, numbered and kept in special aquaria in complete darkness. This was carried out with all snails were dead. The first day of shedding, time of cercarial

incubation, and the periodic cercarial production per an hour were calculated

### Formulation of twelve formulae of hard gelatin capsules

Twelve formulae of hard capsules containing different doses of myrrh extracts and different excipient substances were formulated as shown in table 1. The ingredients of each formula were thoroughly mixed in a mortar. The blend powder was filled in hard gelatin capsules of size 0; each capsule contains 500 mg. Alcohol extract was filled in capsules directly without excipients. Hard shell capsules were filled by using automatic capsule filling machine.

### Disintegration test (USP 31 NF 26)

No significant difference between disintegration of formulae 1, 2, and 3 which contain lactose anhydrous as excipient, and no significant difference between disintegration of formulae 4, 5, and 6 which contain starch as excipient, also no significant difference between disintegration of formulae 7, 8, and 9 which contain calcium carbonate as excipient. Thus formulae 1, 4, and 7 were chosen to study the effect of the excipient type on disintegration. From each formula; six capsules were taken at random. Each capsule was placed in each of the six tubes of the basket with a disk over each capsule. The apparatus was operated using distilled water as the immersion fluid maintained at  $37 \pm 2^\circ\text{C}$ . The time of complete disintegration was determined, and the acceptance criteria of United States Pharmacopoeia were applied. The test was repeated using 0.1 N HCL as the immersion fluid.

**Table 1. Formulations of myrrh extract hard gelatin capsules**

Ingredient (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
N-Hexane extract	100	150	200	100	150	200	100	150	200	-	-	-
Alcohol extract	-	-	-	-	-	-	-	-	-	100	150	200
Lactose anhydrous	395	345	295	-	-	-	-	-	-	-	-	-
Starch	-	-	-	395	345	295	-	-	-	-	-	-
Calcium carbonate	-	-	-	-	-	-	-	395	345	295	-	-
Talc powder ( Lubricant )	5	5	5	5	5	5	5	5	5	-	-	-

**Dissolution study (USP 31 NF 26)**

Formula 1, 4, and 7 were chosen to study the effect of the excipient type on dissolution of myrrh extract from its capsules. Formulae 1, 4 and 7 were evaluated for dissolution using U.S.P. apparatus II. The dissolution medium 900 ml of 0.1 N HCL maintained at  $37 \pm 0.5^\circ\text{C}$  in constant temperature water bath. Sample of 5 ml was withdrawn at certain time intervals (30, 45, 60, 90, and 120 minutes); each sample taken was replaced by an equivalent amount of dissolution medium. The samples were filtered and myrrh extract was assayed calorimetrically <sup>26</sup>.

**Excipients interactions**

To study excipients interactions, mixtures of formula 1 (with lactose anhydrous as an excipient), formula 4 (with starch as an excipient), and formula 7 (with calcium carbonate as an excipient) were kept in tightly closed vials and stored at  $45^\circ\text{C}$  for three months. The samples were examined by IR scanning before and after three months, also examined periodically every week for the following physical characteristics:

1. Caking.
2. Liquefaction.
3. Discoloration or odor: the color of the mixtures was examined for any change of color and/or odor.

**Clinical evaluation of myrrh extracts hard gelatin capsules****Design of study protocol**

The study was approved by the University Protection of Human Subjects Committee, and the protocol complies with the declarations of Helsinki and Tokyo for humans. This study was conducted at Ezbat Amleet, Etay-Elbarood Center; El-Behera Governorate. The results of a survey conducted in Behera Governorate showed that the prevalence of *Schistosoma mansoni* infection was 24 % <sup>27</sup>. All the attendants of our clinical and parasitological evaluation were chosen randomly and screened for active *Schistosoma mansoni* by stool analysis by Kato-Katz method <sup>28</sup> and treated by formulated myrrh extract capsules after taking a written consent. Cases were classified into three categories and 18 groups, 20 cases of each group. The first

category contains 6 groups having mild *Schistosoma mansoni* (*S. mansoni*) infection. The second category contains 6 groups having moderate *S. mansoni* infection. The third category contains 6 groups have heavy *S. mansoni* infection. The six groups of each category were treated by one of formulated myrrh extract hard gelatin capsules according to the following order:

**Section I**

1. n-Hexane myrrh extract capsules 100 mg.
2. n- Hexane myrrh extract capsules 150 mg.
3. n- Hexane myrrh extract capsules 200 mg.

**Section II**

1. Alcohol myrrh extract capsules 100 mg.
2. Alcohol myrrh extract capsules 150 mg.
3. Alcohol myrrh extract capsules 200 mg.

**Exclusion Criteria**

Patients presenting with any of the following signs were not included in the study:

1. Patients who gave history of prior treatment with any anti-bilharzial therapy e.g. praziquantel; during the last three months.
2. Patients with decompensate liver disease.
3. Pregnant or lactating females.
4. Treatment with any investigational drug in the last 4 weeks before study entry.
5. History of drug or alcohol abuse.
6. Mental conditions rendering the patient unable to understand the nature, scope, and possible consequences of participating in the study.
7. Patients unlikely to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits, and likelihood of not completing the study according to the protocol.

**Treatment**

All cases were treated by two capsules from the designed dose one hour before breakfast for three consecutive days. Thorough clinical examination, full history taking and a clinical sheet was filled for all cases.

**Follow up study**

Follow up of cases which received treatment was done clinically and parasitologically by stool analysis by Kato-Katz method after one, two and

three months and any new symptom was considered as side effect. Data entry processing analysis was done using statistical program for social sciences (SPSS program) version 10.1999. Chi-square test for significance was used to compare between different groups and t-test was used to compare between means.

### Results and Discussion

Schistosomiasis remains a public health problem in many regions, including Africa, Middle East, Asia and America. *S. mansoni* infection occurs in 53 countries ranging from the Arabian peninsula, numerous countries in the African continent particularly the Nile valley neighbors, Sudan and Egypt, to the new world, Brazil, Surinam, Venezuela and seven islands in the Caribbean. Schistosomiasis is estimated to infect 200 million people, with an exposed at risk population of about 600 million. The prevalence of *Schistosoma mansoni* was 24 % in Behera Governorate <sup>27</sup>. We choose Ezbat Amleet, Etay El-Barood Center, Behera Governorate to conduct this study. In the present work, most of the cases of mansoniases were 15-30 years old (47.83 %), males (64 %), illiterates (43.67 %). Most of the cases practiced true agricultural work (68.83 %). These results coincide with Abdel-Wahab *et al.*, <sup>29</sup> and El-Khoby *et al.*,<sup>30</sup>. Infection with *S. mansoni* was estimated to cause diarrhea in 0.78 million individuals, blood in stool in 4.4 million and hepatomegaly in 8.5 million <sup>31</sup>.

### Chromatographic analysis of myrrh extract

TLC analysis of crude myrrh, n-hexane extract,

alcohol extract, and volatile oil of myrrh showed violet zones at  $R_f$  0.06-0.7 and at  $R_f$  0.25 which are characteristic of furanosesquiterpenes the main constituents of myrrh and were in agreement with Hanus *et al.*,<sup>32</sup>. GC-MS analysis of myrrh shows the following composition  $\delta$ -Silenene (1.8 %), Pentyl benzene (0.3 %), Ethyl benzene (0.2 %), Valencene (3.6 %),  $\beta$ -Chamigrene (6.4 %), Lingifolene (5.3% ),  $\alpha$ -Himachlene (0.7 %),  $\alpha$ -Guaiene (14.4 %),  $\beta$ -Gurjunene (32.3 %), Alloaromadenderene (0.7%), 2-Benzyl-naphthalene (10.3 %), 3-Isopropyl-1, 2-cyclopentenophenanthrene (16.8 %), 3-Methylpyrene (7.2 %)

### Anti-bilharzial Activity of the eight Fractions

Screening of antibilharzial activity of the different prepared eight extracts of myrrh revealed that the best and powerful anti bilharzial extracts are n-hexane and alcohol extracts which were prepared by maceration (Fraction 1 and Fraction 2) as illustrated in table 2.

N-Hexane and alcohol extracts of myrrh which extracted by maceration showed high anti-bilharzial activity, so they were subjected to formulation and clinical evaluation; in contrast to those which extracted by soxhlet have no or very weak anti-bilharzial activity. Using of heat in extraction by soxhlet may decompose active constituents of myrrh.

### Disintegration test

Formula 1 which contains lactose anhydrous as diluent's gives the best disintegration results, the disintegration of it in 0.1N HCL was 5 minutes

**Table 2. Anti-Bilharzial screening of the eight fractions**

Fraction	Control	1	2	3	4	5	6	7	8
Infected Snails	80%	-	-	3%	5%	6%	8%	9%	10%
Longevity of Shedding Snails (Days)	66.5	-	-	10.5	15.5	18.5	25.6	26.2	30.2
Prepatent Period (Days)	20	-	-	35	33	30	29	25	25
Total Periodic Cercarial Production /Snail	811	-	-	200	311	405	500	601	612
Duration of Shedding of Snails (Days)	50	-	-	21.6	22.1	27.1	35.5	36.4	38

and in distilled water was 25 minutes; while formula 4 which contains starch as diluents disintegrate after 20 minutes in 0.1N HCL and after 35 minutes in distilled water; and formula 7 which contains calcium carbonate disintegrates after 30 minutes in 0.1N HCL and after 45 minutes in distilled water.

### Dissolution study

Figure 1 showed that formula 1 which contains lactose anhydrous as an excipient achieved best release in 0.1 N HCL with average of 77.43 mg (77.43 %) after 120 minutes. The other two formulae gave 43.92 mg (43.92 %) and 44.68 mg (44.68 %) for formula 4 and 7 respectively.

### Excipients interactions

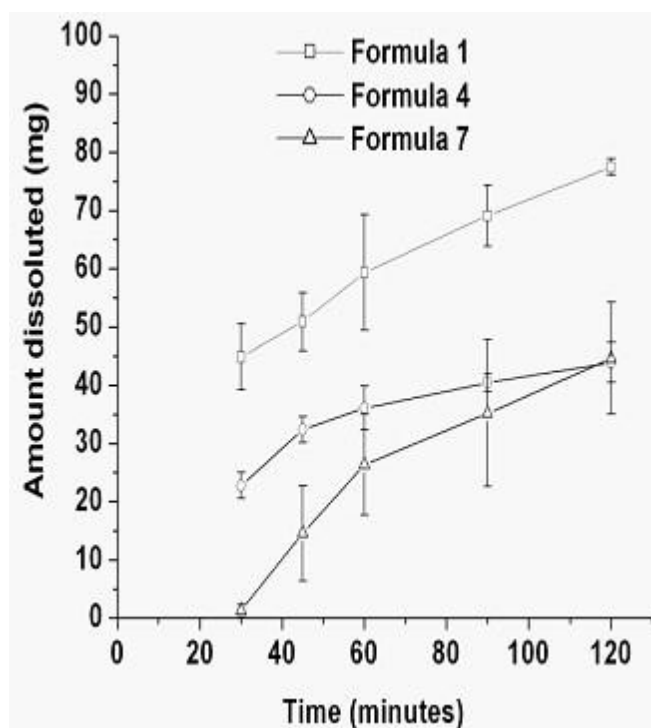
With regard to physical properties of caking, liquefaction, discoloration and odor changes, no change was observed after 3 months storage in air tight containers in oven at 45°C. Infra red (I.R.) scanning of 1, 4, and 7 formulae showed no excipients interactions with regard to lactose

anhydrous, starch, and calcium carbonate. On repeating the I.R. scanning after storage at 45°C for 3 months, there was no change in the characteristic peaks of the prepared formulae from the first curve. These results revealed that, there are no chemical interactions between myrrh extract and the selected excipients, since positions of peaks were not absolutely affected in the stored capsules after three months.

On the basis of the disintegration, dissolution and excipients interactions tests, capsule formulations which contains different doses of n-hexane myrrh extract and lactose anhydrous as an excipient (F 1, 2, 3) and those containing different doses of alcohol myrrh extract without any excipient (F 10, 11, 12) were selected for clinical evaluation study.

### Clinical evaluation of myrrh extracts hard gelatin capsules

The decrease or complete cessation of eggs passing by treatment has been used as criteria for the evaluation of therapeutic activity. The Kato



**Figure 1.** Dissolution profile of n-hexane myrrh extract from its capsules in pH 1.12; formula 1 with lactose anhydrous as diluents; formula 4 with starch as diluents and formula 7 with Calcium carbonate as diluents

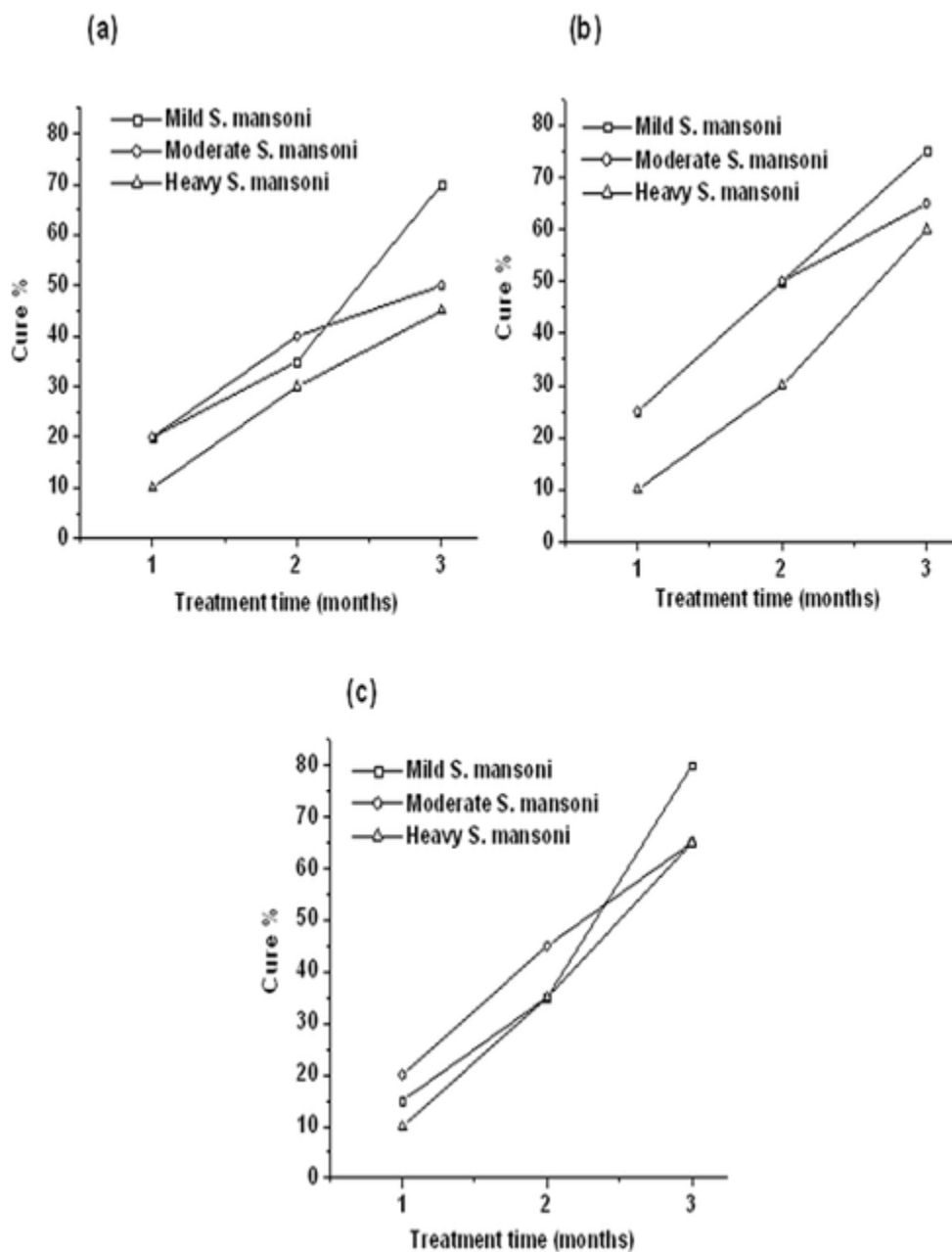
thick smear method is a reliable method for quantitative diagnosis and for the detection of scanty eggs, and also has the advantage of simplicity<sup>33</sup>. The percentage cure was determined by determining the number of patients from the 20 patients that did not show eggs in stool. Patients who still have eggs in stool were considered as positive cases.

Figures 2 and 3 show treatment of *S. mansoni* infected cases with n-hexane myrrh extract capsules 100, 150 and 200 mg (Formula 1, 2, 3). As expected, the cure percent increased by increasing the treatment time from 1 to 3 months.

In addition the number of positive cases decreased by increasing the treatment time as seen from figure 3. On the contrary, increasing the severity of the infection from mild to heavy cases led to decrease the cure percent and increased the number of the positive cases. In cases which incompletely cured, there was significant reduction of infection intensity expressed by number of eggs excreted in stool as seen in table 3. The same results were also observed in case of the treatment of *Schistosoma mansoni* infected cases with alcohol myrrh extract capsules 100, 150 and 200 mg (Formula 10, 11, 12). Figures 4

**Table 3. Reduction of intensity of infection of *S. mansoni* cases which were still positive 1, 2 and 3 months after treatment by n-hexane myrrh extract capsules**

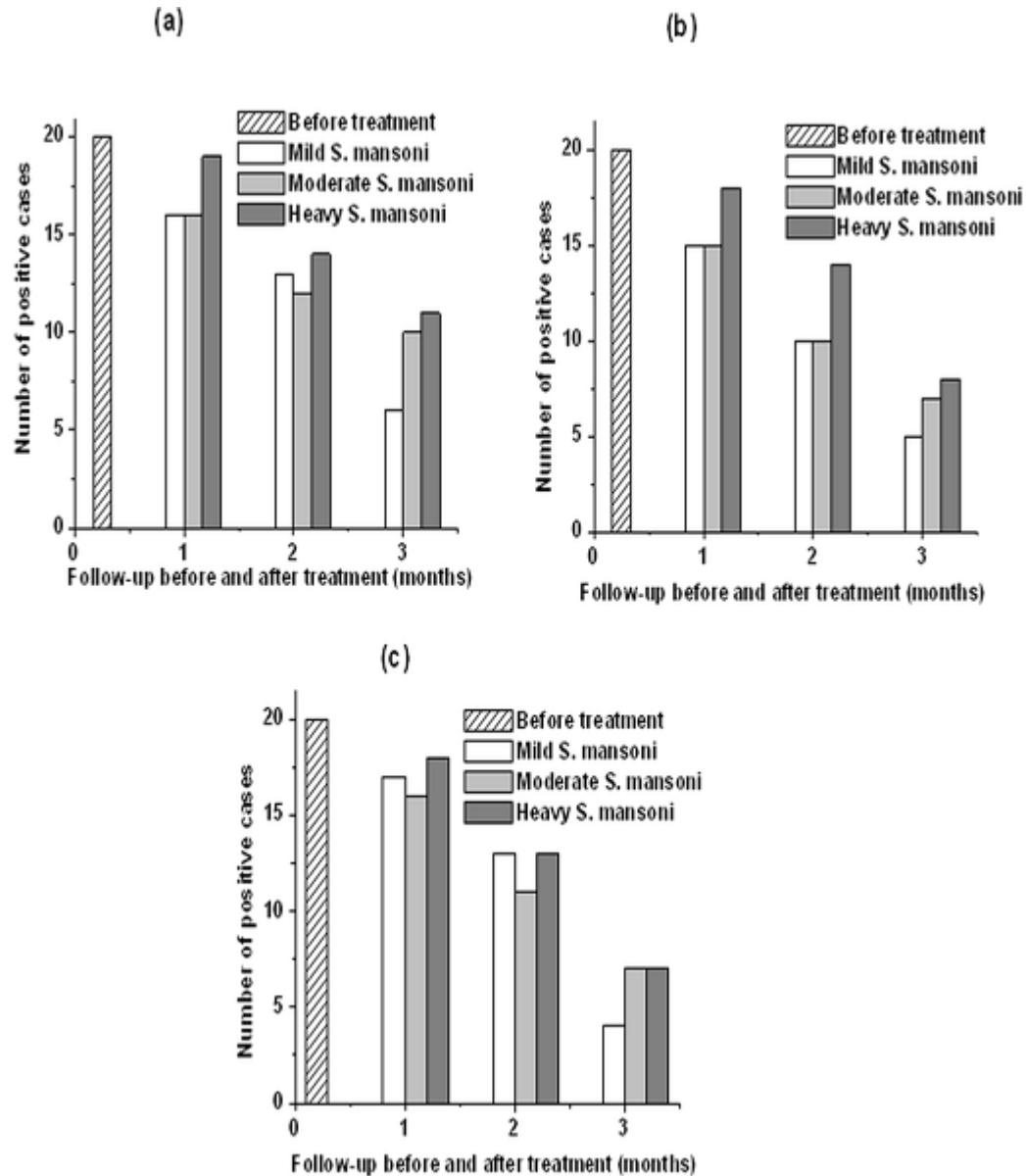
Type of infection	Treatment dose (mg)	Time (months)	Intensity ( $\bar{X} \pm S.D.$ )		t-test
			Pre-treatment	Post-treatment	
Mild <i>S. mansoni</i>	100	1	80.38±12.13	53.06±13.03	6.138
		2	82.31±9.31	37.38±9.10	12.442
		3	78.33±10.01	22.83±2.99	13.007
	150	1	83.72±10.44	46.80±11.28	9.192
		2	80.30±11.26	33.30±7.66	10.917
		3	84.20±12.15	21.80±2.39	11.266
	200	1	79.18±13.74	44.53±9.29	8.616
		2	88.46±14.12	30.77±6.58	11.498
		3	87.50±12.29	19.50±5.45	10.118
Moderate <i>S. mansoni</i>	100	1	205.63±42.32	146.06±44.01	t=3.902
		2	204.00±44.08	96.25±30.84	t=6.939
		3	202.50±48.57	53.90±16.88	t=9.139
	150	1	217.33±41.03	139.53±42.42	5.105
		2	228.10±42.38	80.10±26.39	9.375
		3	224.71±41.45	40.29±10.42	11.417
	200	1	214.63±42.51	151.13±52.77	3.749
		2	219.00±43.37	93.73±35.76	7.392
		3	289.43±46.01	41.86±16.17	9.091
Heavy <i>S. mansoni</i>	100	1	380.37±39.04	229.79±64.29	8.727
		2	381.21±41.45	157.71±46.08	13.479
		3	375.27±43.38	93.18±44.02	15.138
	150	1	386.94±43.03	232.82±50.29	9.761
		2	385.93±45.28	159.79±40.60	13.914
		3	396.63±57.61	87.63±38.91	12.572
	200	1	388.17±42.11	224.61±58.37	9.641
		2	393.00±40.57	134.31±30.76	18.326
		3	382.14±51.83	73.57±14.52	15.168



**Figure 2.** Cure % of *S. mansoni* infected cases after treatment by n-hexane myrrh extract capsules; a)- 100 mg; b)- 150 mg; c)- 200 mg

and 5 show treatment of *S. mansoni* infected cases with alcohol myrrh extract capsules 100, 150 and 200 mg (Formula 10, 11, 12). In case of treatment of heavy *S. mansoni* infected cases with alcohol myrrh extract capsules (100 mg) the cure percent was 0 % after 1 and 2 months as seen from figure 4 and the number of positive cases was 20 cases as illustrated in figure 5. After 3 months, cure

percent elevated to 15 % and number of positive cases reduced to 17 cases. The same results were also observed in case of treatment of heavy *S. mansoni* infected cases with alcohol myrrh extract capsules 150 and 200 mg where the cure percent was 0% after 1 and 2 months as seen from figure 4 and the number of positive cases was 20 cases as illustrated from figure 5. After 3 months, cure

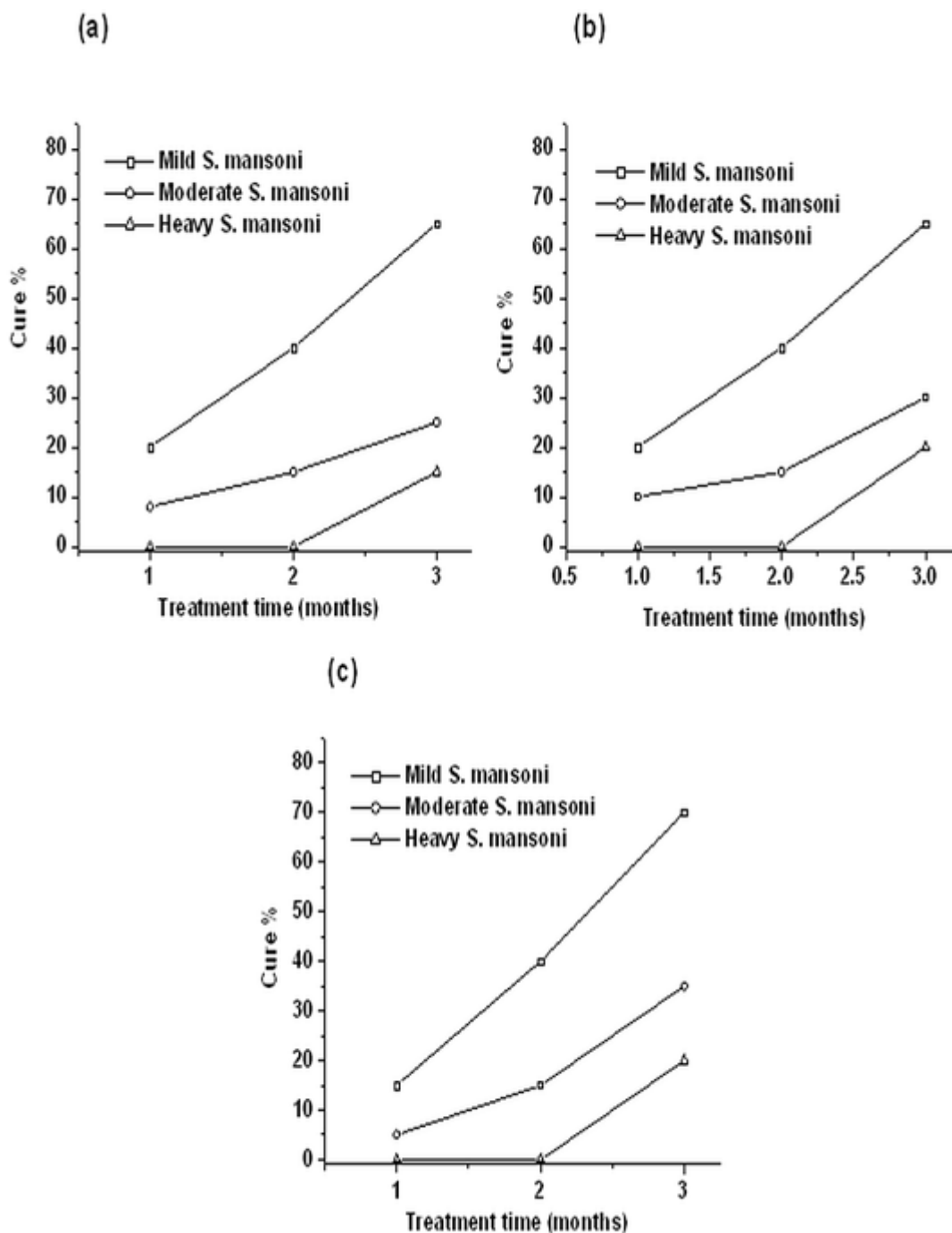


**Figure 3.** Relation between number of *S. mansoni* cases and follow-up before and after treatment by n-hexane myrrh extract capsules; a)- 100 mg; b)- 150 mg; c)- 200 mg

percent elevated to 20 % and number of positive cases reduced to 16 cases with both doses 150 and 200 mg. Although the cure percent was 0% after 1 and 2 months with heavy *S. mansoni*, cases which incompletely cured, there was significant reduction of infection intensity expressed by number of eggs excreted in stool with all alcohol myrrh extract capsules doses as seen from table 4.

In all types of *S. mansoni* infection (mild,

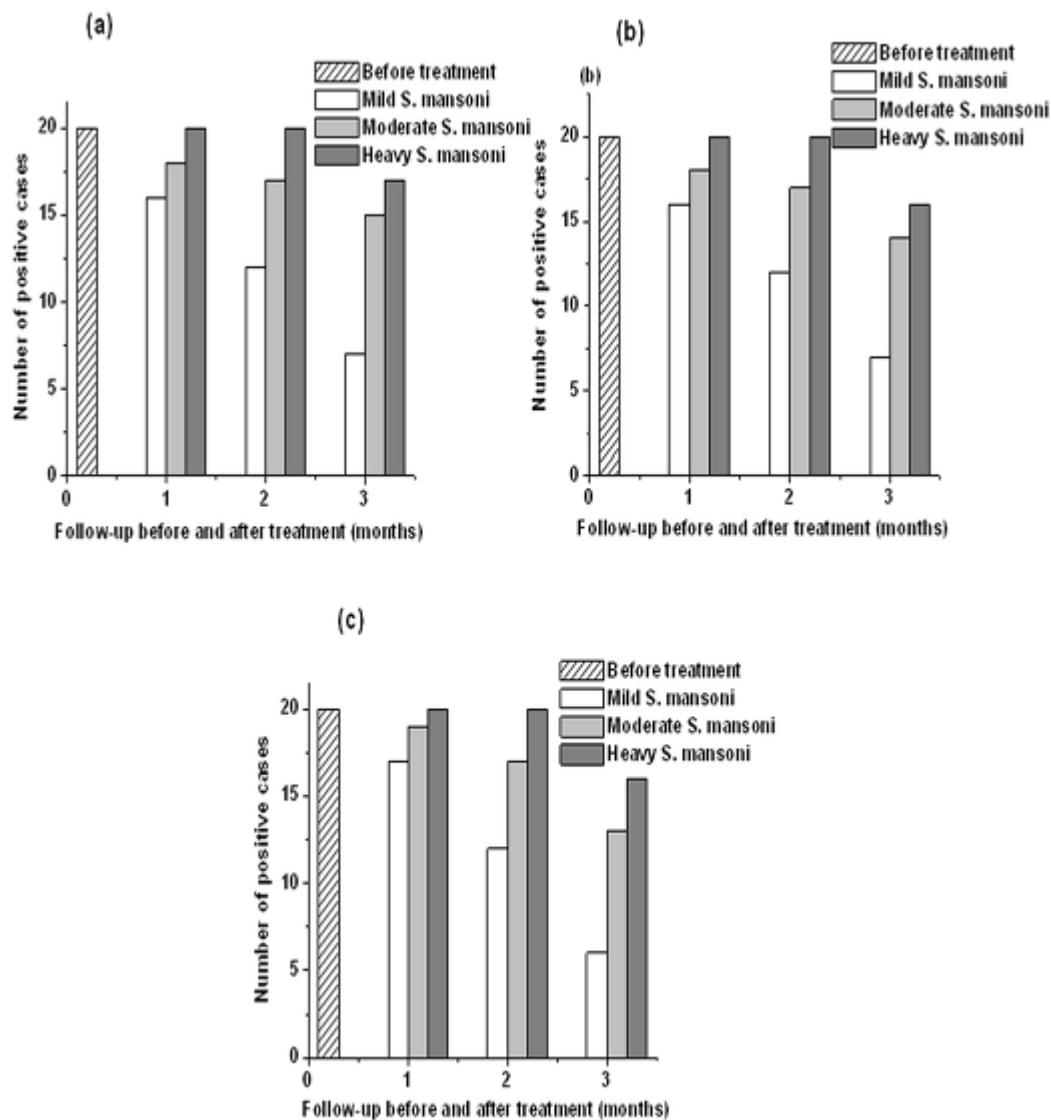
moderate and heavy) it is clear that the efficacy of n-hexane myrrh extract capsules with all doses was higher than the alcohol myrrh extract capsules with the same doses with respect to the cure percent, number of positive cases and infection intensity expressed by number of eggs excreted in stool. P-values were 0.000 in all cases which means that clinical improvement is highly significant (p-value is significant at any value less than 0.05).



**Figure 4.** Cure % of *S. mansoni* infected cases after treatment by alcohol myrrh extract capsules; a)- 100 mg; b)- 150 mg; c)- 200 mg

From the present work there was a markedly significant clinical improvement in the cure percent as regards easy fatigability, abdominal pains and distension. There was complete disappearance of the symptoms of blood in stool, tenesmus or dysentery after treatment by all

doses. All doses and forms were well tolerated by all patients without side effects. Cases with incomplete cure; there was a marked reduction in intensity of infection expressed by number of eggs excreted in stool. The efficacy of n-hexane myrrh extract was higher than the efficacy of



**Figure 5.** Relation between number of *S. mansoni* cases and follow-up before and after treatment by alcohol myrrh extract capsules; a)- 100 mg; b)- 150 mg; c)- 200 mg

alcohol myrrh extract capsules and this could be attributed to the higher ability of n-hexane in the extraction of furanosesquiterpenes which is the main constituent of myrrh<sup>26</sup>.

Furanosesquiterpenes is a volatile oil which is highly soluble in the organic solvent n-hexane in comparison with alcohol which explains this higher ability of n-hexane in the extraction of furanosesquiterpenes. On the other hand, alcohol may extract the hydrophilic constituent from myrrh and thus the concentration of furanosesquiterpenes in the alcoholic myrrh

extract was lower than its concentration in n-hexane extract and this may explain the higher efficacy of n-hexane myrrh extract in comparison with alcohol extract.

### Conclusion

Myrrh extract is a safe, potent and effective treatment, and can be used in community-based prevention and control of Schistosomiasis. Hard gelatin capsules of myrrh extract are highly effective as a pharmaceutical dosage form against Schistosomiasis.

**Table 4. Reduction of intensity of infection of *S. mansoni* cases which were still positive 1, 2 and 3 months after treatment by alcohol myrrh extract capsules**

Type of infection	Treatment dose (mg)	Time (months)	Intensity ( $\bar{X} \pm S.D.$ )		t-test
			Pre-treatment	Post-treatment	
<b>Mild</b> <i>S. mansoni</i>	100	1	70.38±16.98	45.81±14.98	4.352
		2	71.00±16.49	30.75±8.07	7.596
		3	66.00±17.03	16.43±5.00	7.390
	150	1	79.63±13.83	49.75±9.93	7.018
		2	79.50±14.32	36.17±6.87	9.448
		3	77.57±18.05	20.29±4.39	8.158
	200	1	73.47±15.70	48.12±12.05	5.282
		2	74.58±13.94	32.83±7.59	9.110
		3	75.00±11.31	22.83±4.75	10.414
<b>Moderate</b> <i>S. mansoni</i>	100	1	187.78±42.60	143.11±40.16	3.237
		2	188.06±43.90	110.53±42.18	5.251
		3	186.13±45.39	81.53±32.54	7.254
	150	1	201.72±45.06	137.94±42.65	4.361
		2	203.82±45.53	82.12±27.15	9.467
		3	210.79±44.57	55.21±21.75	11.737
	200	1	194.63±34.98	141.79±37.06	4.528
		2	194.88±37.08	92.53±28.30	9.047
		3	192.62±27.73	69.00±25.82	11.764
<b>Heavy</b> <i>S. mansoni</i>	100	1	377.50±34.96	299.70±50.18	5.689
		2	377.50±34.96	232.25±56.29	9.804
		3	385.59±31.11	163.59±45.68	16.560
	150	1	375.15±38.74	299.85±58.45	4.802
		2	375.15±38.74	223.05±53.76	10.265
		3	377.38±41.29	174.81±38.36	14.377
	200	1	379.00±38.47	308.95±48.39	5.067
		2	379.00±38.47	225.60±55.93	10.106
		3	387.94±31.44	165.63±48.95	15.286

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