Acute and sub acute toxicity study and randomized clinical trial of polyherbal coded drug candicure in the management of acute vulvo-vaginal candidiasis

Article *in* Pakistan journal of pharmaceutical sciences · January 2019

CITATIONS
0

READS
214

6 authors, including:

Sabira Sultana
The Islamia University of Bahawalpur
24 PUBLICATIONS **303** CITATIONS
SEE PROFILE

Muhammad Asif
The Islamia University of Bahawalpur
84 PUBLICATIONS **873** CITATIONS
SEE PROFILE

Muhammad Akram
Government College University Faisalabad
365 PUBLICATIONS **1,214** CITATIONS
SEE PROFILE

Syed Muhammad
London School of Business and Finance
32 PUBLICATIONS **85** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Research and articles on teaching models and strategies based on constructivism theory. View project

Computer Science and Engineering View project

Acute and sub acute toxicity study and randomized clinical trial of polyherbal coded drug candicure in the management of acute vulvo-vaginal candidiasis

Sabira Sultana[1], Tasneem Qureshi[2], Hafiz Muhammad Asif[3], Muhammad Akram[1], Sultan Ayaz[1], and Syed Muhammad Ali Shah[1]

[1]Directorate of Medical and Health Sciences, Department of Eastern Medicine, Government College University, Faisalabad, Pakistan
[2]Department of Surgery and Allied Sciences, Faculty of Eastern Medicine, Hamdard University, Karachi, Pakistan
[3]University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, Islamia University Bahawalpur, Pakistan

Abstract: Vulvo-Vaginal Candidiasis is common gynecological disorder. Clinical trials are designed to evaluate the effect of medical intervention with allopathic and herbal medicine to treat vulvo-vaginal candidiasis. The toxicity index and the safety profile of test drug were assessed on animals' model. Toxicity study of polyherbal formulation was done in order to consider it safe before use. In acute toxicity study, a single dose of 2000 mg/kg was given to Swiss Albino mice and in sub-acute toxicity study three different doses were given to Wistar rats. Randomized Controlled Clinical Trials was conducted. Inclusion and exclusion criteria and informed consent from the patients were to be monitored. In acute toxicity study no morbidity and mortality noticed on single administration of dose 2000mg/kg/day. In sub -acute toxicity study no significant changes were observed in consumption of food and water, body weight, hematological parameters, Liver function test, renal function parameters and lipid profile. The statistical analysis showed *C. albican* was eradicated in 59 participants (78%) out of 75 participants by the use of Candicure (Test drug) and in 56 participants (74%) out of 75 participants by the use of Fluconazole therapy (Control drug). Chi-square test was applied and p-value was calculated 0.3101 which is greater than 0.05 showed that Candicure therapy and Fluconazole therapy is equally significant in eradication of *C. albican*. The acute and sub-acute toxicity study of polyherbal formulation on experimental animals is safe at a dose of 2000mg/kg/day. The clinical findings of randomized controlled trial revealed that the effect was almost comparable to control drug in eradicating the micro-organism.

Keywords: Clinical trials, antifungal activity, *in vitro* activity, poly herbal formulation.

INTRODUCTION

Vaginal candidiasis, a most prevalent gynecological condition seen in primary health care services. Disease is more frequently associated with substantial morbidity, discomfort, healthcare cost and painful intercourse; conversely, it is hardly ever life threatening. This problem must affect 75% of women once in their whole lifetime (Swaminathan *et al.,* 2017). *C. albicans* is frequent colonizer and responsible for vulvo-vaginal candidiasis. *C. albicans* exhibits the ability to stay alive and propagate in physiological extremes of pH, osmolarity, accessibility of nutrients and temperature. This adaptability may account for the flourishing ways of *C. albicans* both as a commensal colonizer of vagina and as a pathogen (Alves *et al.,* 2016). Even though varieties of antifungal agents have been available, but pathogenic microbes develop a strong resistance to these antifungal agents. In addition, several antifungal agents have undesirable side effects, showing drug-drug interactions or causing resistance in the body; some showily poor efficacy, resulting less efficacious in the therapeutic managements of acute vulvo-vaginal candidiasis (Vladareanu *et al.,* 2018). Therefore, it is essential to search more efficacious and less toxic antifungal compound that would overcome the problems. With the intention to overcome the problem of limited availability of drugs required to treat candidiasis, folk medicine derived from plants are still being used. This motivates the search for new and active anti-*C. albicans* agents from the plant sources. Medicinal plants contain valuable therapeutic agents that have beneficial effects on the human body (Karo *et al.,* 2016). Owing to all these benefits, medicinal plants continue to be a most important resource of novel compounds. Currently, the non-as systematic use of available antimicrobial drugs has caused serious drug resistance in human pathogenic microorganisms. These circumstances lead scientists to seek out new and valuable antimicrobial compound to replace the present regimens (Sheidaei *et al.,* 2017). Oriental herbology is practiced, in which different herbs are combine to form combination so that maximum therapeutic effects achieve. For instance, a polyherbal formulation Diakyur is used in the management of type II diabetes. This formulation contains six herbs. Similarly, another polyherbal formulation Soshiho-tang, used in China, Japan and Korea consists of seven herbs. Soshiho-tang has several pharmacological effects, including anti-

**Corresponding author:* e-mail: drsabirachishti12@gmail.com

inflammatory, anti-oxidant, immunomodulatory and hepatoprotective (Shin *et al.,* 2012).

The tested drug is polyherbal formulation containing seven herbs viz. *Quercus infectoria* (Mazo), *Bambusa arundinacea* (Tabasheer), *Punica granatum* flower (Gulanaar*), Areca catechu* (Sapari dakne), *Eletaria cardamom* (Ilaichi khurd*), Woodfordia fruiticosa* (Gul dahawa). The study is designed to assess the efficacy of trial drug Candicure to cure the vulvovaginal candidiasis and to compare the effectiveness of Polyherbal formulation Candicure vs. Standard allopathic antifungal (fluconazole 150 mg) in treatment of vaginal candidiasis. Clinical trials are designed to understand the nature of disease, its associated symptoms, and patient's response towards management. The study has aim to evaluate the effect of medical intervention with allopathic and herbal medicine to treat vulvo-vaginal candidiasis. The main objectives of the study are; to determine the efficacy of test drug Candicure to cure the vulvo-vaginal candidiasis, to compare the effectiveness of polyherbal formulation Candicure vs. Standard allopathic. Antifungal (fluconazole 150 mg) in treatment of vaginal candidiasis, to assess the safety of trial drug. The toxicity index and the safety profile of test drug is assessed. Toxicity study of polyherbal formulation is important in order to consider them safe before use. The toxicity study of test drug is conducted on animals' model. The purpose of the toxicity study is to establish the therapeutic index, i.e. the ratio LD50: ED50. The narrow the margin, the more likely the drug produce adverse effects, the higher the index the safer the compound. Acute toxicity study is usually conducted to assess the lethality and lethal dose.

MATERIALS AND METHODS

Toxicity study of Polyherbal Coded Drug formulation

Experimental animals

Swiss albino mice and Wister rats of either sex were used in the study. The mice weigh was 23-24g while Wister rats weigh were 145-250 g. The animals were kept in standard environment (23-25°C, 12h/12h light/dark cycle). The animals were fed standard pelleted diet, water ad libitum. Animals were acclimatized one week prior to start of study. The study protocol was approved by the Animal Ethical Committee of Hamdard University Faculty of Eastern Medicine Karachi and the Reference No. is ERB-18-07.

Experimental design dose

The 50 mg/kg/day was chosen conferring to dose administered in adult human beings on daily bases. The higher doses (100 and 200 mg/kg/day) were chosen for sub-acute toxicity study. The dose of individual rats in all groups was calculated on the base of body weights before the start of the study.

Acute toxicity study

Before the start of the study the mice were kept fasted for 24 hours. Ten mice of either sex were used. A single dose of polyherbal product at a rate of 2000mg/kg were given to each mouse. Strictly observation has been done. The animals were observed for first 30min after dosing and thereafter observed during first 24 hrs. and for 72 hours. The change in autonomic effects (salivation, lacrimation, piloerection) were observed. The change in central nervous system (Drowsiness, convulsion, tremors), skin, weight of body, consumption of food, consumption of water and mortality was observed (Afolabi *et al.,* 2012; Balogun *et al.,* 2016).

Sub-acute toxicity study

The animals were divided in to 4 groups of five animals each. First three groups were given experimental dose at a rate of 500mg/kg/day, 1000mg/kg/day, 2000mg/kg/day respectively and the fourth group was control. The normal food and water were given to fourth group. The weight of animal checked weekly and behavioral changes, morphological changes were observed. On the 28th day of treatment, the animals were anaesthetized by injecting 5ml/kg of a solution of 1% chloralose in 25% urethane (w/v) by i.p. Cardiac puncture was done. Blood was collected in EDTA sample tubes and heparinized tubes for hematological analysis and biochemical analysis. After sacrificing the mice, the kidneys and liver were harvested. The tissue piece of the organ was sent to laboratory for histopathology. Mortality was recorded during the 28th period of treatment (Ishtiaq *et al.,* 2017).

Hematological analysis

Blood samples were examined by using reputable procedures and CBC machine by Medonic. The content includes; RBCs count WBCs count, PLT count, HCT, Hb, MCV, MCH and MCHC.

Biochemical parameters

Biochemical parameter blood urea, creatinine, uric acid, triglycerides, cholesterol, HDL, AST, VLDL, bilirubin, and SGPT were checked from serum sample.

Histopathology

Kidney and Liver tissues separated from rat were placed in 10% formal-saline. Graded alcohol is used to dehydrate the tissues. Paraffin is added. The tissues cut in to thick pieces about 3-4µm. These were stain with hematoxylin-eosin. Photo microscopic assessment is carried out. Under 40X and 100X objectives slides were observed (Habu *et al.,* 2008; Barnes, 2003).

STATISTICAL ANALYSIS

Results were recorded as mean ± SEM. One-way ANOVA was applied on the data with posthoc Tukey's HSD test using SPSS version 21. Significance was measured at values of $p<0.05$ and $p<0.01$.

Table 1: Acute study with dose of 2000 mg/kg/day on mice for three days

Sr. No	Parameters	Animals							
		Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6	Mice 7	Mice 8
1	Salivation	No	No	No	No	No	No	No	No
2	Lacrimation	No	No	No	No	No	No	No	No
3	Drowsiness	No	No	No	No	No	No	No	No
4	Fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Weight	No	No	No	No	No	No	No	No
6	Tremors	No	No	No	No	No	No	No	No
7	Piloerection	No	No	No	No	No	No	No	No
8	Body weight	24g	23g	23g	24g	25g	27g	24g	23g
9	Water consumption	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Food Consumption	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Table 2: Observations of physical signs during sub-acute toxicity study

Parameters	Mice			
	Control	50mg/kg/day	100mg/kg/day	200mg/kg/day
Nasal bleeding	-	-	-	-
Paralysis	-	-	-	-

Table 3: Effect of test doses of formulation on hematological parameters in animals

Parameters	Mice			
	50mg/kg/day	100mg/kg/day	200mg/kg	Control
WBCs (10*3/ul)	3.86±0.02	3.88±0.03	3.90±0.04	3.87±0.03
RBCs (10*6/ul)	5.48±0.00	5.43±0.05	5.44±0.098	5.50±0.01
PLT (10*3/ul)	642.69±0.45	644.02±1.84	641±1.32	643.67±1.42
Hb (g/dl)	12.05±0.00	12.04±0.00	12.06±0.01	12.05±0.00
HCT (%)	40.20 ± 0.01	40.39 ± 0.04	40.42 ± 0.01	40.17 ± 0.04
MCV(fl)	74.30 ± 0.43	74.46 ± 0.06	74.56 ± 0.12	74.71 ± 0.13
MCH (pg)	20.56 ± 0.08	20.57 ± 0.11	20.81 ± 0.007	20.8 ± 0.01
MCHC (g/dl)	40.20 ± 0.01	40.42 ± 0.01	40.39 ± 0.04	40.17 ± 0.04

The data is expressed as Mean ±SEM

Table 4: Effect of test doses of formulation on AST, ALT and bilirubin

Parameters	Control	50mg/kg/day	100mg/kg/day	200mg/kg/day
Bilirubin (mg/dl)	1.05 ± 0.051	1.65 ± 0.024	0.56 ± 0.12	1.57 ± 0.04
ALT (µ/ml)	38.51 ± 0.21	38.20 ± 0.25	38.21± 0.01	38.27 ± 0.04
AST (µ/ml)	39.11 ± 0.30	38.38± 0.30	38.38 ± 0.30	38.72 ± 0.26

Table 5: Effect of test doses of formulation on blood urea, creatinine and uric acid in animals

Parameters	Control	50mg/kg	100mg/kg	200mg/kg
Blood Urea mg/dl	23.7 ± 0.275	23.45 ± 0.20	23.98 ± 0.27	23.87 ± 0.20
Creatinine mg/dl	0.39 ± 0.007	0.416± 0.007	0.39 ± 0.007	0.37 ± 0.04
Uric acid mg/dl	3.41 ± 0.18	3.321± 0.067	3.37± 0.06	.42 ± 0.017

Table 6: Effect of test doses of formulation on TG, Cholesterol, HDL and VLDL

Parameters	Control	50mg/kg	100mg/kg	200mg/kg
Triglycerides	40.20± 0.01	41.1± 0.84	40.39 ± 0.04	42.08 ± 0.41
VLDL	6.14 ± 0.26	6.13 ± 0.21	6.70 ± 0.50	09.86 ± 0.80
HDL	38.04 ± 0.394	38.20 ± 0.25	39.11± 0.30	38.70 ± 0.210
Cholesterol	67.54 ± 0.375	68.1 ± 0.01	65.77± 1.33	67.54 ± 0.30

Table 7: Disease wise presenting complaints in total patients at baseline

Presenting complain at base line		Treatment group		Total	P-value
		Test	Control		
Vaginal discharge	Yes	75	75	75	-
	No	0	0	0	
	Total	75	75	150	
Itching	Yes	62	66	128	.020
	No	13	09	22	
	Total	75	75	150	
Soreness	Yes	43	44	87	0.86
	No	32	31	63	
	Total	75	75	150	
Edema	Yes	16	11	27	0.28
	No	59	64	123	
	Total	75	75	150	
Erythema	Yes	7	12	19	0.21
	No	68	63	131	
	Total	75	75	150	

Table 8: Over all Comparative Evaluation in improvement of sign/symptoms by Candicure and Fluconazole therapy

Sign/Symptoms	Treatment Groups						P-value
	Candicure			Fluconazole			
	Improved	Not Improved	Result	Improved	Not Improved	Result	
Vaginal Discharge	59/75	16/75	78.6%	44/75	31/75	74%	0.0082
Vaginal Itching	59/62	03/62	95.16%	59/62	16/62	79.33%	0.0073
Vaginal Soreness	39/43	05/43	90.6%	33/44	11/44	75%	0.097
Edema	12/16	04/16	75%	4/11	07/11	63.3%	0.04
Erythema	06/7	01/7	85%	11/12	01/12	91%	0.03

Table 9: First follow up for all the symptoms

	Test Group		Control Group		P-value
	No.	%	No.	%	
Complete Cured	53	70.66%	51	68%	0.001
Improvement	06	11.32%	05	11.32%	
Failure	16	21.33%	19	25.33%	

Table 10: Second follow up for relapse

	Test Group		Control Group		P-value
	No	%	No	%	
Relapse	06	11.32%	09	17.64%	0.001
No Relapse	47	62.66%	42	56%	

Clinical Efficacy of herbal coded formulation (Candicure)

Study Design

This is the case control, multicenter study, conducted on patients living nearby Shifa-ul-Mulk Memorial Hospital at Hamdard University Karachi, Naseem ul Sehat, Eastern Medicine Clinic, Bahawalpur (PHC: Reg-No-13349). Hakeem Muhammad Said Shaheed Memorial Research Center, Bahawalpur, and Bahawalpur Civil Hospital from June 2016 to April 2018.

Diagnostic technique

Patients having the symptoms of Vulvo-vaginal candidiasis were clinically examined by taking complete history and general physical examination, per speculum examination and high vaginal swab. A clinical trial proforma consisting of patient bio data, clinical features and investigation and patient consent form was filled before the initiation of treatment and repeatedly filled up during the course of treatment.

Study design

Study was Multicentral Randomized Controlled Clinical Trials with an open intervention. The patients were checked by General physician and given test drug (Polyherbal formulation) and control drug (Allopathic) for vulvo-vaginal candidiasis. The patients were categorized in two groups i.e. control group and test group. The test group was administered polyherbal formulation Candicure that formulated by different medicinal plants. The control group was administered allopathic Fluconazole capsule. A total number of 150 participants of female sex coming the OPD were be enrolled in the study according to inclusion criteria. Only those females were included in the study who are willing to signed the consent form.

Subject inclusion criteria; Women aged 18 to 50 years with symptoms of acute vulvovaginal candidiasis, only those participate included who are willing, only married patient included, only those participants included who do not take any topical or systemic antifungal one month prior the start of study. Evidence of acute vulvovaginal candidiasis by diagnostic technique as per speculum examination and high vaginal swab. Subject exclusion criteria; Pregnant patient excluded Unmarried females with candidiasis were excluded, Patients allergic to antifungal drugs, Patient with recurrent vaginal candidiasis, Patients suffering in liver disease, Patients taking hormonal therapy, menopausal women, Patients suffering in diabetes mellitus and immunocompromised patients as these patients had recurrent attacks of candidiasis excluded from the study, Women suffering in venereal disease, Chlamydia infection, trichomoniasis, or vaginitis other than candidiasis also excluded. Patients withdrawn were those who are not willing to continue, patient suffering in any systemic disease during treatment were withdrawn from study, drug intolerance, If the participant is not regular in taking treatment during the course of study, any serious adverse effects or allergic reactions occurs during treatment. All patients either gave verbal or written, informed consent for the participation in the study, and the study protocol was approved by independent Ethical Committee in Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan. Trial was conducted on approximately 150 patients from both groups (75 patient from control and 75 from experimental group) between ages of 18-50 years regardless of socioeconomic status. After the diagnosis of participants, the participants were randomly separated in two groups of 75 each. Patients in Group 1(control group) were prescribed capsule fluconazole 150mg stat (single dose) and those in Group 2 (Test group) were prescribed Candicure 500mg capsule thrice daily for seven days. Short term efficacy was measured by the eradication of organism on the follow up visit after the one week and the long-term efficacy were assessed by the negative high vaginal swab after 2 weeks of the 2nd visit. Clinical effectiveness was record as cure, improvement, failure and relapse. The side effects were recorded during therapy. Liver function test, blood complete examination and urine complete examination were also be examined at baseline and after treatment to analyze and record any drug adverse action on these systems. Data were collected by the filling of Clinical trial Proforma by personal interview, use of case record, personal observation file and documents.

STATISTICAL ANALYSIS

Data will analysis using SPSS version 17. Relevant descriptive statistics, frequency and percentages were computed on present symptoms and findings of clinical examination before and after the treatment. Chi square test were applied to test the hypothesis. Time measurement was presented by mean standard deviation.

RESULTS

Acute toxicity study

In acute toxicity study all the animals given dose of 2000mg/kg, b.w. of polyherbal formulation. The animals were observed for 72 hours for an interval of 30 minutes. Abnormal behavior was observed. It was showed that the drug showed on toxic effects and no abnormal sign in clinical parameters was observed. The result showed that the LD_{50} of polyherbal drug is more than 2000mg/kg/day (table 1).

Sub-acute toxicity study

Sub-acute toxicity study was of 28 days duration. Three different doses were administered; 50mg/kg/day, 100mg/kg/day, 200mg/kg/day, while one group were considered control. Clinical parameters observed were consumption of water and food, body weight, hematological contents (WBCs, PLT, Hb, MCV, MCHC, HCT, RBCs, MCH. Liver function test (AST, ALT, Bilirubin), Renal function parameters (creatinine, uric acid, blood urea) and lipid profile (cholesterol, High density lipid protein, triglycerides and VLDL) were noted.

Effect on body weight

No variations in weight were observed in all groups throughout the observed period in animals.

Clinical signs

No nasal bleeding and paralysis effect were observed in all group administrating 50mg/kg/day, 150mg/kg/day and 200mg/kg/day on 21st and 22nd day (table 2).

Results of hematological parameters

The result of hematological parameters at a dose of 50mg/kg/day, 100mg/kg/day, 200mg/kg/day showed no significant variations in WBCs, RBCs, PLT count. No declined observed in hemoglobin value when compared

the treatment group with control group. Similarly, HCT, MCV, MCH, MCHC hematological parameters remained normal in all groups

Effect on biochemical liver parameters

The biochemical liver parameters of animal of treatment groups showed that levels of liver enzymes were in normal range when compared to control. No significant alteration ($p>0.05$) recorded in liver biochemical parameters (table 4).

Kidney parameters

The biochemical kidneys parameters of animal of all treatment groups showed that the levels of blood urea, uric acid and creatinine were in normal range when compared to control. No significant alteration ($p>0.05$) recorded in kidney biochemical parameters.

Lipid profile

The lipid profile of animal of all treatment groups showed that the levels of cholesterol, triglycerides, HDL and VLDL were in normal range when compared to control. No significant alteration ($p>0.05$) recorded in the blood lipid profile of animals (table 6).

Results of clinical efficacy of poly coded drug (Candicure)

The average age of female patient of test group was 35.24 and standard deviation was 9.8. The mean age of female patient of control group was 35.40 and standard deviation was 9.82. The clinical presentation of participants was documented at baseline and after the treatment of both test and control group. The relative analysis was done among the test group and control to observe the improvement level in both groups.

According to the statistical analysis *Candida* species was eradicated in 56 patients (74%) out of 75 patients by the intake of Fluconazole allopathic therapy (Control drug) and in 59 patients (78%) out of 75 patients by the use of Candicure (Test drug). Chi-Square Test was applied and p-value was calculated as 0.562 which is greater than 0.05 indicating that Candicure and fluconazole therapy are equally significant in *Candida* species eradication.

At the base line all the participants of Test (Candicure) and Control group (Fluconazole) have vaginal discharge. After giving the treatment in Test group out of 75 participants 59 (78%) participants showed complete improvement. In control group (Fluconazole) out of 75 patients 56 (74%) showed complete improvement. The statistical difference between two drugs was calculated and p-value was 0.0082. The effect of test drug was comparable to control drug.

Vaginal soreness was diagnosed in 43 patients out of 75 in Test group along with other complaints. Test drug (Candicure) was prescribed to the participants and found that out of 43 participants 39 participants showed complete improvement and five participants showed no improvement. In control group (Fluconazole) was prescribed to 44 participants 33 participants showed complete improvement and 11 participants showed no improvement. The statistically significant difference was 0.097 which showed that Candicure therapy has good efficacy in treating vaginal soreness.

Vaginal itching was present in 62 patients before the start of Candicure treatment. After prescribing Candicure (Test drug) 59 patients showed complete improvement while 3 patients still had complaint of vaginal itching. Vaginal itching was present in 66 patients before the start of fluconazole treatment. After prescribing Fluconazole (Control Drug) 49 patients showed complete improvement while 13 patients were still had complaint vaginal itching. The statistically significant difference was 0.007 which showed that Candicure therapy has good efficacy in treating vaginal soreness.

Edema was diagnosed in 16 patients out of 75 in Test group along with other complaints. Test drug (Candicure) was prescribed to the participants and found that out of 16 participants 11 participants showed complete improvement and five participants showed no improvement. In control group (Fluconazole) was prescribed to 11 participants 09 participants showed complete improvement and 2 participants showed no improvement. The statistically significant difference was 0.622 which showed that Candicure therapy has good efficacy in treating Edema.

Erythema was diagnosed in 07 patients out of 75 in Test group along with other complaints. Test drug (Candicure) was prescribed to the participants and found that out of 07 participants 05 participants showed complete improvement and 2 participants showed no improvement. In control group (Fluconazole) was prescribed to 12 participants 04 participants showed complete improvement and 08 participants showed no improvement. The statistically significant difference was 0.03 which showed that Candicure therapy has good efficacy in treating Erythema.

At the 7th day follow up for all the symptoms, 53 (70.66%) of total patients in Group 1, and 51 (68%) patients of Group 2 had been completely cured; 6 (11.32%) patients in Group 1 and 5 (11.32%) patient in Group 2 showed only improvement in sign and symptoms; 16 (21.33%) patients in Group 1 and 19 (25.33%) patients in Group 2 showed no response. Relapse was seen in 6 (11.32%) out of 53 cured patients in Group 1, and 9 (17.64%) patient of the 51cured patients in Group 2 on day 21. Treatment response was significantly better in Group 1 compared to Group 2.

DISCUSSION

Current studies on the prevalence of vaginal candidiasis infections showed that approximately half of the women population of the world has been suffering from this disease. It is most common gynecological disorder. This disease causes discomfort, pelvic pain, painful sexual act, healthcare cost and involve in considerable morbidity (Achkar *et al.,* 2010). Different antifungal agents available such as imidazole, triazole, clotrimazole, fluconazole for its treatment. The antifungal agent available but they are limited in number, have adverse effects, resistance of candida to these agents and relapse of the candida infections (Karo *et al.,* 2016).

It is calculated that, yearly, 2,821,440 females of reproductive age suffer from repetitive vulvovaginal candidiasis in Pakistan. Due to intake of self-medication and over- and under-diagnosis with over-the-counter topical antifungal agents, population-based figure regarding the incidence and prevalence of vulvovaginitis in Pakistan is lacking. Our judgment in the study was determined using data by Foxman et al., who describe vulvovaginitis in 9% of unselected women based on internet questionnaires. Jabeen *et al* have used a 6% rate, of 'yeast' infection in women. A study estimates the load of reproductive tract infection in urban females in Pakistan showed vaginal candidiasis as second most frequently occurred genital infection, with a prevalence of 7-12% (Jabeen *et al.,* 2017; Foxman *et al.,* 2013).

In order to overcome the problem of less availability of drugs needed to treat candidiasis, researchers have been directed towards the research of herbal formulations having therapeutic efficacy. This encouraged the search for new and dynamic anti-*C. albicans* agents from plant sources. Therefore, systematic analysis of alternative treatment on vulvo-vaginal candidiasis was determined and clinical evaluation of Unani/Herbal formulation as compared to allopathic medicine was carried out. Epidemiological assessment, laboratory investigation, treatment and prevention of acute vulvo-vaginal candidiasis infection as open comparative prospective were the main objective of the study. The toxicity index and the safety profile of test drug polyherbal formulation was assessed. Toxicity study of polyherbal formulation was important in order to consider it safe before use. The toxicity study of test drug is conducted on animals' model. Acute toxicity study is usually conducted to assess the lethality and lethal dose. All animals survived by the end of the study; Clinical signs symptoms did not reveal any major findings. The LD50 of the Candicure was greater than 2000mg/kg and hence it is practically nontoxic. In acute toxicity study no morbidity and mortality noticed on single administration of dose 2000mg/kg/day. In sub -acute toxicity study no significant changes were observed in consumption of food and water, body weight, hematological contents (WBCs, PLT, RBCs, Hb, MCV, MCH, HCT, MCHC). Liver function test (AST, ALT, Bilirubin), Renal function parameters (creatinine, uric acid, blood urea) and lipid profile (cholesterol, HDL, triglycerides, (VLDL). After the assessment of toxicity of formulation, a clinical study was conducted to determine effect of herbal formulation treatment on vaginal candidiasis versus allopathic medicine. The statistical analysis showed *C. albican* was eradicated in 59 participants (78%) out of 75 participants by the use of Candicure (Test drug) and in 56 participants (74%) out of 75 participants by the use of Fluconazole therapy (Control drug). Comparisons between two groups Test group and Control group showed on significant difference ($p>0.05$). Chi-square test was applied and p-value was calculated 0.3101 which is greater than 0.05 showed that Candicure therapy and Fluconazole therapy is equally significant in eradication of *C. albican*. Study stated that the highest prevalence of Vulvo-vaginal candidiasis was present among women in aged between 35.24 and 36. 35 years. The result of this study has shown that cheesy vaginal discharge was the commonest presentation in patients with acute Vulvo-vaginal candidiasis occurring in all cases. This was followed by vaginal itching that was present in 82.66% of cases. Vaginal soreness in 57.33% of cases and erythema occurred in 10.85% cases. At the first follow up at the seventh day 53 (70.66%) of total patients cured by Polyherbal formulation candicure, and 51 (68%) patients completely cured by standard drug Fluconazole. Treatment response, was significantly better in Group 1 taking Candicure test drug compared to Group 2 taking Fluconazole standard drug. Comparative analysis of drugs in the improvement of associated sign and symptoms of vulvo-vaginal candidiasis infection associated showed that herbal drug Candicure (Test drug) showed overall superior outcomes in the improvement of subjective sign and symptoms as compared to Control drug Fluconazole therapy. The results from this study have clearly revealed the evidence of efficacy in real terms. Herbal medicine can assist gynecological practitioner to choose the best way for treating vulvo-vaginal candidiasis. Furthermore, the use of herbal medicines can prevent the side effects of synthetic medicines (Sheidaei *et al.,* 2017). Taking into consideration the prevalence of vaginal candidiasis, the treatment of this disease indorses women's health. The use of herbal medicines as a complimentary method for the treatment of vaginal candidiasis promote the quality of obstetrics and gynecology health services.

CONCLUSION

The clinical findings of randomized controlled trial revealed that the test drug Candicure is effective at 500mg thrice a day in the treatment of Vulvo-vaginal Candidiasis. The effect was almost comparable to control drug in eradicating the micro-organism. There was no untoward manifestation was associated with the test drug Candicure

and found good in the treatment of Vulvo- Vaginal candidiasis and its associated symptoms. The acute and sub-acute toxicity study of polyherbal formulation on experimental animals is safe at a dose of 2000mg/kg/day.

ACKNOWLEDGEMENT

The authors are thankful to Dean, Chairman and Principle of Hamdard University Karachi for supporting and providing the laboratories facilities. The authors are thankful to Shifa Ul Mulk Memorial Hospital, Hamdard University Karachi for giving opportunity to conducting clinical trials.

REFERENCES

Afolabi SO, Akindele AJ, Awodele O, Anunobi CC and Adeyemi OO (2012). A 90-day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats. *BMC Complement Altern Med.,* **12**(1): 79.

Achkar JM and Fries BC (2010). Candida infections of the genitourinary tract. *Clin. Microbiol. Rev*., **23**: 253-73.

Aliero A, Aliero BL and Buhari U (2008). Preliminary phytochemical and antibacterial screening of *Scadoxus multiflorus*. *Int. J. Pure Appl. Sci.,* **2**: 13-17.

Alves BG, Ferreira C, Alves CT, Henriques M, Azeredo J and Silva S (2016). Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors *Crit. Rev. Microbiol*., **42**(6): 905-927.

Anibal PC, Peixoto TA, Foglio MA and Hofling JF (2013). Antifungal activity of the ethanolic extracts of *Punica granatum* L. and evaluation of the morphological and structural modifications of its compounds upon the cells of *Candida* spp. *Braz. J. Microbiol*., **44**(3): 839-848.

Balogun FO and Tom Ashafa AO (2016). Acute and subchronic oral toxicity evaluation of aqueous root extract of *Dicoma anomala* Sond. in Wistar rats. *Evidence Based Complemen. Altern. Med*., Article ID 3509323, 11 pages.

Barnes J, Anderson LA, Phillipson JD (2003). Herbal medicines: A guide for healthcare professionals, Pharmaceutical Press, London, UK.

Foxman B, Muraglia R, Dietz JP, Sobel JD and Wagner J (2013). Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: Results from an internet panel survey. *J. Low Genit Tract Dis.,* **17**: 340-345.

Habbu PV, Shastry RA, Mahadevan KM, Joshi H and Das SK (2008). Hepatoprotective and antioxidant effects of *Argyreia speciosa* in rats. *Afri. J. Tradit Complement Altern. Med*., **5**: 158-64.

Ishtiaq S, Akram M, Kamran SH and Hanif U et al., (2017). Acute and sub-acute toxicity study of a Pakistani polyherbal formulation. *BMC Complement. Altern. Med.,* **17**(387): 1-13.

Jabeen K, Farooqi J, Mirza S, Denning D and Zafar (2017). A Serious fungal infection in Pakistan. *Eur. J. Clin. Microbiol. Infect Dis.,* **4**: 1-8.

Karo MB, Kamelia E, Miko H , Simanjuntak TP and Hatta M (2016). Effects of Herbal Plants on Candidiasis Vulvovaginalis Therapy. *American Journal of Laboratory Medicine*, **1**(3): 65-68

Khan A, Izhar V, Khan MA and M Amaar (2015). Frequency of dyspareunia associated with vaginal *candida* in young sexually active females. *Pak. J. Mol. Med.,* **2**(1): 35-37.

Parveen N[1], Munir AA, Din I, Majeed R. (2008). Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic. *J. Coll. Physicians Surg. Pak*., **18**(3): 154-157.

Sheidaei S, Sadeghi T, Jaafarnejad F, Rajabi O, Javad M and Najafzadeh (2017). Herbal medicine and vaginal candidiasis in Iran. *A Review Evidence Based Care Journal*, **7**(2): 71-77.

Shin IS, Lee MY, Kim Y, Seo CS, Kim JH and Shin HK (2012). Subacute toxicity and stability of Soshiho-tang, a traditional herbal formula, in Sprague Dawley rats. *BMC Complement. Altern. Med.,* **12**(1): 266.

Swaminathan KR, Mridula Devi M, Gerald S, Swathi C, Prakesh and Thomas BM (2017). Prevalence of vulvovaginal candidiasis in the women of the reproductive age, in rural India. *International Journal of Clinical Obstetrics and Gynaecology*, 1(2): 37-39.

Vladareanu R, Mihu D, Mitran M, Mehedintu C, Boiangiu A, Manolache M and Vladareanu S (2018). New evidence on oral *L. plantarum* P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): A randomized double-blind placebo-controlled study. *Eur. Rev. Med. Pharmacol. Sci.,* **22**(1): 262-267.