Clinical study on the efficacy of Amoebex (coded herbal drug) compared with Metronidazole for the treatment of Amoebic dysentery

Syed Muhammad Ali Shah[1], Khan Usmanghani[2], Naveed Akhtar[3], Muhammad Akram[4]*, Hafiz Muhammad Asif[4] and Muhammad Mohtasheemul Hasan[5]

[1]Department of Eastern Medicine and Surgery, Govt. College University, Faisalabad, Pakistan
[2]Department of Basic Clinical Sciences, Faculty of Eastern Medicine, Hamdard University, Karachi, Pakistan
[3]Faculty of Pharmacy, Islamia University of Bahawalpur, Pakistan
[4]Department of Eastern Medicine and Surgery, The University of The Poonch, Rawalakot, Azad Jammu & Kashmir, Pakistan
[5]Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: Amoebiasisis an infectious disease, which originated with the single-celled parasitic protozoan *Entamoeba histolytica*. The parasitic amoeba infects the liver and intestine and may cause mild diarrhea and serious dysentery with bloody and mucoid stool. A study was conducted to evaluate the efficacy of Amoebex (400mg), a herbal formulation for the treatment of amoebiasis infections as compared to that of Metronidazole (400mg). The therapeutic evaluations of these medicines were carried out on 184 clinically diagnosed cases of the amoebiasis infection. Sample sizes of Ameobex for this study included a total of 93 patients and for Metronidazole a total of 91 were registered and treated. Comparison of the data recorded for the participants relating to sign and symptoms variables showed significant differences of efficacy between test and control groups ($p<0.0357$) and no side effects were at all recorded in test group. According to observation, there was a difference in the overall clinical success of both treatment groups, however, the efficacy of the test treated medication (Amoebex) was superior to that of Metronidazole as ($p<0.03$), and on the basis of the statistical analysis done by the chi square test, the null hypothesis was rejected. `It is clearly evident that Amoebex possesses therapeutic value for the treatment of amoebiasis associated symptoms but also the eradication rate of amoebiasis is superior by Amoebex as compared to that of Metronidazole (Control drug).

Keywords: Amoebic dysentery, amoebex, metronidazole, clinical study.

INTRODUCTION

Amoebiasis is contagious disease caused by *Entamoeba histolytica"*. The symptoms of mild diarrhea and dysentery with bloody and mucoid stool occur when amoeba infects the liver and intestines. *Entamoeba histolytica* may be life threatening that leads to intestinal bleeding if left untreated. In tropical and temperate regions *over* 50 million of population affected with amoebiasis, and each year about 100,000 deaths occur Worldwide (Pritt & Clark 2008, Grecu *et al*., 2006). It is spread by ingestion of water and foods infected with feces including *Entamoeba histolytica* cysts. In allopathic system of medicine first-line amoebiasis treatment is an anti-amoebic drug such as Metronidazole and Nidazole that have various side effects (Ravdin, 1995). The use of these drugs for couple of decades has developed resistance in parasite. It has been cited that Metronidazole can also cause chromosomal defect for the treatment of amoebiasis (Rosas *et al*., 2008). Due to resistance and side effects, a herbal formulation developed to control this parasitic infection.

Aims and objectives

Evaluation of the efficacy of herbal coded formulation (Amoebex 400mg) in comparison to allopathic medicine (metronidazole 400mg) to treat amoebic dysentery.

Methodology

The study was based on an experimental clinical trial of herbal formulation Amoebex for amoebiasis infection in which patients were randomly assigned to receive either herbal medicine or control allopathic treatment. Proper history and clinical examination were recorded on follow up. This was a case control, double blind, multicenter evaluation based study, conducted at the Shifa-ul-mulk Memorial Hospital Hamdard University Karachi, Hakeem Muhammad Said Shaheed Memorial Research Center, Bahawalpur and Bahawalpur Victoria Hospital (BVH), Bahawalpur from March 2010 to February 2012.

Methods/Design

The research based clinical study was prospective randomized case controlled trial in primary care with an open intervention. All patients examined by the general physician and given either herbal or allopathic medicine for amoebiasis. All patients were divided into two groups, control group and test group on the basis of treatment.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS and

**Corresponding author:* e-mail: makram_0451@yahoo.com

Microsoft excels 2010 software and Wilcoxon Sign Ranks test was performed for data manipulation. Likert scale was used to analyze the intensity of symptoms.

Inclusion criteria

The patients suffering from amoebiasis infection
Patients having no previous record of treatment against amoebiasis
Patients living in Bahawalpur and Karachi division
Patients without any chronic ailments
All socioeconomic including lower, middle and higher classes

Exclusion criteria

Patient with concurrent physical illness, for example uncontrolled hypertension and diabetes mellitus
Patients having surgical history of stomach or intestine were excluded
Any drug interaction or hypersensitivity
Pregnant females were also excluded due to safety measures
Chronic diseases such as tuberculosis and cardiac myopathies
Patients hospitalized for any serious diseases.

This clinical trial has been conducted to compare the efficacy and safety of two different treatment groups. Amoebex 400mg tablet as test drug and metronidazole 400mg tablet as control drug was given for the treatment of Amoebiasis. The stool D/R and antibody detection tests were performed for the diagnosis and confirmation of amoebiasis infection. Different parameter i.e. age, sex, and other clinical sign and symptoms were studied and compared between two treatment groups (Control and Test groups) at baseline and end of therapy. The written consent of patient was taken at the start of therapy. Out of 184 total patients enrolled only 93 were treated with coded herbal formulation Amoebex and 91 with the control therapy. The primary outcome of this study was to eradication of *Entamoeba histolytica* and secondary outcome to treat the sign and symptoms of amoebiasis. The frequency of male patients was 103 while 81 female

Table 1: Bioactivity studies on medicinal plants for the treatment of amoebic dysentery

Study [Ref.]	Medicinal Plants	Outcome measures and conclusion
Salimuzzaman S & Shamshuddin BA, 1989.	H. antidysenterica	Abdominal cramps, diarrhea, nausea and vomiting. Isolated a new alkaloid holarrifine effective for amoebiasis
Longanga AO *et al*., (2001)	Psidiumguajava	Antidiarrheal activity of root extracts form Roureopsis obliquifoliolata and Epinetrum illosum. Significantly reduced castor oil induced diarrhea in mice.
Rosa Martha P´erez *et al*., (2006).	Psidium guajava	Psidium guajavaposses antispasmodic and antimicrobial properties in the treatment of diarrhea and dysentery.
Vidal *et al*., (2007)	Mentha piperita Linn	Giardia lamblia: The effect of extracts andfractions from Mentha piperita Lin. (Lamiaceae) on trophozoites
Calzada *et al*., (2006)	Plants used in Mexican Traditional Medicine	*In vitro* susceptibility of Entamoeba histolytica and Giardia lamblia to plants used in Mexican traditional medicine for the treatment ofgastrointestinal disorders
Paul *et al*., (2005)	Fifty five medicinal plants belonging to different families	*In vitro* amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). 14 exhibited an antiamoebic activityat a dose of 100µg/ml from the second to thefourth day of incubation.

Table 2: Mean distribution of age

Treatment groups	Sex	Mean	Number (n)	Standard Deviation
Test drug (Amoebex)	Male	28.14	55	7.43
	Female	27.82	38	7.01
	Total	26.55	93	7.40
Control drug (Metronidazole)	Male	27.95	48	6.80
	Female	29.49	43	6.82
	Total	28.37	91	6.83
Total	Male	27.75	103	6.91
	Female	28.30	81	6.71
	Total	27.46	184	6.56

Table 3: Distribution of age

Age groups	Treatment groups		Total
	Test (n)	Control (n)	
15-24Years	39	41	80
25-34 Years	32	34	66
35-45 Years	22	16	38
Total	93	91	184

Table 4: Stool D/R in total patients at baseline and after treatment

		Treatment groups		Total (n)	
		Test	Control		
Stool D/R At Baseline	Positive	78	67	145	P value 0.03
	Negative	00	00	00	
	Total	78	67	145	
Stool D/R After treatment	Negative	58	39	97	
	Positive	20	28	40	
	Total	78	67	145	

Table 5: Abdominal pain in total patients at baseline and treatment

		Treatment groups		Total (n)	P value
		Test	Control		
Abdominal pain At Baseline	Yes	72	68	140	0.3992
	No	21	23	44	
	Total	93	91	184	
Abdominal pain After treatment	Improved	67 (93.05%)	50 (73.33%)	117	0.0027
	Not improved	05 (6.94%)	18 (26.47%)	23	
	Total	72	68	130	
McNemar Test calculations in improvement in abdominal pain by use ofAmoebex and Metronidazole					
P value		One Tailed		<0.000001	
		Two Tailed		<0.000001	
Odd ratio Larger/smaller		10			
95% Confidence Interval (CI)		Upper limit		25.0759	
		Lower limit		3.9869	

The *p*-value calculated by the chi-square test is shown in these tables, which indicate that overall a larger number of patients showed improvement in reducing the complaint of abdominal pain when treated with Amoebex as compared to Metronidazole.

patients enrolled in this study. These patients were randomly selected, at the time of enrollment there were total of 208 patients. After screening and selection criteria only 184 patients complied with criteria for inclusion to be treated. Out of remaining 24 patients, 08 patients did not agree to participate in the clinical trial, 06 patients dropped out due to poor response in follow up, 04 patients excluded due to some serious side effects and remaining 04 patients were dropped due to allergic reaction during the course of treatment. During treatment physician filled up a clinical evaluation proforma for every patient designed on the basis of clinical evaluation and assessment of improvement in clinical signs and symptoms and record of the side effects encountered during the treatment. A weekly record of sign and symptoms was maintained for analyzing the improvement in Amoebiasis associated symptoms. Disappearance of abdominal pain, diarrhea, stool with blood and mucus, nausea and vomiting were especially noted. Routine examination of different investigations mentioned in clinical trial protocol especially stool D/R was done for assessing the improvement. All this data was statistically analyzed by Chi-Square and the level of significance were applied. In order to validate the results, alternative statistical analysis such as Exact Fisher test was applied to confirm the efficacy of the treatment groups both in test and control group as Amoebex and Metronidazole treatment respectively.

Patient characteristics

The mean age of patients prescribed for Amoebex was 28.14 and 27.82 years of males and females respectively. The mean age of patient prescribed for Metronidazole was 27.95 and 29.49 years of males and females respectively (table 2).

Table 6: Diarrhea at baseline and after treatment

		Treatment groups		Total	P vaue
		Test	Control		
Diarrhea at baseline	Yes	75	74	149	0.500
	No	18	19	37	
	Total	93	91	184	
Diarrhea after treatment	Improved	61 (81.33%)	51 (59.45%)	112	0.0586
	Not improved	14 (18.66%)	23 (40.54%)	37	
	Total	75	74	149	
McNemar Test calculations in Improvement in Diarrhea (Amoebex and Metronidazole).					
P value		One Tailed		0.00002	
		Two Tailed		0.00001	
Odd ratio Larger/ Smaller		3.6429			
95% Confidence Interval (CI)		Upper limit		6.5808	
		Lower limit		2.0166	

Overall, 81.33% of patients prescribed Amoebex showed complete improvement for diarrhea and 59.45% of patients prescribed Metronidazole showed complete improvement.

Table 7: Stool with blood in total patients at baseline and after treatment

		Treatment groups		Total	P value
		Test	Control		
Stool with blood at base line	Yes	31	29	60	0.4783
	No	62	62	124	
	Total	93	91	184	
Stool with blood after treatment	Improved	25 (80.64%)	20 (29.73%)	45	0.228
	Not improved	06 (19.35%)	09 (31.03%)	15	
	Total	31	29	60	
McNemar Test calculations in Improvement in Stool with blood (Amoebex and Metronidazole).					
P value		One Tailed		0.009355	
		Two Tailed		0.004678	
Odd Ratio Larger/Smaller		3.3333			
95% Confidence Interval (CI)		Upper limit		8.3003	
		Lower limit		1.3386	

The 80.64 % of patients prescribed Amoebex had complete improvement and 29.7% of patient prescribed Metronidazole had complete improvement in treating stool with blood (table 7).

Table 8: Stool without blood in total patient at baseline and after treatment

		Treatment groups		Total	P value
		Test	Control		
Stool without blood at baseline	No	68	71	139	0.3634
	Yes	25	20	45	
	Total	93	91	184	
Stool without blood after treatment	Improved	60 (88.23%)	58 (81.69%)	45	0.2008
	Not improved	08 (13.33%)	13 (18.30%)	15	
	Total		71	60	
McNemar Test calculations in improvement in Stool without blood (Amoebex and Metronidazole).					
P value		One Tailed		<0.000001	
		Two Tailed		<0.000001	
Odd Ratio Larger/Smaller		7.25			
95% Confidence Interval (CI)		Upper limit		15.1836	
		Lower limit		3.4618	

The 88.23% of patients prescribed Amoebex had complete improvement and 81.69% of patient prescribed Metronidazole had complete improvement (table 8).

Table 9: Nausea and vomiting in total patients at baseline and after treatment

		Treatment groups		Total	P value
		Test	Control		
Nausea and vomiting	Yes	39	32	71	0.2134
	No	54	59	113	
	Total	93	91	184	
Nausea and vomiting after treatment	Improved	33 (84.61%)	21 (65.62%)	54	0.0564
	Not improved	06 (15.38%)	11 (34.37%)	17	
	Total	39	32	61	
McNemar Test calculations in Improvement in nausea and vomiting Amoebex and Metronidazole).					
P value		One Tailed		0.005925	
		Two Tailed		0.002962	
Odd Ratio Larger/Smaller		3.5			
95% Confidence Interval (CI)		Upper limit		8.6717	
		Lower limit		1.4126	

The 84.61% of patients were prescribed Amoebex showed complete improvement and 65.62% of patients were prescribed Metronidazole showed complete improvement (table 9).

Table 10: Stool with mucus in total patients at baseline and after treatment

		Treatment groups		Total	P value
		Test	Control		
Stool with mucus at baseline	Yes	45	42	87	0.4382
	No	48	49	97	
	Total	93	91	184	
Stool with mucus after treatment	Improved	38 (84.44%)	29 (69.04%)	67	0.0732
	Not improved	7(15.55%)	13 (30.95%)	20	
	Total	45	42	87	
McNemar Test calculations in Improvement in Stool with mucus (Amoebex and Metronidazole).					
P value		One Tailed		0.000313	
		Two Tailed		0.000156	
Odd Ratio Larger/Smaller		4.1429			
95% Confidence Interval (CI)		Upper limit		9.4573	
		Lower limit		1.8149	

The 84.44% of patients prescribed Amoebex had complete improvement and 69.04% of patients prescribed Metronidazole had complete improvement (table 10).

Table 11: Overall comparative evaluations in improvement of sign/symptoms by Amoebex and Metronidazole

Sign/ Symptoms	Treatment Groups						P value
	Amoebex (Herbal)			Metronidazole			
	Improved	Not Improved	Results	Improved	Not Improved	Results	
Abdominal Pain	67/72	05/72	93.05%	50/68	18/68	73%	0.0027
Diarrhea	51/75	14/75	80.35%	51/74	23/74	59.45%	0.0586
Stool with Blood	25/31	06/31	80.64%	20/29	09/29	29.73%	0.228
Stool without blood/ Loose stool	60/68	08/68	88.23%	58/71	13/71	81.69%	0.2008
Nausea/vomiting	33/39	06/39	84.61%	21/32	11/32	65.62%	0.0564

The age was classified in different class interval 15 to 45 years range as 15-24, 25-34 and 35-45. Between 15-24 years of age, the total number of patients was 78, between 25-34 years of age, the total number of patients was 52 and between 35-45 years of age, the total recorded patients were 45.

Treatment assignment and follow-up

One hundred and eighty three patients consented to participate in the study. The two treatment groups were compared for efficacy results and side effects of the medicine administered. In a trial, effect of Amoebex 400mg 2 tablets after meal thrice daily compared to

Table 12: Overall improvement in severity of symptoms in Test group by Wilcoxon

	Overall severity of symptoms			
Baseline			After treatment	
(T0)			(T1)	
Median	IQR	Median	IQR	p value
11	7-14	4	3-7	0.006

Table 13: Improvement in intensity of symptoms with Amoebex tablet using Wilcoxon Signed Rank Test

Symptoms	Intensity of symptoms				
	Baseline (T0)			After treatment (T1)	
	Median	IQR	Median	IQR	*p* value
Abdominal	2.5	2-3	1	1-2	0.05
Pain					
Diarrhea	2	1-3	0.5	0-1	0.02
Stool with	2	1-3	1	1-2	0.01
Blood					
Stool without	2.5	2-3	0.5	1-3	0.003
Blood					
Nausea/Vomiting	2.5	2-3	1	0-1	0.03
Stool with	2	2-3	1	1-2	0.06
Mucus					

Table 14: Overall severity of symptoms in control group by Wilcoxon Signed Rank Test

Overall severity of symptoms				
Base line (T0)		After one month of treatment (T1)		
Median	IQR	Median	IQR	P value
11	8-13	5	3-8	0.0113

Table 15: Improvement in intensity of symptoms with Metronidazole

	Intensity of symptoms				*p* value
Symptoms	Baseline (T0)			After treatment (T1)	
	Median	IQR	Median	IQR	
Abdominal	2.5	2-3	1.5	1-2	0.05
Pain					
Diarrhea	2	1-3	1	0-1	0.02
Stool with	2	1-3	1	1-2	0.01
Blood					
Stool without	2.5	2-3	1	1-3	0.003
Blood					
Nausea/Vomiting	2.5	2-3	1	0-1	0.03
Stool with	2	2-3	1	1-2	0.06
Mucus					

Metronidazole was investigated on a total of 184 patients suffering from Amoebiasis.

RESULTS

According to the statistical analysis, *Entamoeba histolytica* was eradicated in 52patients (57.14%) out of 91 patients with Metronidazole (Control drug) and in 66 patients (70.96%) out of 93 patients with Amoebex (Test drug). In test group, the evaluation of *Entamoeba histolytica* eradication was significantly high. Moreover, there was a significant improvement in Amoebiasis in test group as compared to control group. Chi-Square Test was applied and the p value calculated below 0.0357 which is below 0.05 and it indicates that the efficacy of the Amoebex in *Entamoeba histolytica* elimination and

Table 16: Comparison in intensity of symptoms between two treatment groups by Wilcoxon Signed Rank Test

Amoebex					Metronidazole				
Before		After treatment (T1)			Before		After treatment (T1)		
Treatment (T0)					Treatment (T0)				
Median	IQR	Median	IQR	P value	Median	IQR	Median	IQR	p value
11	7-14	4	3-7	0.006	11	8-13	5	3-8	0.011

associated symptoms is significant and has pronounced effects.

Laboratory investigations

Stool D/R test

In control group, 67 patients were evaluated by stool D/R test before treatment. After treatment, 39 patients recorded as negative stool D/R and 28patients were remained positive, whereas in test group, 78 positive patients recorded by Stool D/R test before treatment, in which 58 patients recorded as negative stool D/R and 20 patients were detected as positive after treatment (Table 4).

Clinical features

Abdominal pain

After treatment test group patients (Amoebex) has 93.05% improvement as compared to control (Metronidazole) 83.33% improvement. Chi-Square Test was applied and p value was calculated as 0.0072. According to McNamara's calculation, one tailed and two-tailed *p*-value showed significant results with odd ratio 10 while upper limit was 25.07 and lower limit was as 3.9 with confidence interval (table 5).

Diarrhea

Amoebex was prescribed to 75 patients who were suffering from diarrhea. The 61 patients showed complete improvement and 14 patients showed no improvement after the treatment with herbal drug Amoebex. Metronidazole was prescribed to 74 patients with complaint of diarrhea. After the treatment with Metronidazole 44 out of 74 showed complete improvement and 30 patients showed no improvement. After treatment, test group patients with Amoebex have 76% improvement whereas control group with Metronidazole showed 20% improvement. Chi-Square Test was applied and found p value as 0.0586. According to McNamara's calculation, one tailed and two-tailed *p*-value showed significant results with odd ratio 3.64 while upper limit was 6.5 and lower limit was 2.01 with confidence interval.

Stool with blood

Amoebex was prescribed to 31 patients with complaint of stool with blood. After the treatment with Amoebex, 25 patients out of 31 showed complete improvement and 6 patients showed no improvement. Metronidazole was prescribed to 29 patients with complaint of stool with blood. After the treatment with Metronidazole, 20 out of 29 showed complete improvement and 9 patients showed no improvement. After treatment, test group (Amoebex) has 80.64% improvement as compared to control (Metronidazole) 29.37% improvement. According to McNamara's calculation, one tailed and two-tailed *p*-value shows significant results with odd ratio 3.33 while upper limit was 8.33 and lower limit was 1.30 with confidence interval.

Stool without blood/loose stool

Amoebex was prescribed to 68 patients with complaint of stool without blood. After the treatment with Amoebex, 60 patients out of 68 showed complete improvement and 8 patients showed no improvement. Metronidazole was prescribed to 71 patients with complaint of stool without blood. After the treatment with Metronidazole, 58 out of 71 showed complete improvement and 13 patients showed no improvement. After treatment, test group (Amoebex) has 88.64% improvement as compared to control (Metronidazole) 81.37% improvement. According to McNamara's calculation, one tailed and two-tailed *p*-value showed significant results with odd ratio 7.25 while upper limit was 18.15 and lower limit was 3.46 with confidence interval.

Nausea and vomiting

Amoebex was prescribed to 39 patients with complaint of nausea and vomiting. After the treatment with herbal drug Amoebex, 33 out of 39 showed complete improvement and 6 patients showed no improvement. Metronidazole was prescribed to 21 patients with complaint of nausea and vomiting. After the treatment with Metronidazole, 21 out of 32 showed complete improvement and 11 patients showed no improvement. After treatment in test and control group, test group (Amoebex) has 84.61% improvement and control (Metronidazole) showed 65.62% improvement as shown in table 9.

Stool with mucus

Amoebex was prescribed to 45 patients with complaint of stool with mucus. After the treatment with herbal drug Amoebex, 38 out of 45 showed complete improvement and 2 patients showed no improvement. Metronidazole was prescribed to 42 patients with complaint of stool with mucus. After the treatment with Metronidazole 10 out of 29 showed complete improvement and 13 patients showed no improvement. After treatment, test group (Amoebex) has 84.44% improvement as compared to

control (Metronidazole) 69.04% improvement as shown in the table 10. According to McNamara's calculation, one tailed and two tailed *p*-value shows significant results with odd ratio 4.14 while upper limit was 9.45 and lower limit was 1.81 with confidence interval as shown in the table 10.

The objective of this study was to compare herbal medicine Amoebex with that of Metronidazole and to see whether these may result to determine the efficacy to cure diarrhea and its symptoms. It was observed that there is a marked improvement in overall subjective signs and symptoms when treated with Amoebex as compared to Metronidazole. There was a noticeable improvement in abdominal pain, diarrhea, nausea/vomiting, stool with/without blood and stool with mucus, which are the most common symptoms of active amoebiasis infection. This may be the striking findings of present stud and this effect could be due to the presence of *Holarrhena antidysenterica* which is major active ingredient of Amoebex.

Intensity of symptoms

Intensity of symptoms is a signal of infection accessing the inflammatory process in the intestine. In this study, the intensity of symptoms was recorded as absent: 0, mild: 1, moderate: 2 and severe: 3 at baseline (T0) and after the completion of treatment (T1). Median values, interquartile ranges were determined and p values calculated by using Wilcoxon signed rank test to record the indirect effects of Amoebex in the reduction of inflammatory process and severity of infection.

Improvement in symptoms with Amoebex

There was a statistically significant decrease in the overall symptom score from baseline (T0: median 11, IQR 7-14) to after completion of treatment (T1: median 4, IQR 3-7).

Wilcoxon signed rank test

Different sign and symptoms record when analyzed through Wilcoxin Signed Rank test exhibited value as follows. Abdominal pain (T0: median 2.5, range 2-3; T1: median 1, range 1-2), diarrhea (T0: 2, range 1-3; T4: median 0.5, range 1-2), stool with blood (T0: 2, range 2-3; T1: median 1, range 1-2), stool without blood (loose stool) (T0: 2.5, range 2-3; T1: median 0.5, range 1-3), nausea/vomiting (T0: median 2.5, range 2-3; T1: median 1, range 0-1) and stool with mucus (T0: median 2, range 2-3; T1: median 1.5, range 1-2) all showed statistically significant improvement after treatment with Amoebex.

Improvement profile with control therapy

Metronidazole also exhibited a statistically significant decrease in the over all symptom score from baseline (T0: median 11, IQR 8-13) to after treatment (T1: median5, IQR 3-08). Abdominal pain (T0: median 2.5, range 2-3; T1: median 1, range 1-2), diarrhea (T0: 2, range 1-3; T1: median 0.5, range 1-2), stool with blood (T0: 2, range 2-3; T1: median 1, range 1-2), stool without blood (loose stool) (T0: 2.5, range 2-3; T1: median 0.5, range 1-3), nausea/vomiting (T0: median 2.5, range 2-3; T1: median 1, range 0-1) and stool with mucus (T0: median 2, range 2-3; T1: median 1.5, range 1-2) all showed statistically significant improvement after treatment with Amoebex.

Comparative analysis of intensity of symptoms between treatment groups

A comparative analysis was done in the level of intensity of symptoms between two treated groups i.e. test and control groups before and after the treatment. Wilcoxon signed rank test was applied to see the statistical difference after calculating the median values and interquartile ranges. It was concluded from this statistical analysis, that Amoebex (Test) possesses greater value to lower down the intensity of symptoms as compared to Metronidazole therapy (Control).

DISCUSSION

In order to overcome problem of treatment for amoebic dysentery, there is a need to design and develop new poly herbal formulation that has better efficacy with less or no adverse effects. Herbal medicine could be another choice to treat amoebiasis and relieving its clinical sign and symptoms. So considering this option, a formulation has been designed based on literature search. Therefore, one of the viable candidates to treat amoebic dysentery could be a coded anti-diarrheal preparations used in Kinshasa, Congo and the extract of medicinal plants used in the formulation inhibited *Entamoeba histolytica* growth with MAC<10ug/ml. Youvraj *et al.,* (1995) reported anti-amoebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica in vitro* and *in vivo* and showed about 89% efficacy as compared to metonidazole at cure rate of 89%. Julia *et al.,* (1986) reported the epidemiology of ameobic dysentery and concluded that in 1981 that about 480 million people suffered with *Entamoeba histolytica* and 36 million patients proceed to amoebic colitis or extra intestinal abscesses. Okpekona *et al.,* (2004) reported about 17 medicinal plants collected from Ivory Coast for the screening of anti-parasitic activity *in vitro* and among these medicinal plants, *Anogeissusleio carpus* and *Terminalia glaucescens* were highly active against *Entamoeba histolytica.* It has been previously reported that *Holarrhena antidysenterica*, *Althaea officinalis*, *Aegle marmelos* and *Helicteres isora* are commonly used for the treatment of amoebiasis. The clinical efficacy of some herbal medicine for amoebiasis was determined on patient treated with amoebin cap (herbal formulation) showed remarkable efficacy than Entamizole DS. After applying the statistics significant p value was found while

in randomized clinical study parasite cure rate was more than control drug metronidazole that is 57.4% (Shahabuddin *et al*, 2006). *In vitro* and *in vivo* study conducted by Sheela *et al.,* (1996) to investigate the anti-amoebic activity of *Piper longum,* the crude ethanolic extract exhibited efficacy with almost 90% cure rate, while piperine a pure compound derived *Piper longum* exhibited 40% bioactivity (Sheela *et al*., 1996). Salvador *et al.,* (1999) investigated the *in-vitro* determination of the amebicidal activity of *Buddleia cordata* (Loganiaceae) on several strains of *Acanth amoeba.* Aqueous and methanolic extracts of *Buddleia cordata* were screened against antiamoebic activity. The extracts of 29 strains of free-living amoebae were screened and about 14, 15 were amebostatic respectively but only linarin was amebostatic. Linarin value ranged from 31.25mg/ml to 4 mg/ml (Salvador *et al*., 1999). McGaw *et al.* (2000) reported anti-amoebic activity in South African medicinal plants (McGaw *et al.,* 2000). Fernando *et al.* (2005) cited *in-vitro* antiprotozoal activity in *Geranium mexicanum on Entamoeba histolytica* and *Giardia lamblia* and they were found active against both protozoa. Paul *et al.,* (2005) investigated *in vitro* amoebicidal activity of some medicinal plants of the Bamun region (Cameroon) and fifty five medicinal plants selected on the basis of their traditional use in different diseases. Polyxenic culture of *Entamoebia histolytica* showed antiamoebic activity at a dose of 100µg/ml on the second to the fourth day of incubation. Leaves extract of *Codiaeumvariegatum* showed antiamoebic activity (EC_{50}=10,74 the second day), and was more active than metronidazole as reference product. The literature citations on the different biological studies on medicinal plants for the treatment of amoebic dysentery are presented in table 1. It was observed that there is a marked improvement in overall subjective signs and symptoms when treated with Amoebex as compared to metronidazole. There was a noticeable improvement in abdominal pain, diarrhea, nausea/vomiting, stool with blood/without blood and stool with mucus, which are the most common symptoms of active *Entamoeba histolytica* infection. Misra *et al*., (1977) investigate symptomatic intestinal amoebiasis randomly in sixty patients, which were treated for 3 days with a single dose of 2g of either tinidazole or metronidazole respectively. Patient cured with metronidazole were 53% that is (16/30) while in present study cured was 57.4% that is somehow equal response to above study (Misra & Gupta., 1977). In a previous study conducted by Pehrson P *et al.,* (1984) non-invasive amoebiasis verses treatment with metronidazole 800 mg three times daily or tinidazole 600 mg twice daily for five days to observe the parasite cure rate in a small pilot study. Both treatments were seen unfavorable with parasite cure rate of 44 and nil respectively, in contrast to previous published results showing PCR over 80%. While in present study, 57% of the patient showing parasite cure rate when metronidazole was given two tablets thrice a day for five days (Pehrson & Bengtsson, 1984). Swami *et al.,* (1977) investigated the efficacy of metronidazole and tinidazole in sixty adult patients with symptomatic intestinal amoebiasis with positive *Entamoeba histolytica* present in stool D/R. Both drugs were given to the patients at dose of 1g once daily for 3 days, Twenty nine on tinidazole and 27 on metronidazole out of 56 patients, completed the trial as per the protocol. Cure rate in tinidazole was 96.5% and 55.5% on metronidazole. No toxic effects were seen on any one of the drug. Tinidazole showed highly cure rates than metronidazole in the treatment of symptomatic intestinal amoebiasis ($p>0.01$). Botero (1974) studied on 115 patients with symptomatic intestinal amoebiasis, 56 were treated with Ro 7-0207 and 59 with metronidazole in a double blind study. Similar results were seen in both groups and mild side effects were noted except in one patient who received Ro 7-0207 and symptoms include numbness of hands and tongue, difficulty in speaking, and headache. These symptoms terminated when treatment was stopped. While in present study, metronidazole outcomes were seen 57.4% with mild side effects in some patients showed nausea and vomiting.

CONCLUSION

The finding from this study demonstrated that there was statistically significant difference when comparing the efficacy of herbal medicine Amoebex to Metronidazole for the treatment of Amoebiasis. This is clearly evident that Amoebex possesses a therapeutic value for the treatment of Amoebiasis and eradication rate of Amoebiasis is superior with Amoebex as compared to Metronidazole. Chi-square test and Wilcoxon Signed Rank Test analysis displayed the statistical difference between the both treatment groups. The results obtained described that test drug is more effective than control for management of Amoebiasis associated symptoms, the effect being confirmed by physicians and patients alike.The results from this research study have clearly revealed the evidence of efficacy of test drug Amoebex for the improvement of Amoebiasis associated symptoms as compared to Metronidazole. The approach of this scientific clinical study validates the Unani medicine, so in its ultimate result, it leads to new class of therapeutics.

REFERENCES

Botero D (1974). Double blind study with a new nitroimidazole derivative, Ro 7-0207, versus metronidazole in symptomatic intestinal amebiasis. *Am. J. Trop. Med. Hyg.*, **23**: 1000-1001.

Calzada F and Aguilar A (2006). *In vitro* susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *J. Ethnopharmacol.*, **108**: 367-370.

Fernando C, Antonio CJ and Lilian YM (2005). *In vitro* antiprotozoal activity from the roots of *Geranium mexicanum* and its constituents on *Entamoeba histolytica* and *Giardia lamblia. J. Ethnopharmacol*., **98**: 191-193.

Grecu F, Bulgariu T, Blanaru O, Dragomir C, Lunca C, Stratan I, Manciuc C and Luca V (2006). Invasive Amebiasis. *Chirurgia,* **101**: 539-542.

Julia A (1986). Problems in recognition and diagnosis of Ameobiasis. *Clin. Infect Dis.*, **8**: 228-238.

Longanga AO, Vercruysse A and Foriers A (2001). Antidiarrhoeal activity of root extracts from Roureopsisobliquifoliolata and Epinetrumillosum. *Fitoterapia*., **72**: 291-294.

McGaw LJ, Ja¨ger AK and Staden J (2000). Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *J. Ethnopharmacol.*, **72**: 247-263.

Misra P and Gupta C (1977). A comparison of a short course of single daily dosage therapy of tinidazole with metronidazole in intestinal amoebiasis. *J. Int. Med. Res.*, **5**: 434-437.

Okpekona T, Yoloua S, Gleyeb C, Roblotb F, Loiseauc P, Boriesc C, Grellierd P, Frappiere F, Laurensb A and Hocquemillerb R (2004). Antiparasitic activities of medicinal plants used in Ivory Coast. *J. Ethnopharmacol.*, **90**: 91-97.

Paul F, Moundip, Kamini G, Melanie F, Charles F, Bilong B and Iris B (2005). *In vitro* amoebicidal activity of some medicinal plants of the bamun region (cameroon). *Afr. J. Tradit. Complement. Altern. Med*., **2**: 113-121.

Pehrson P and Bengtsson E (1984). Treatment of non-invasive amoebiasis-a comparison between tinidazole and metronidazole. *Ann. Trop. Med. Parasitol.*, **78**: 505-508.

Pritt BS and Clark CG (2008). Amoebiasis. *Mayo. Clin. Proc.*, **83**: 1154-1159.

Ravdin JI (1995). Amebiasis (Review). *Clin. Infect Dis*., **20**: 1453-1466.

Rosa P, Sylvia M and Rosario V (2008). Pisidiumguajava: A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.*, **117**: 1-27.

Rosas P, Arteaga-Nieto P, Reynoso R, Villagómez JC and Sabanero M (2008). *Burseraf agaroides*, effect of an ethanolic extract on ornithine decarboxylase (ODC) activity *in vitro* and on the growth of *Entamoebahistolytica*. *Exp. Parasitol.*, **119**: 398-402.

Salimuzzaman S and Shamshuddin BA (1989). Isolation & structure of holarrifine, anew alkaloid from the bark of *H. antidysenterica*. *Pak. J. Sci. Ind. Res.*, **32**: 1-3.

Salvador RZ, Cinthya O, Guillermo A, Jose´ Luis M, Amira A and Alfonso RV (1999). *In vitro* evaluation of the amebicidal activity of *Buddleia cordata* (Loganiaceae, H.B.K.) on several strains of *Acanthamoeba. J. Ethnopharmacol*., **66**: 327-334.

Shahabuddin S, Usmanghani k, Muhammad SS and Ejaz M(2006). Clinical evaluation of some herbal medicine for amoebiasis. *Pak. J. Pharmacol.*, **23**: 9-12.

Sheela G. Krishna N and Lakshmi V (1996). Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolyticain vitro* and *in vivo*. *J. Ethnopharmacol.*, **50**:167-170.

Swami B, Lavakusulu D and Devi CS (1977). Tinidazole and metronidazole in the treatment of intestinal amoebiasis. *Curr. Med. Res. Opin.*, **5**:152-156.

Tona L, Kambu K, Ngimbi N, Cimanga K and Vlietinck AJ (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J. Ethnopharmacol.*, **61**: 57-65.

Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, Cimanga K, Bruyne T, Apers S, Totte J, Pieters L and Vlietinck AJ (2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa Congo. *Phytomed.*, **7**: 31-38.

Vidal F, Vidal JC, Gadelha APR, Lopes CS and Coelho MGP (2007). Monteiro-Leal LH. *Giardia lamblia*: The effects of extracts and fractions from *Mentha piperita* Lin. (Lamiaceae) on trophozoites. *Exp. Parasitol.*, **115**: 25-31.

Youvraj R, Sohni PK and Ranjan MB (1995). Antiamoebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica in vitro* and *in vivo. J. Ethnopharmacol.*, **45**: 43-52.