Original Research Article

A pilot study of the use of Picrorhiza kurroa in patients with malaria

ABSTRACT

Background and Purpose: Malaria is potentially a severe disease caused by infection of red blood cells with protozoan parasites of the genus Plasmodium. Malaria is an important cause of death, illness in children and pregnant women, especially in Africa The present study aimed to develop an alternative treatment that at may be effective and safe against *falciparum* & *vivax* malaria and easily available locally and culturally acceptable. To confirms the claims of traditional herbal medicinal plants Picrorhiza kurroa was analyzed by using water and alcoholic extracts.

Methods: The present study was conducted on 45 subjects, 24 with aqueous extract and 21 with alcohol extract to assess the clinical efficacy of the medicinal plant Picrorhiza kurroa against malaria caused by Plasmodium falciparum and P.vivax in Dera Ismail Khan. The roots of P.kurroa were extracted by a Soxhlet extractor using triple distilled water and ethanol as a solvent to obtain both aqueous and alcoholic extracts of P.kurroa. Both extracts are formulated in a capsule of 500mg.

Results: The efficacy was determined clinically and pathologically in patients from 14 to 50 years of both sexes, two capsules of 500 mg stat followed by one capsule twice daily for three days consecutively were given. After the treatment 44.44 % of patients recovered, among them 85% (17/45) were male and 15% (3/45) were female. 44.44% efficacy of the drug is considered for further research on the same plant.

Conclusion: The study concluded that P. kurroa qualified as an active compound to undergo further investigation for its antimalarial activity and its active constituents should be investigated for better outcomes in the field of traditional medicines.

Key Words: *Picrorhiza kurroa, Malaria, Plasmodium falciparum, Plasmodium vivax*

INTRODUCTION

Malaria is potentially a serious disease caused by infection of red blood cells with protozoan parasites of the genus *Plasmodium*. The parasites are inoculated into the human host by a feeding female anopheline mosquito. It is an important cause of death and illness in children and adults especially in tropical countries (WHO, 2010). The disease results from the multiplication of malaria parasites within red blood cells causing symptoms like recurrent cycles (every one to three days) of fever, chills, muscle aches, headaches, nausea, vomiting and jaundice also may occur (Acremont *et al.*, 2010). The four *Plasmodium* species that infect humans are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Increasingly, human infections with the monkey malaria parasite, *P. knowlesi*, have also been reported from the forested regions of South East Asia (WHO, 2010).

Picrorhiza kurroa is a well known herb in Ayurvedic system of medicine and has traditionally been used to treat disorders of liver and upper respiratory tract, reduce fevers, and to treat dyspepsia, chronic diarrhea and scorpion sting. It is a low more or less hairy perennial herb from the Scrophulariaceae family; the plant is naturally distributed in alpine and temperate regions of Himalaya from 2500 to 3500 m. It is a perennial creeping herb, which spreads by stolons. A whorl of radical leaves arise from rhizome tip. The flowering scape attains an average height of 16.0 to 17.5 cm. Majorly output from Tibetan towns of China,such as Nie La Mu,Ya Dong,Cuo Na,Bo Mi,etc.and West north regions of Yunnan Province,west of SiChuan province of China, other origins including Nepal, Sikkim, Bhutan and India (http://www.mdidea.com/products/new/new04801.html)
The leaves of the plant are flat, oval, and sharply serrated. The flowers, which appear June through August, are white or pale purple and borne on a tall spike; manual harvesting of the plant takes place October to December. The roots are 0.5 to 2 mm thick (Fig. 1) dusky grey in colour without a growing bud. Microscopically, the cortex is composed of only thick parenchymatous cells, cortical bundles are absent, xylem is exarch showing 4-7 arcs and the pith is absent (Mitra & Prasad, 1972). The research on P. *kurroa* has focused on its hepatoprotective, antipyretic, anticholestatic, anti oxidant and immune modulating activity (Sharma *et al.*, 1986), (Subedi, 2000)

The active constituents are obtained from the roots and rhizomes. The plant is self-generating but unregulated over-harvesting has caused it to be threatened to near extinction. The root is also used in diseases of liver and spleen including jaundice and anaemia (Uniyal & Isaar, 1969). Nomadic commodities from Himalaya keep this drug at their place for use as home remedy for various stomach disorders. In Nepal root paste is applied for speedy healing of wounds. The decoction of roots in water is given with salt to febrile cattle as antipyretic, also roots are used in dysentery or jaundice caused by damp-heat; hemorrhoids, consumptive fever and fever in infantile malnutrition due to digestive disturbance. 3-4 gm of drug is generally given as antiperiodic and 0.6-1.2 gm as bitter tonic. Typical adult dosage is 400 to 1500 mg/day, with dosages up to 3.5 g/day sometimes being recommended for fevers between 400 and 1,500 mg of powdered, encapsulated Picrorhiza per day has been

UNDER PEER REVIEW

recommended. One author considers this equivalent to the use of 1~2 ml of fluid extract twice per day. Picrorhiza tastes quite bitter. Combining with ginger root powder capsules or taking as tea can improve palatability.

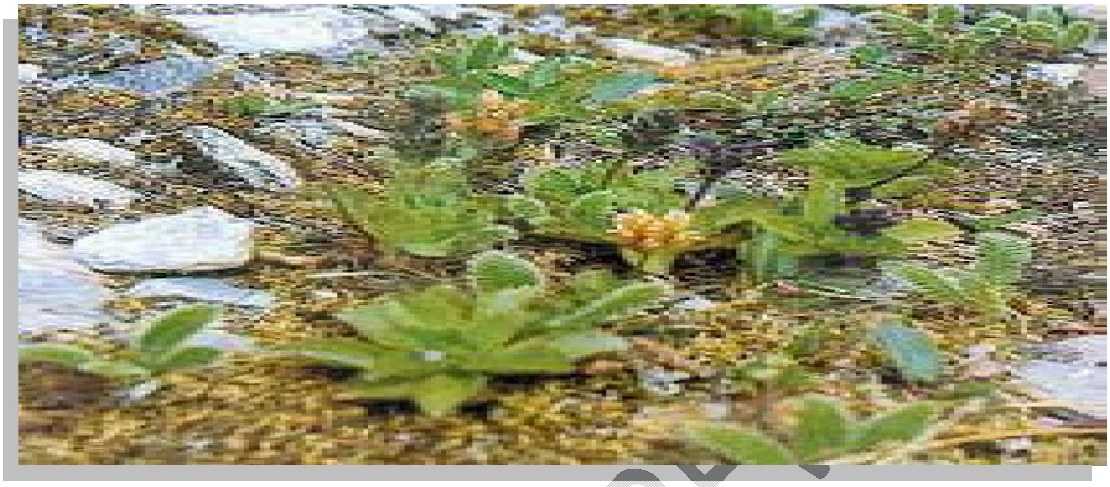


Fig. 1.1 Picrorhiza kurroa Plant

Source: *http://www.mdidea.com/products/new/new04801.html*

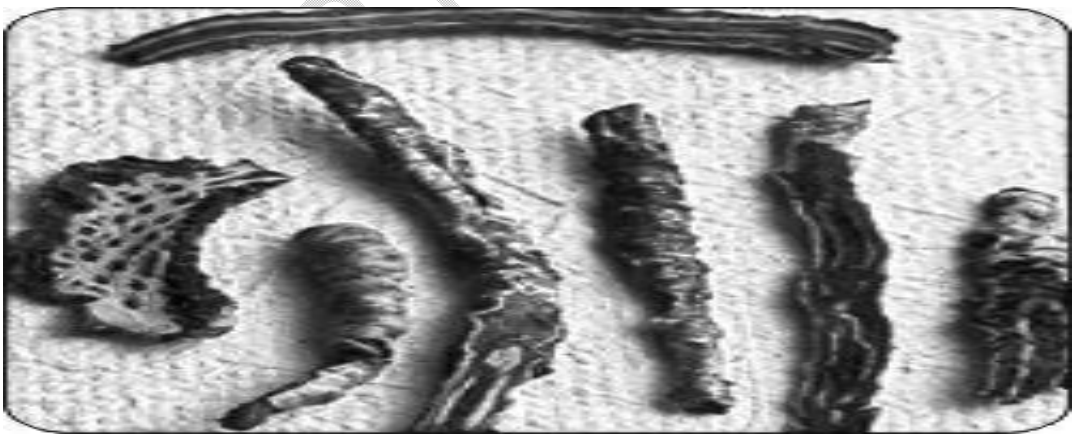


Fig. 1.2 Picrorhiza kurroa Roots

Source:*http://www.lookfordiagnosis.com.images.php?term=Picrorhiza&photo_id=4638330945&lang=3*

Active constituents

Kutkin is the active principal of *P. kurroa* and is comprised of kutkoside and the iridoid glycosides which have been further subdivided as Picroside I, II, and III. Other identified active constituents are apocynin, drosin,and nine cucurbitacin glycosides. (Weinges *et al.*, 1972), (Stuppner and Wagner, 1989). Apocynin is Cacetechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti inflammatory agent (Simons *et al.*, 1990), while the cucurbitacins have been shown to be highly cytotoxic and possess anti tumour effects (Stuppner and Wagner, 1989).

Dry root Rhizome contains Kutkin 3.4% and D-Mannitol 0.5%, Vanillic acid 0.1%,Kutkiol,Kutkistero1,0.18% and Apocynin. Latest research findings shows that ARVENIN-III has been isolated from roots and rest of the followings namely Apocynin, D-Mannitol,Glucose,Glucosido-Vanilloyl-Glucose,Kurrin,Kuthinol, Kuthirterol,Kutkin,Kutkiol,Kutkisterol, Kutkoside, Picrorhizin, Picroside-I,Picroside-II,Tripalmitin and Vanillic-Acid are isolated from rhizome:

(http://www.mdidea.com/products/new/new04801.html.

The mechanism of action of kutkins appears to the same as that of silymarin (active constituent and hepatoprotective constituent of Silybum marianum). Studies have shown that kutkins are more potent than silymarin as far as hepatoprotective activity is concerned (Sing AP, Alternative Medicine)

Objectives

The purpose of present study was

- To develop an alternative treatment which may be effective and safe against *falciparum* & *vivax* malaria.
- Which may be easily available locally and culturally acceptable.
- To confirms the claims of traditional herbal medicinal plants.

Keeping these objectives in mind, *Picrorhiza kurroa* was analyzed by using water and alcoholic extracts.

MATERIALS AND METHODS

2.1 Materials

The medicinal plant used in this research was *Picrorhiza kurroa* (Kutki). It is a low more or less hairy perennial herb from the Scrophulariaceae family, found in the Himalayan region growing at elevations of 3000 to 5000 meters (Said, 1972). Its roots were dried and processed to determine its medicinal (antimalarial) activity. The current study had been approved by competent authority i.e. Board of Advance Studies, Gomal

UNDER PEER REVIEW

University, Dera Ismail Khan when synopsis was submitted before commencement of the study.

It was identified with help of Herbarium of Biological Sciences, Quaid-e-Azam University, Islamabad.

Reagents

1 Glass triple Water

2 Alcohol (BDH>99.7-100% v/v)

2.2 Extractions Method

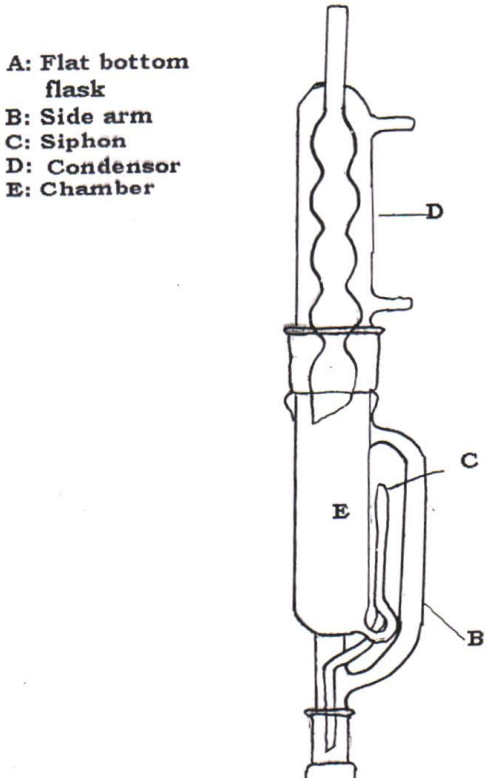
The Soxhlet extraction method was used for plant extraction at the Drugs Control & Traditional Medicine Division, National Institute of Health, Islamabad;

It was the best method of extraction by which most of compounds could be extracted.

2.3 Procedures

Soxhlet Extraction

The extraction is an important process in the preparation of medicine from a medicinal plant. This process removes constituents from one phase bringing into contact with a second immiscible liquid phase. Crude drugs prepared from plant are usually assayed for their content of active ingredient in this way and extraction of active components from solid forms is often the first step in their analysis. In this study Soxhlet Extractor (figure below) was used. This extractor comprised of flat bottom flask, chamber to which side arm and siphon tube are attached and a condenser. (Kenneth, 1975)



UNDER PEER REVIEW

Fig.2.1 Soxhlet Extractor

Source: Kenneth, A.C.1975. A textbook of Pharmaceutical analysis 2nd edition.pp.342- 343

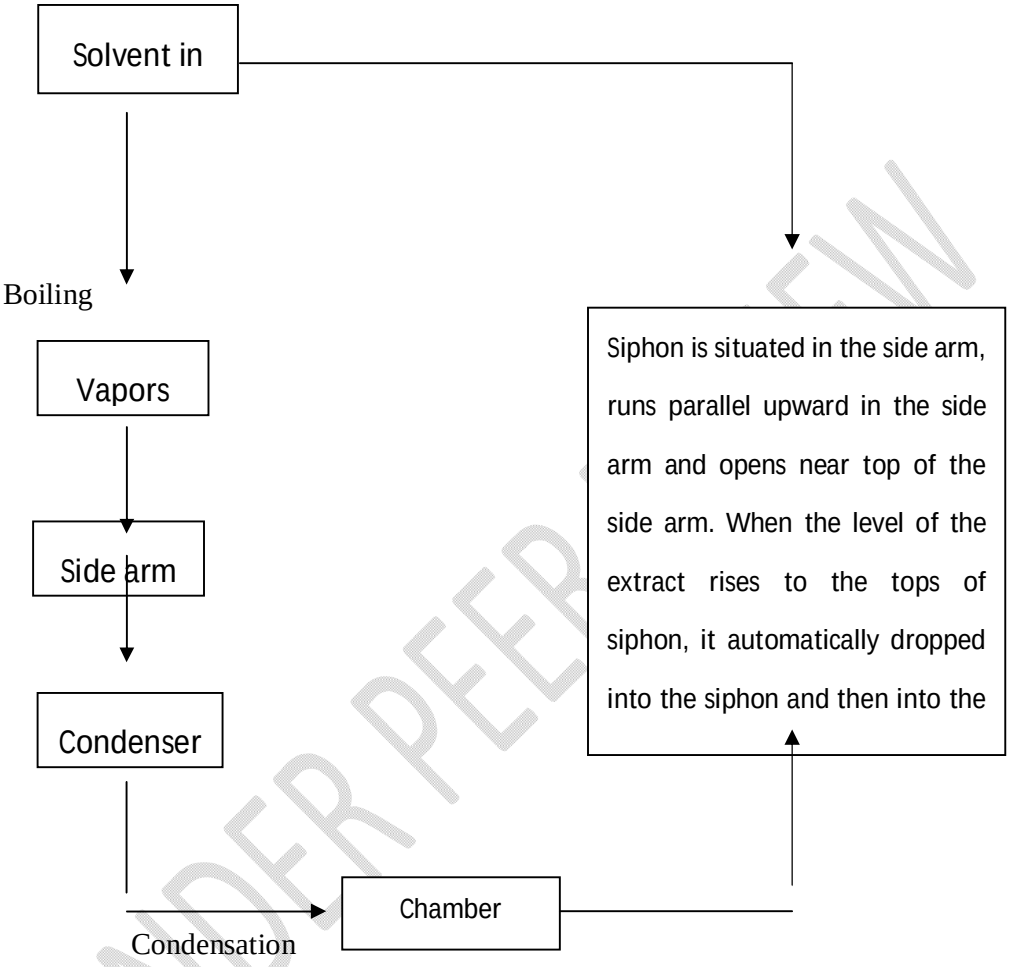


Fig. 2.2 Flow Diagram showing Extraction Process

Soxhlet Extractor is a piece of Laboratory apparatus (Harwood and Moody) invented in 1879 by Franz Von Soxhlet. (Dingler's, 1879) It was originally designed for extraction of lipids, however; a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet

extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated and vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon_side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

2.2.1 Soxhlet Aqueous Extraction

Sufficient quantities of properly grounded roots of Kutki were introduced into the thimble separately. This was then placed in the chamber; triple distilled water was boiled in the flask. The vapors produced entered into the condenser through side arm of Soxhlet extractor. After condensation the condensed solvent entered into the chamber containing crude plant material until level of liquid in the chamber reached to the top of siphon which then passed through the siphon and was collected into flask. This process of extraction of active ingredients was completed in 6-8 hours. The extracted material was concentrated under reduced pressure by rotary vacuum evaporator. The extract was a paste like material that was completely dried on water bath and was kept in refrigerator until use.

2.2.2 Maceration or Cold Extraction

The dried parts of Kutki roots were finely ground and taken in caped sterile flasks, each containing three litres of absolute alcohol. The flasks were shaken properly to mix the plant material and alcohol thoroughly. A sterile magnet bar was added in each flask and placed on magnetic stirrers. Complete extraction of active ingredients by maceration was done in 07 to 14 days. Each extract was filtered by Whatman filter paper (No. 01) and was concentrated under reduced pressure by rotary Vacuum evaporator and was dried on water bath at temperature 67.9 ºC. For complete drying a dessicator containing Silica was used for 24 hours. These were then kept at

04ºC until use.

UNDER PEER REVIEW

2.2.3 Soxhlet Hot Alcoholic Extraction

The finely ground sufficient quantity of Kutki was extracted by Soxhlet extractor using alcohol as a solvent. The vapors produced entered into the condenser through side arm of Soxhlet extractor. After condensation the condensed solvent entered into the chamber containing crude plant material until level of liquid in the chamber reached to the top of siphon which then passed through the siphon and was collected into flask. This process of extraction of active ingredients was completed in 6-8 hours. The extract was concentrated under reduced pressure by rotary vacuum evaporator and was transferred to sterile Petri plate. It was then dried and kept at 04ºC until use.

2.4 Formulation and Preparation of dosage forms

The dried extracts thence obtained were introduced into hard gelatin capsules and were

designated as Drug 'A' (Distilled Water extract capsules, blue in colour) and Drug 'B' (Alcohol extract capsules, purple red in colour). The traditional medicines are either given in form of decoctions, powder forms or capsules. To make these drug extracts palatable, capsule formulation was used. The paste of active ingredients obtained by extraction was aseptically filled into hard gelatin capsules of "0" number sizes (Remington, 2000) such that each capsule was having 500 mg of P. kurroa extract (http://www.wrc.net/wrcnet_content/di.../appendix1.html)

2.5 Selection of Patients

The patients were introduced into the study as per following inclusion, exclusion criteria;
(http://www.scribd.com/doc/325999051/Malaria-Action-Research-Project)

Inclusion criteria: Patients clinically suggestive of malaria and peripheral smear positive for *Plasmodium falciparum* and / or *P. vivax*, with informed consent.

Exclusion criteria:

- Patients less than 14 years and more than 50 years of age.
- Pregnant and lactating women.
- Severe Malaria: Malaria with unconsciousness, convulsions, severe anemia, respiratory diseases, bleeding from any site, auxiliary temperature above 105^0 F patients with any chronic disease such as Diabetes, HIV AIDS, Tuberculosis.
- History of having taken antimalarials drug with in last 7 days.

2.6 Development of Protocols

Following Protocol was developed for this study

1 Patient Diagnosis

UNDER PEER REVIEW

2 Confirmation of Malaria

3 Physical Examination

4 Signs & Symptoms

5 Follow-Up

The patients who have given written consent at time of commencement of the study were physically examined by the Physician. On the basis of symptoms and history of fever, microscopy was performed in the laboratory as per conventional method. Once the patient was diagnosed either with *P. falciparum* and / or *P. vivax*, patients were randomly treated with two drugs i.e. Drug 'A' (aqueous extract) and Drug 'B' (alcoholic extract) and one patient was treated with one drug only. Drug 'A' was given with dosage regimen as Two capsules (500mg each) in Stat and then one capsule twice daily with meals for next 3 days to 24 patients (20 males, 4 females) and the Drug 'B' was given on same dose regimen to 21 patients (16 males, 5 females) The overall recovery rate was 44.44%. Non recovered 25 patients were switched immediately by the physician to conventional antimalarials.

(This work was done by a team of Professionals i.e. Pathologist, Pharmacist, Physician, Traditional Medicine Practitioner and Laboratory Technician.)

RESULTS

The Clinical efficacy of alcoholic and aqueous extracts of medicinal plant *Picrorhiza kurroa* against malaria caused by *Plasmodium falciparum* and *P. vivax* was determined at Rural Health Centre Kotjai, Tehsil Paharpur, Dera Ismail Khan.

Microscopically diagnosed patients of malaria who had single infection of *P. falciparum*, and / or *P. vivax* aging from 14-50 years (both sexes) without any complications and pregnancy were selected for the study.

From the Peripheries of Dera Ismail Khan, fifty patients were screened and only five of them could not followed the inclusion criteria i.e. without any complications, microscopically diagnosed (MP positive), no pregnancy, aged between 14-50 years and follow up on day 3 for data compilation. (Only 3 days treatment limited to avoid any complication and to ensure patient safety). So, forty five patients (9 female, 36 male) were included in the study. After microscopy to confirm malaria either *P. falciparum* or *P. vivax*, these were physically examined by the physician, history taken, treated for three days and followed up for data compilation.

Patients came from following peripheries of D.I. Khan

Kotjai:	17	D I Khan (city):	01
Jara:	04	Khanokhel:	05
Dhakki:	11	Khushrana:	01
Kirri Khisore:	01	Kath Garh:	03

Kotla Lodhian: 01 Total: 45

Teer Garh: 01

Table 3.1 Summary of Results

Variables	Number of subjects	%age	Symptoms Grade	Status	Result
Total No. of Patients:	45	100%			
Total No. of Male Patients:	36	80%	3 High		
Total No. of Female Patients:	9	20%	2 Medium		
			1 Low		
Total Patients Recovered	20	44.44%			
Total Patients Not Recovered	25	55.55%			
Percentage of F. Negative after drug		41.66%			
Percentage of V.Negative after drug		55.55%			
Pl. Vivax Cases	9	20%			
Pl. Falciparum Cases	36	80%			
Female with P. falciparum	6	Recovered	1	Switched	5
Female with P. vivax	3	Recovered	2	Switched	1
Male with P. falciparum	30	Recovered	14	Switched	16
Male with P. vivax	6	Recovered	3	Switched	3
Total patients treated with Drug 'A'	24	Recovered	6	Switched	18

UNDER PEER REVIEW

Total patients treated with Drug 'B'	21	Recovered	14	Switched	7
Percentage of Drug A	53.33%	Success	25%	Fail	75%
Percentage of Drug B	46.66%	Success	66.66%	Fail	33.33%
	100%				

(Note: Switched mean patients not recovered from the treatment were switched to conventional antimalarials)

Important indicators of the study are shown in following figures (graphical presentation):

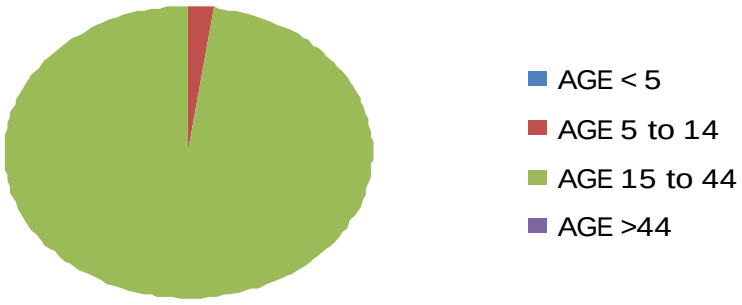


Fig. 4.1: Age Grouping (5 to 14 2.2%, 15 to 44 97.8%)

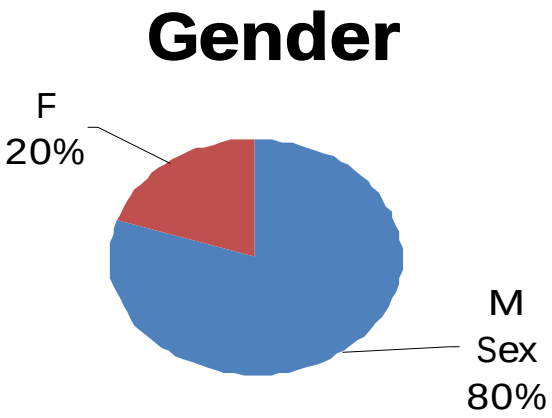


Fig 3.2: Gender of Patients (Male 80%, Female 20%)

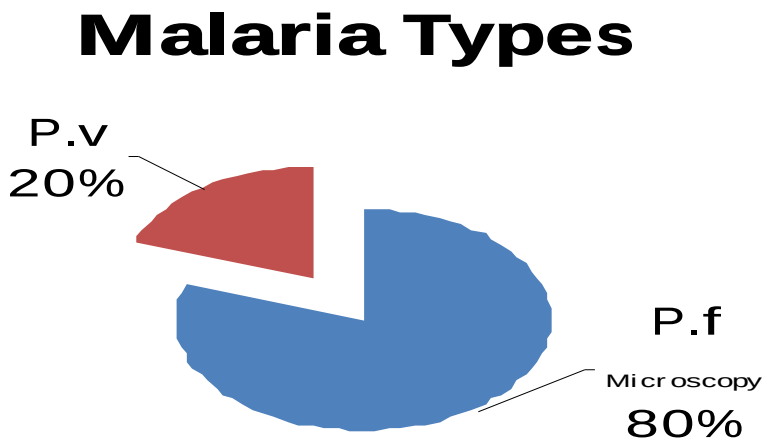


Fig 3.3: Malaria Types

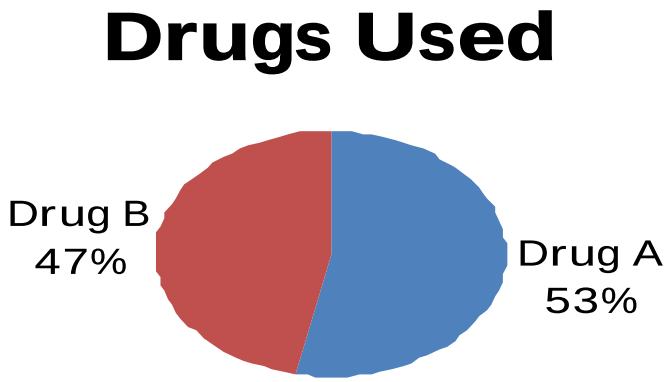
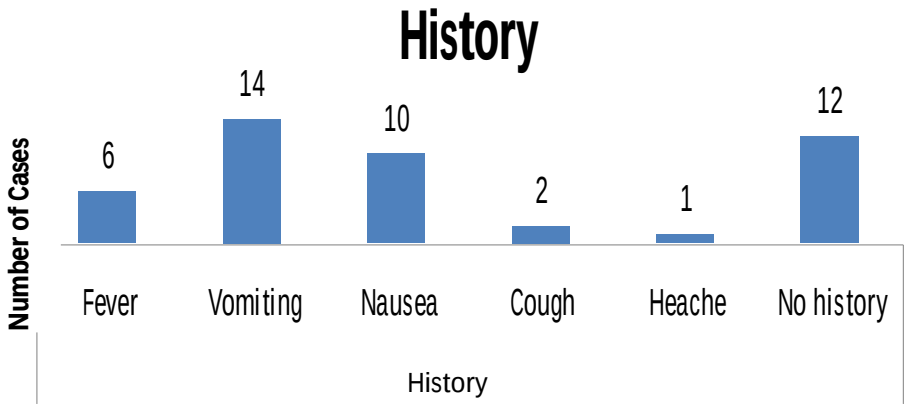


Fig 3.4: Drugs used to treat Malaria



Symptoms

Fig 4.5: Number of patients having history of various symptoms

Table 4.2 Patients Symptoms before and after treatment with recovery Percentage

Variables	Patients Tested			Patients Recovered		
	Male	Female	Total	Male	Female	Total
Fever	36	9	45	17(47.3%)	3 (33.4%)	20(44.45%)
Chills	36	9	45	17(47.3%)	3 (33.4%)	20(44.45%)
Sweating	36	9	45	17(47.3%)	3 (33.4%)	20(44.45%)
Headache	36	9	45	17(47.3%)	3(33.4%)	20(44.45%)

UNDER PEER REVIEW

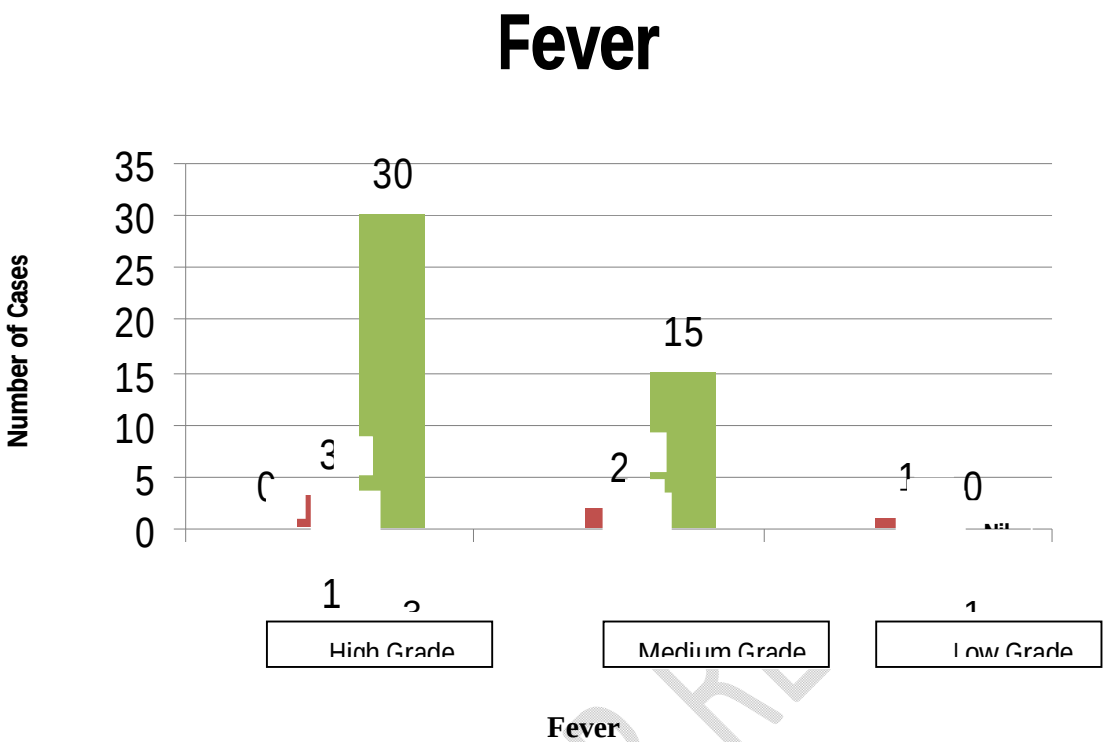


Fig 3.6: Patients with Fever (High, Medium, Low Grade)

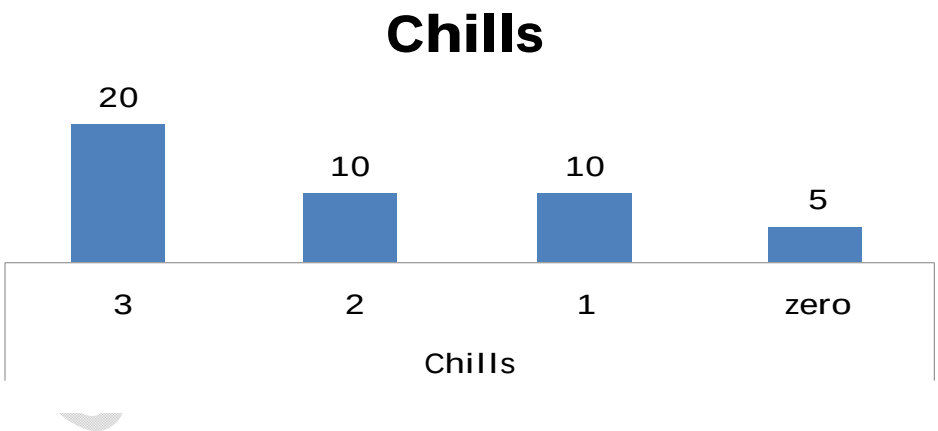


Fig 3.7: Patients with Chills

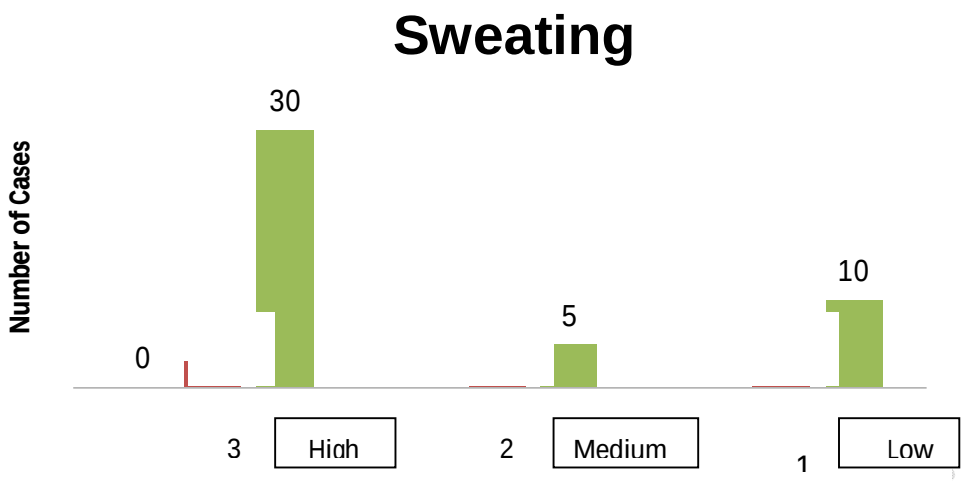


Fig 3.8: Patients with Sweating

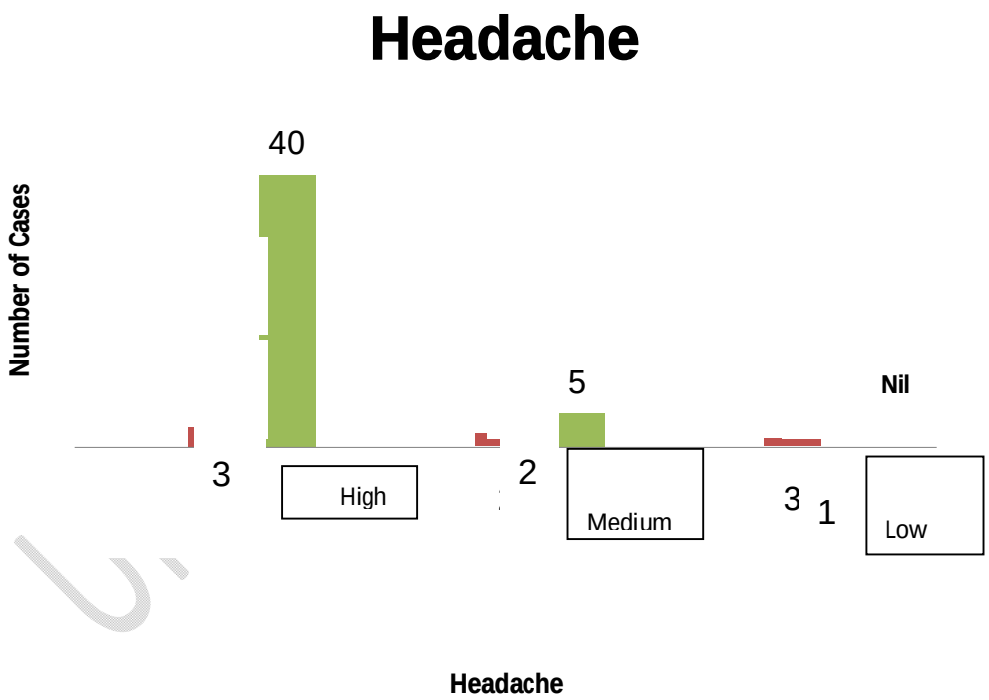
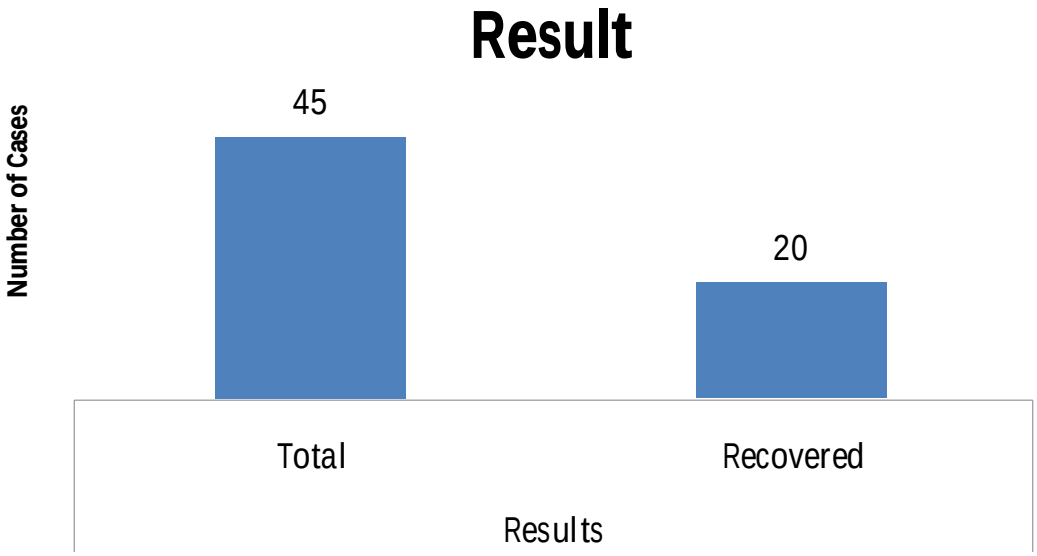


Fig 3.9: Patients with Headache



Recovery

Fig 4.10: 44.44% Patients Recovered

The patients were divided into 4 groups (WHO, 2009) i.e. < 5, 5-14, 15-44, >44. Only one patient (2.2%) felled in 5-14 years group whereas, 44 patients (9[illegible] 15-44 years group. Less than 5 years old children were not enrolled [illegible] order to avoid complications and ensure patient safety. It was found that adults are susceptible to malaria in this region. Out of total 45 patients, 36 (80%) were male and 09 (20%) were female patients.

The four important indicators i.e. symptoms of malaria (fever, chills, sweating and headache) were monitored. Out of 45 patients, 30 patients (22 males, 8 females) had high grade fever (66.66%) and 15 patients (14 males, 8 females) had medium grade fever (33.33%) and no patient was found with low grade fever. 20 patients (13 males, 7 females) had high grade chills (44.44%), 10 patients (8 males, 2 females) had medium grade chills (22.22%), 10patients (10 males) had low grade chills, whereas 5 male patients were found without chills (11.11%). Out of 45 patients, 30 patients (22 males, 8 females) had high grade sweating (66.66%), 5 patients (5 males) had medium grade sweating (11.11%) and 10 patients (9 males, 1 female) had low grade sweating (22.22%). Out of total 45, 40 patients (31 males, 9 females) had high grade headaches (88.88%) while 5 patients (5 males) had medium grade headaches (11.11%).

The patients with malaria symptoms were microscopically confirmed, it was found that 36 patients (30 males, 6 females) had *falciparum* malaria (80%) and 9 patients (6 males, 3 females) had *vivax* malaria (20%).

UNDER PEER REVIEW

The history was taken from patients and found that 6 patients (4 males, 2 females) had only fever, 14 patients (11 males, 3 females) had fever with vomiting (for last 4-7 days), 10 patients (9 males, 1 female) had fever with nausea (for last 4-7 days), 2 patients (both males) had fever with cough, 1 male patient had fever with headache and 12 patients (9 males, 3 females) had no history of illness.

Out of 24 patients (20 males, 4 females) treated with Drug 'A', only 6 patients (25%) were recovered and had no symptoms of malaria on their follow up visit on day 3, and out of 21 patients (16 males, 5 females) treated with Drug 'B', only 14 patients (66.66%) were recovered.

The overall recovery rate was 44.44%. Non recovered 25 patients were switched immediately by the physician to conventional antimalarials.

DISCUSSION

Global malaria prevalence reported 441 million cases in 2009 by World Health Organization (WHO, 2010) which is 1.40 cases per 1000 population per year. In comparison in Pakistan, total cases reported were 2 million in 2009 and API is 1.93 per 1000 per year (Kakar *et al*, 2010) which showed less prevalence but the data is insufficient and further investigations are suggested.

The *Plasmodium falciparum* proportion in total confirmed cases in 2009 in Pakistan was 20.3 per 1000 cases (Kakar *et al*, 2010). The malaria caused by P. falciparum is the most serious form of disease accounting up to 80% malaria cases worldwide. It is the main species found in Tropical and subtropical Africa and parts of Central America and South America, Bangladesh, Pakistan, Afghanistan and Nepal (Cheesbrough, 1998).

The *P.vivax* on the other hand is most frequent and widely distributed cause of recurring (tertian) malaria. *P. vivax* is one of four species of Plasmodium that is less virulent and is seldom fatal. Overall it accounts for 65% of malaria cases in Asia and South America. Chloroquine remains the drug of choice for vivax malaria (WHO, 2010) except in Indonesia region where chloroquine resistance is common and alternatively artesunate is drug of choice. Eradication of liver stages is achieved by giving Primaquine after checking patient's G6PD status to reduce the risk of hemolysis (Baird and Hoffman, 2004). At least a 14 day course of Primaquine is required for the radical treatment of *P. vivax*. (WHO, 2010).

The treatment of malaria depends upon the severity of the disease; whether patients who can take oral drugs have to be admitted depends on the assessment and the experience of the clinician. Uncomplicated malaria is treated with oral drugs. The most effective strategy for *P. falciparum* infection recommended by WHO is the use of artemisinins in combination with other antimalarials artemisinin-combination therapy (ACT), in order to avoid development of drug resistance against artemisinin based therapies. The signs and symptoms of uncomplicated malaria are non specific and therefore, malaria is suspected clinically mostly on basis of fever or history of fever (WHO, 2010).

UNDER PEER REVIEW

In sub Saharan Africa where malaria is endemic and in other parts of the world, plants are extensively used for the treatment of periodic fevers and malaria. The spread of multi-drug resistant *P. falciparum* has highlighted the urgent need to develop new antimalarial drugs, preferably inexpensive drugs that are affordable for developing countries, where malaria is prevalent (Miller, 1992). Four crude organic extracts obtained from medicinal plants used in Nigerian folk medicine have been tested in vitro against *P. falciparum*. The most active extract was obtained from *E. chlorantha* that showed appreciable inhibition of the parasites at all concentrations used in the study. Treatment with antipyretic agents like *A. indica* would lead to early relief of fever and pyrexia to eliminate the parasite thereby helping the body's immune system. Fever is a host response associated with schizont rupture and is the most common clinical manifestation of malaria (Gatton and Cheng, 2002). It has been reported that chemosuppresive and prophylactic activities existed in the medicinal herbs used in the study (Kimbi *et al*., 1998)

In this present study, *P. kurroa* used in traditional medicine (Indigenous System) was evaluated for its antimalarial activity *in vivo*.

The results generally showed that this plant possessed antimalarial activity against *P. falciparum* (Khokkar, 2002) and *P. vivax*. The alcoholic extracts seemed to have better effect than aqueous extract. 66.66% patients were recovered from malaria by using alcoholic extract of *P. kurroa*. *Picrorhiza* is not readily water-soluble and is therefore not usually taken as a tea. While it is ethanol soluble, the bitter taste makes tinctures unpalatable, so it is therefore usually administered as a standardized (4% kutkin) encapsulated powder extract. Typical adult dosage is 400 to 1500 mg/day, with dosages up to 3.5 g/day sometimes being recommended for fevers. *Picrorhiza* root extracts are widely used in India with no adverse effects having been reported. The (LD.sub.50) of kutkin is greater than 2600 mg/kg in rats with no data available for humans (CSIR, 1989-1990).

It was reported that *P. kurroa* roots were effective against intermittent fevers. In China and Malaya, its rhizome is a favorite remedy for bilious dyspepsia accompanied by fever (Baquar, 1989). The roots of *P. kurroa* contains Picrorhizin, glucose wax (Nadkarni, 1976), picrorhizetin, apocynin (Rastogi & Mehrotra 1993), cathartic acid, berberine, alcoholic extract (Nadkarni, 1976 & Duke and Auensu 1985), kutkin. Kurrin (non-bitter), kutkisterol sesquiterpene. Excellent antiparasitic activity of ethanol extracts showed more solubility of active constituents in ethanol than distilled water.

The hepatoprotective action of Picrorhiza kurroa is not fully understood but may be attributed to Picrorhiza's ability to inhibit the generation of oxygen anions and to scavenge free radicals.(Russo et al., 2001) Picrorhiza's antioxidant effect has been shown to be similar to that of superoxide dismutase, metal-ion chelators, and xanthine oxidase inhibitors.(Chander *et al.*, 1992a) In rats infected, with malaria, Picrorhiza restored depleted glutathione levels, thereby enhancing detoxification and

UNDER PEER REVIEW

antioxidation, and helping maintain a normal oxidation-reduction balance.(Chander *et al.*, 1992b).

Sauza and Gloria (1998) also obtained higher yield of alkaloids while extracting oleoresins with ethanol. The reason being solubility of active ingredients increased with rise in temperature resulting in higher extraction. It may also be probably due to fresh proportion of solvent coming in contact with sample after an interval of time. Dieterle and Kaiser (1932) also obtained similar results while working on extraction of essential oil with Soxhlet apparatus. The decoction of P. *kurroa* when administered to rats with carbon tetrachloride-induced liver injury showed lesser structural damage in the hepatic tissue, as compared to the control rats (Chaturvedi & Singh, 1965). The alcoholic extract of *P. kurroa* showed protective action against hepatotoxicity induced by carbon tetrachloride in rabbits as evidenced by marked regression of serum transaminases (SGOT and SGPT) and serum alkaline phosphatase levels. The percentage retention of bromosulphathalein also showed comparatively lower values in the drug treated group (Pandey & Chaturvedi, 1968)

Truly, no in vitro drug sensitivity test can entirely mimic the in vivo situation, but in vitro methods should ideally utilize both uniform drug exposure and the medium approximating the in vivo milieu. (Sixsmith et al., 1984).Here results showed 80% of *P. falciparum* and 20% of *P.vivax* cases which is indicating an increasing trend of *falciparum* malaria.

CONCLUSION

Despite the considerable progress in malaria control over the past dacades, malaria remains a disease of priority. Plant materials remained an important resource to combat serious diseases in the World (Tshibangu *et al.*, 2002). *P. kurroa* qualified as active compound to undergo further investigation for its antimalarial activity and its active constituents should be investigated for better outcomes in the field of traditional medicines.

Ethical Approval and Consent

As per international standard, patients' written consent and ethical approval has been collected and preserved by the author(s).

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UNDER PEER REVIEW

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