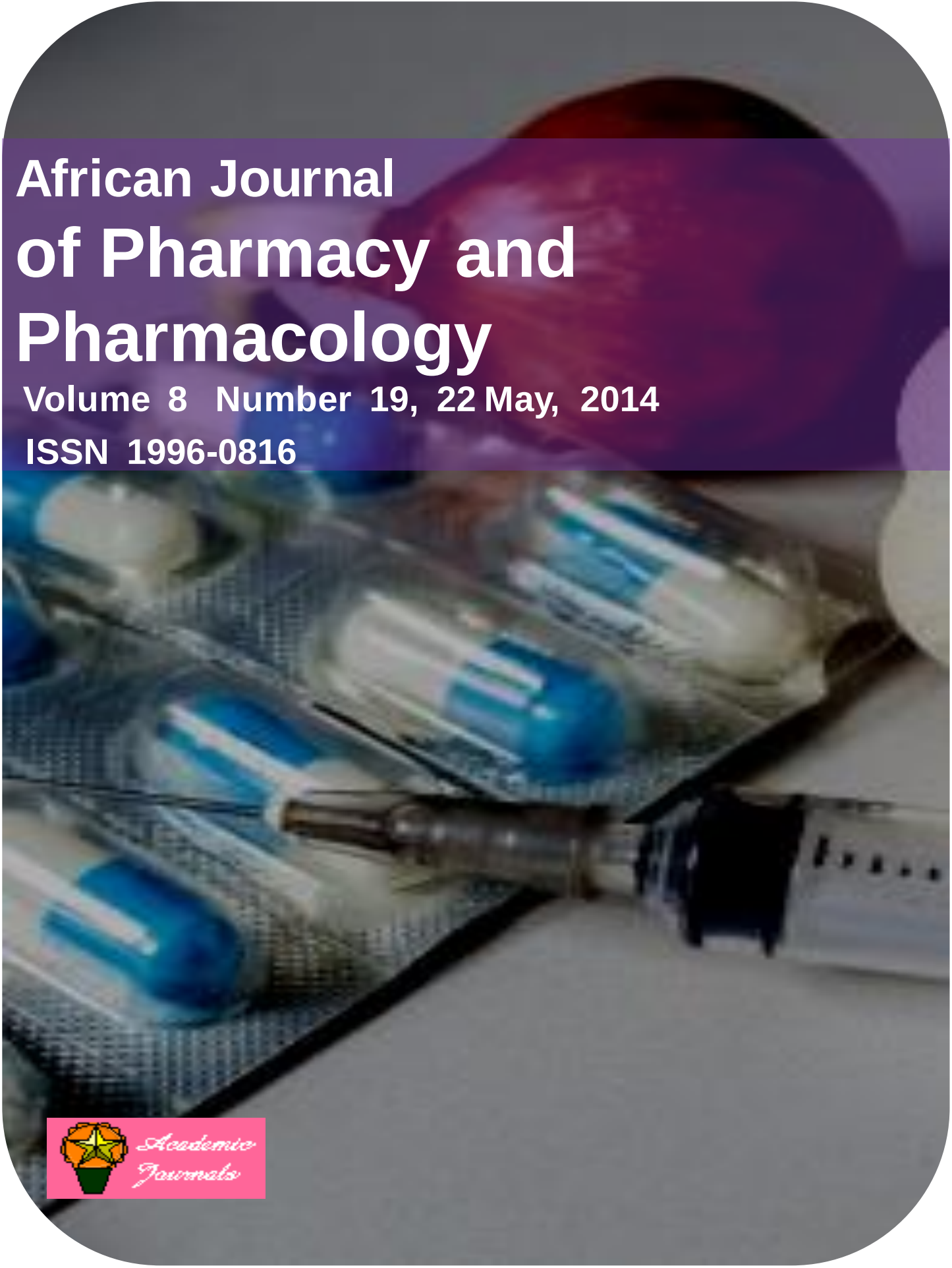

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Full Length Research Paper

Evaluation of topical antinociceptive effect of *Artemisia absinthium* extract in mice and possible mechanisms

Fatemeh Zeraati[1]*,Farzaneh Esna-Ashari[2], Malihe Araghchian[1], Amir Hossein Emam[3], Majid Vafaei Rad[3], Sadegh Seif[3] and Kaivan Razaghi[4]

[1]Department of Pharmacology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
[2]Community Medicine Department, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
[3]School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
[4]Research Center for Agriculture and Natural Resources, Hamadan, Iran.

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This study, evaluated the topical antinociceptive effect of dried extract of *Artemisia absinthium* in mice, and some mechanisms underlying these effects were also investigated. Four concentrations (1, 2.5, 4, and 6% W/V) of dried extract of the plant in normal saline were evaluated for topical analgesia in tail flick model test. The mice tail was immersed in normal saline supplemented with different concentrations of extract as well as normal saline as control for 2 min before tail flick test. Atropine (5 mg/kg), metoclopramide (1 mg/kg), ondansetrone (0.5 mg/kg) and naloxone (4 mg/kg) were injected intraperitoneally (IP), 20 min before tail immersion in normal saline containing the extract at 4% concentration. Subsequently, maximum possible effect percentage (MPE%) was calculated for each dose. It was found that the plant extract produced antinociceptive effect at (4 and 6% W/V) concentration in tail flick model. Furthermore, analgesic effect of extract at 4% W/V concentration was significantly attenuated by pretreatment with atropine, metoclopramide, ondansetrone and naloxone. These results suggest that the extract produced antinociception in tail flick model probably through cholinergic, serotoninergic, dopaminergic, and opiodergic system.

Key words: *Artemisia absinthium*, antinociceptive effect analgesia, mechanism of action.

INTRODUCTION

The traditional medicine has used medicinal plants to relieve pain without noticeable side effect. Therefore, it can introduce novel drugs with less complications and cost. Pain is described as a discomforting feeling in a certain part of the body. The obligation of medical science is to maintain human health and ease the pain. Understanding the concept of pain, therefore, is one of the most essential tools in realizing these goals. Since pain is considered an indicator of disease, all around the world and in all cultures, it is the most common symptom that compels patients to visit doctors (Kruger, 2001). Different classes of drugs have been considered and used for pain relief. Opioids and non-steroidal anti-inflammatory drugs were included. Currently, the side effects of these drugs are

*Corresponding author. E-mail: zeraati@umsha.ac.ir. Tel: +98-918-312-2063. Fax: +98-811-8226035.

causing problems in treatment (Katzung et al., 2007). Analgesics, particularly medicinal plants, which have less side effects and addictive characteristics, can gain importance. Localized treatments have many advantages compared to systemic drugs. In limiting the drugs contact to location, central side effects may reduce significantly. As for opioids, this effect can limit side effects such as reduced irritability (sedation), respiratory depression and nausea. Also, for non-steroidal anti inflammatory drugs (NSAID), side effects of the gastro intestinal system reduces (Katzung et al., 2007). One of these medicinal plants is *Artemisia absinthium* (Wormwood). The medical use of this plant dates back to at least Roman times (Lachenmeier, 2010). The plant was used as anti-diarrhea (Zargari, 1989), antihelmintic (Lopes-Lutz et al., 2008; Tariq et al., 2009; Tariku et al., 2011), and antimicrobial (Lopes-Lutz et al., 2008). Anti-proliferative effects on human breast cancer cells were recently studied by Shafi et al. (2012), who showed that absinthium possess potent antioxidant properties, and may be used as a protective agent against disorders associated with oxidative stress (Canadanovic-Brunet et al., 2005; Bora and Sharma, 2011), neuroprotective and may prove to be useful adjunct in the treatment of stroke (Bora and Sharma, 2010).

Its use has been claimed to remedy cure indigestion and relieve gastric pain (European Medicines Agency, 2009). A recent double blind study by Omer et al. (2007) suggested that Wormwood may help patients with Crohn's disease, because of its steroid-sparing effects.

The systemic analgesic and anti inflammatory effects of this drug have been previously evaluated either by Fayyaz et al. (1992) and Sadeghifard and Zareian (2008). This study assessed its topical analgesic effect in mice. The mechanism of action was also discussed. The local name of tested plant is Afsantin.

METHODOLOGY

Plant and preparation of plant extract

A. absinthium L. is a member of the Asteraceae or Compositae family. The plants were planted and grown in Avicenna Medicinal Plants Research Station of Hamadan Agricultural and Natural Resources Research Center, Hamadan, Iran. The plant recognition and identification was carried out by herbarium of the research center. The leaves and inflorescence of the plants were harvested in two plant growth stages including: before opening flowers in May and last stage of flower maturity in late August. The selected parts of these plants were then dried in shade at temperatures between 21 and 30°C for 15 to 30 days, after which these parts of plants were chopped and ground.100 g of the plant was mixed with 120 ml of 8% acetic acid for two weeks and the extract was obtained through pressing and grinding of the plant using stainless steel press. The extract was concentrated at room temperature. Different concentrations (1, 2.5, 4, and 6%) of the extract were prepared in normal saline.

Drugs

The drugs used were as follows: morphine, naloxone (T.D., Iran), metoclopramide (Tehran Chemie, Iran), ondansetron (EXIR, Iran), and atropine (Darou Pakhsh, Iran). All drugs were freshly dissolved in 0.9% saline for intraperitoneal (IP) injection. Morphine (10 mg/ml) was used topically.

Animals

Male Albino N-MR (Institute of the Razi, Tehran) weighing 25 to 32 g was used in the experiments. All experiments were performed in accordance with institutional animal use guidelines. The animals were housed in standard stainless steel cages in a controlled room temperature (22±2°C) with a 12/12 h light/dark cycle. Mice were divided into 9 groups. Each group contains 8 animals.

Nociceptive assay

Antinociception was assessed using the tail flick apparatus. The tail withdrawal latency (s) was measured before administration of any drug or vehicle. Normal response latencies were usually between 2.5 and 3.0 s. An 8-s cut-off was used to prevent tissue damage. The response was tested at 10, 20,30,60,90 and 120 minutes after drug administration, respectively. All drugs were injected (IP, 0.1 ml/10 g) 20 min before extract administration. After drug injection (Naloxone 1 to 5 mg/kg, ondansetrone 0.5 mg/kg, atropine 5 mg/kg, metoclopramide 1 mg/kg), 3 cm of tail was put in the extract as treatment group or normal saline as control group for 2 min. Anti-nociception was quantified as the percentage of maximum possible effect (%MPE) using the method of Keil and Delander (2011).

The following formula was used to calculate %MPE:

$$MPE\ (\%) = 100 \times [(\text{test} - \text{control latency}) / (\text{cutoff} - \text{control latency})]$$

Statistic analysis

Statistical analysis was carried out using Statistical package for Social Sciences (SPSS) for Windows, version 16. The parametric test Mann-Whitney U and Kruskal-Wallis tests were used to compare different groups with control and different groups with each other, respectively. Trend analysis test was used for comparison between times in different groups.

RESULTS

Antinociceptive effect of *A. absinthium* was studied at different concentrations. It was found that at 1 and 2.5 concentrations, the extract did not show any analgesic effect until 4 and 6% concentrations. The difference of effects were significant between 4.6% concentration and lower doses and control ($P<0.05$) (Figure 1). There is a significant linear trend for mean of MPE% to increase across the different time in both groups ($P<0.05$) (Figure 1). Topical analgesic effect of morphine (10 mg/ml) was compared with absinthium 4 and 6%. There was no significant difference between them ($P>0.05$) (Figure 1).

Analgesic effect of 4% concentration of absinthium was reduced after injection of naloxone, metoclopramide,

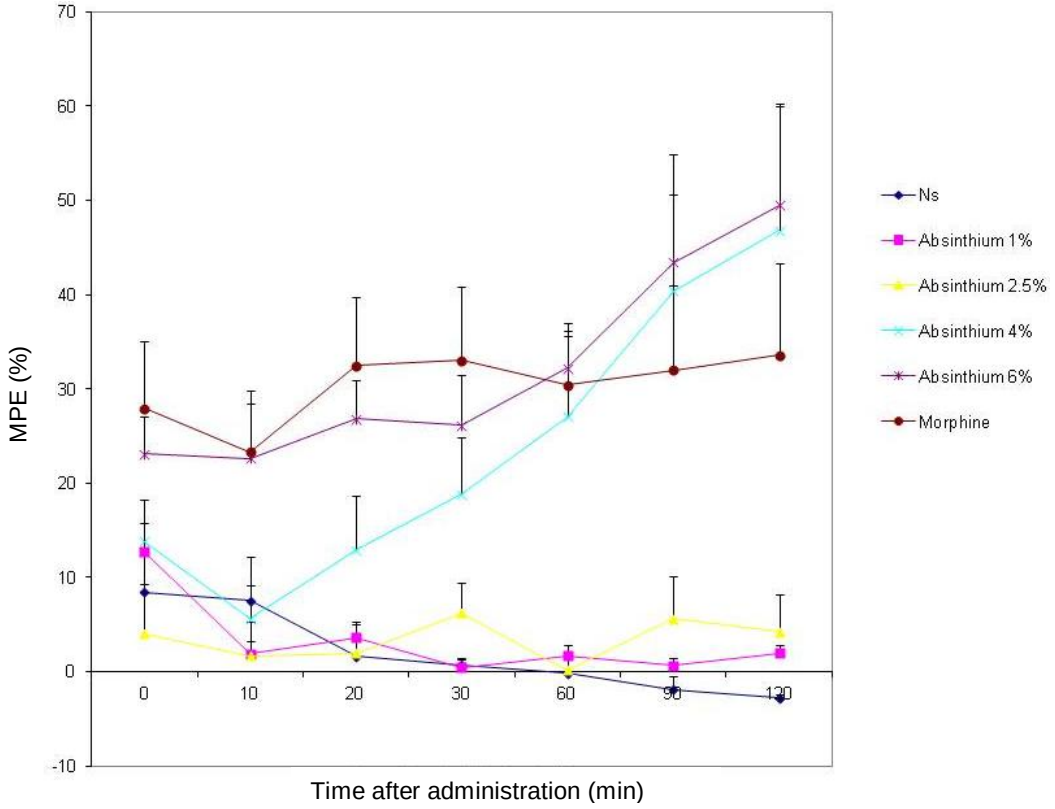

Figure 1. Comparison of different concentration of the extract with morphine10 mg/ml and normal saline in mice by tail flick test.
MPE: Maximum possible effect.

ondansetrone and atropine (P<0.05) (Figure 2). Naloxone inhibited analgesic effect of herbal extract at the 60, 90, and 120 min and metoclopramide at the same time with naloxone (Figure 2). Ondansetrone had inhibitory effect at 30, 60, 90, and 120 min and atropine at the 90 and 120 min (Figure 2).

DISCUSSION

To evaluate the topical antinociceptive effect of *A. absinthium* extract on mice, different concentrations of the plant were prepared and tested. The plant is already known to remedy indigestion and lessen gastric pain (European Medicines Agency, 2009). The systemic analgesic effects of this extract have been previously evaluated by Fayyaz et al. (1992). The tail-flick response is believed to be a spinally mediated reflex (Chapman et al., 1985). Moreover, Grumbach (1966) has shown that the effectiveness of analgesic agents in tail-flick pain model is highly correlated with relief of human pain.

In this study, the extract showed antinociceptive effect at 4.6% concentration (P<0.05), but did not show antinociceptive effect in lower doses. Thus, antinociceptive effect of the extract was dose dependent. The non-significant effect between morphine (10 mg/ml) and the extract shows that it has antinociceptive effect as well as morphine. Fayyaz et al. (1992) had compared the systemic effect of absinthium extract with acetylsalicylic acid. In spite of rapid onset of analgesic action, the plant extract, even at high dose, showed less potent analgesia (both in terms of intensity and duration) when compared with acetylsalicylic acid. In this study absinthium extract showed similar effect (both in terms of onset and intensity) of topical analgesia when compared with morphine (P>0.05). The anti-inflammatory effect of absinthium extract was assayed (Fayyaz et al., 1992). In the other study, it resulted that this extract may inhibit inflammatory cytokines such as TNF-alpha and NF kappa B (Krebs et al., 2010).

Antinociception exerted by *A. absinthium* extract in tail flick test appears to depend upon opioidergic neurotransmission, since the effect of these compounds was partially antagonized by the opioid receptor antagonist naloxone. The antinociceptive effects elicited by the extract were antagonized by atropine. This antagonism indicates a muscarinic receptor-mediated interaction in the antinociceptive activity induced by herbal extract and suggests a very important involvement of muscarinic cholinergic mechanisms in the expression of the antinociceptive

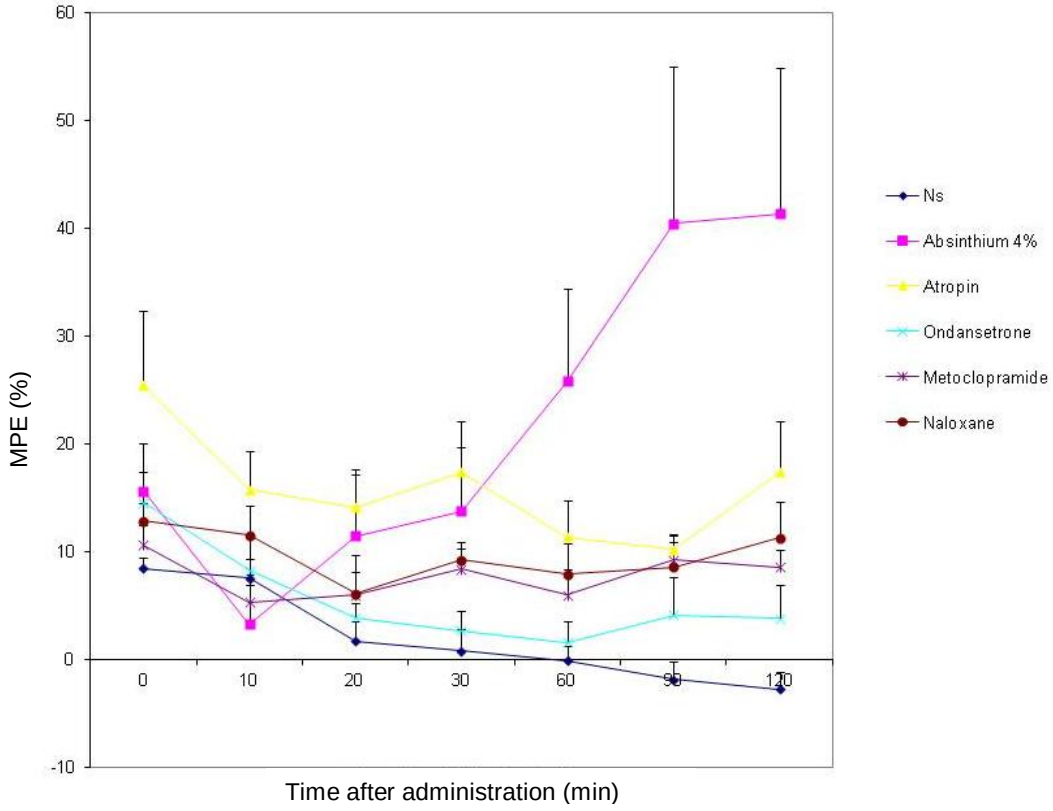

Figure 2. Comparison of absinthium 4% analgesia effect before and after pretreatment with drugs (naloxone 1 to 5 mg/kg, ondansetrone 0.5 mg/kg, atropine 5 mg/kg and metoclopramide 1 mg/kg) by IP injection 20 min before the extract administration in mice.
MPE: Maximum possible effect.

effect.

Several reports support a role for acetylcholine (ACh) in the inhibition and modulation of the nociceptive information transmission (Eisenach, 1999). Studies have shown the involvement of the dopaminergic system in mechanisms of antinociception. Of note, dopamine receptors agonists were described as facilitating analgesic response (Michael-Titus et al., 1990; Suaudeau and Costentin, 1995).

The present results show that the antinociceptive action of *A. absinthium* extract was attenuated by metoclopramide. Pivotal studies have shown a spinal analgesic action of 5-HT released from brainstem structures (Yaksh and Tyce, 1979; Yaksh and Wilson, 1979). In this study analgesic effect of the extract was inhibited by ondansetrone, a serotonin antagonist.

Conclusion

A. absinthium extract has topical analgesic effect. Pretreatment with naloxone 1 to 5 mg/kg, ondansetrone 0.5 mg/kg, atropine 5 mg/kg and metoclopramide 1 mg/kg reduced analgesic effect of the extract ($P<0.05$). It can result that these pathways may have important role in analgesic effect of *A. absinthium* extract.

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Full Length Research Paper

Effect of oral supplementation of *Aloe vera* extract on haematology indices and immune cells of blood in rabbit

Amjad Ali Channa[1]*, Izhar Hyder Qazi[2], Saeed Ahmed Soomro[3], Atta Hussain Shah[4], Jameel Ahmed Gandahi[5], Roshan Ali Korejo[1], Imtiaz Ahmed Shah[1], Nawab Ali Kalhoro[1] and Band-e-ali Khaskeli[1]

[1]Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan.
[2]Department of Veterinary Anatomy and Histology, Faculty of Bio-Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan.
[3]Department of Veterinary Physiology and Biochemistry, Sindh Agriculture University, Tandojam, Pakistan.
[4]Department of Animal Products Technology, Sindh Agriculture University, Tandojam, Pakistan.
[5]Department of Veterinary Anatomy and Histology, Sindh Agriculture University, Tandojam, Pakistan.

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Aloe vera **is being widely used in herbal medicine as antibacterial, antiviral, antifungal, and antiinflammatory agent. It is also used as topical application for many skin diseases; its antidiabetic effect in rats is also reported. Looking at its enormous medicinal effects, the present study was conducted to investigate the effects of oral supplements of** ***Aloe vera*** **extract on hemotology indices and immune cells of rabbit. Total of twenty (n=20) rabbits were selected and divided into 4 group's i-e A, B, C and D with n=5 in each group. Group A was kept as control while B, C and D were given 400, 500 and 600 mg/kg of** ***Aloe vera*** **extract, respectively daily for 21 days. Blood samples were collected from central ear vein transferred into test tubes containing ethylene diamine tetra acetate (EDTA) on 0 day, 7th day, 14th day and 21st day and analysed for complete blood count (CBC). Results revealed that oral supplementation of** ***A. vera*** **extract significantly increase mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and lymphocytes at level (p< 0.05) and decrease neutrophils in normal domestic rabbits. Results of this study concluded that** ***A. vera*** **has hematopoetic and immunomodulatory effects; thus its extract can be used for the treatment of anemia and immune deficiency problems.**

Key words: *Aloe vera*, biological active, anaemia, immunodeficiency.

Introduction

Aloe vera, derived from an Arabic word "Alloeh" means shinning bitter substance and *vera* means 'true' (Ombrello, 2008). It is a succulent cactus like perennial plant originated from arid climates of North Africa (Akinyele et al., 2007*).* There are about 275 species worldwide (Saeed et al., 2003-2004; Nandal and Bhardwaj, 2012). *A.*

*Corresponding author. E-mail: brainboss@ymail.com.

vera contains 99·5% water and 0.5% solids (Hamman, 2008). It contains 200 chemica compounds out of which 75 are biological active, that is, aloin, *Aloe* polysaccharide (Jun et al., 2005), reducing sugars, organic acids, enzymes, metallic cations and lecithin (Eshun and He, 2004). Due to antibacterial properties, *A. vera* is used against commonly found bacteria including *Staphylococcus, Streptococcus, Klebsiella, Pseudomonas, Escherichia coli, Salmonella*, etc., (Lawrence et al., 2009). It is also used to treat many viral diseases and enhance immune system by acting as effective antiviral agent against human immunodeficiency virus (HIV) and hepatitis (Rabe et al., 2005). Research has investigated that *A. vera* have positive influence on central nervous system, especially on the ependymal cells at ventricular zone (Rengin et al., 2008).

Besides antibacterial and antiviral effects, *A. vera* has antifungal, antineoplastic activities and widely used for topical applications as an antiaging agent (Ivan, 2006). *A. vera* significantly influences the function of liver and pancreas by influencing aspartate amino transferase (AST) and alanine transferase (ALT) (Iji et al., 2010). It has been reported that treatment of normal as well as diabetic rats with *A. vera* significantly reduces its blood sugar level indicating that it has antidiabetic effects (Rehman et al., 2011).

Keeping in view the clinical importance of *Aloe vera*, the present study is conducted with objectives to determine: (a) effects of oral supplementation of *A. vera* on haematology indices and immune cells in blood of rabbits, (b) the safe and effective therapeutic dose of *A. vera* through oral route of administration.

MATERIALS AND METHODS

A. vera leaves were used in this study. Fresh leaves of *A. vera* were splitted into two parts after removing spiny margins; white transparent pulp was scraped out and weighed. Hundred grams (100 g) of *A. vera* was blended and boiled in 200 ml of distilled water for 10 min, after boiling it was cooled at room temperature and filtered with muslin cloth. The extract thus obtained was stored in a refrigerator at 4°C until use. A total of 20 male rabbits of an average body weight: 1·4 kg were used in this study. Rabbits were kept at animal house under control environment. The rabbits were given 0.25 kg rice and 0.25 kg green grass, especially *Alpha alpha* and water was given as *ad-libitum*. Rabbits were divided into four groups, that is, A, B, C and D consisting of 5 in each group. Group A was kept as control, while groups B, C, and D were given *A. vera* extract orally at the dose of 400, 500 and 600 mg/kg body weight, respectively daily for 21 days. Blood was collected before treatment day 0, and then days 7, 14, and 21st post treatment. Blood was collected from central ear vein, and transferred to test tubes containing anticoagulant (EDTA: Ethylene diamine tetra acetate), and brought to Post Graduate Laboratory of Veterinary Physiology, Sindh Agriculture University, Tandojam for CBC analysis.

RESULTS

Mean corpuscular hemoglobin (MCH)

As shown in Figure 1, MCH of rabbits that received oral supplementation of *A. vera* extract increased from day 0 to 21st post treatment. MCH of groups A, B, C and D was 15.42, 15.34 14.94 and 15.24 pg and 15.46, 21.18, 21.14 and 21.54 pg day 0 and 21st day post treatment, respectively. Results revealed gradual increase in MCH values of treatment group. The statistical analysis of mean MCH values of treatment and control group indicated significantly ($P<0.05$) higher values in treatment than MCH values of the control groups.

Mean corpuscular volume (MCV)

Results shown in Figure 2 indicate gradual increase in values of MCV in the blood of rabbits in treatment group. MCV of groups A, B, C and D were 58.48, 58.558, 58.48 and 58.54 cu and 58.56, 65.72, 65.04 and 65.66 cu, on day 0 and day 21st, respectively. Statistical analysis showed significantly higher ($P<0.05$) MCV of rabbit's blood in treatment group than control.

Neutrophils

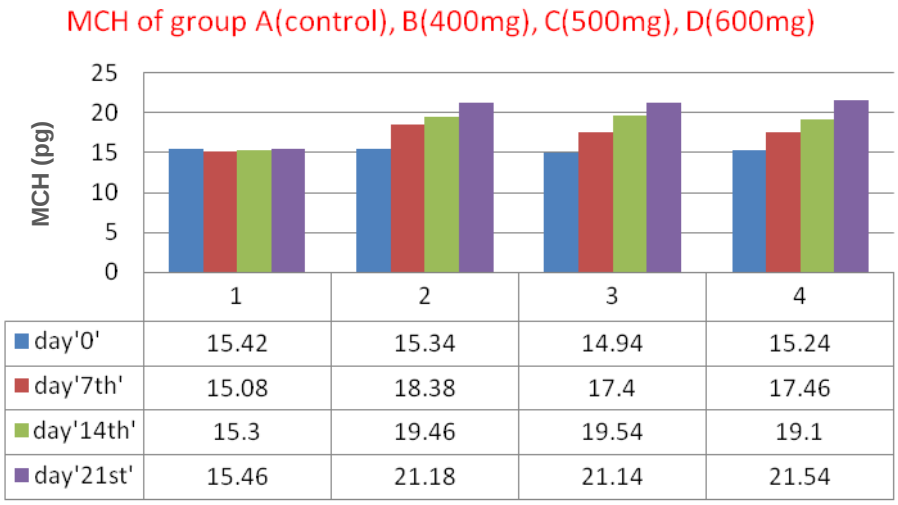
The neutrophil counts in the blood of rabbits in treatment group, that is, B, C and D significantly decreased ($P<0.05$). As shown in Figure 3, the neutrophil count of groups A, B, C and D was 45.94, 45.46, 45.5 and 45.38 and 45.76, 29.18, 24.52 and 23.38 on day 0 and 21st day. There was significantly lower ($P<0.05$) neutrophil in groups B, C, D as compare to control group A.

Lymphocytes

Oral supplementation of *A. vera* significantly increased lymphocyte in treatment groups B, C, and D, that is, 43.66, 43.86, 43.68 and 57.84, 60.32, 61.74 (Figure 4) from day 0 to 21st post treatment, respectively.

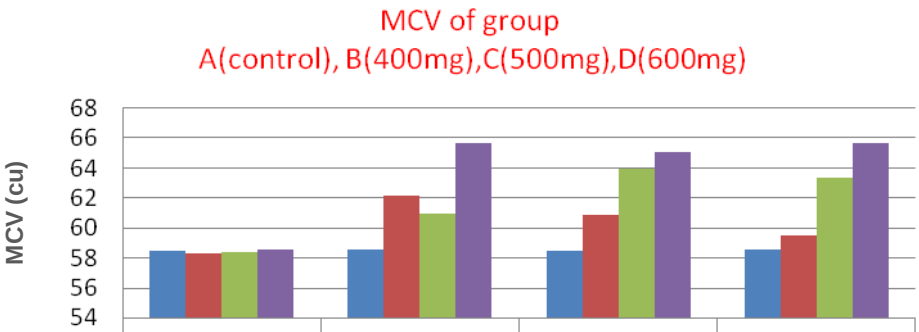
DISCUSSION

Haematology parameters such as red blood cell count, hematocrit, hemoglobin concentration and derived hemotology indices, e.g MCV, MCH and MCHC are important blood marker for diagnosis of various blood diseases. The excessively decreased values of these parameters indicated different types of anaemia in human as well as animals caused by loss of blood through hemorrhage, bone marrow disease, iron deficiency, vitamin B12 deficiency, or folic acid deficiency, etc. Various therapeutic medicines are used for the treatment of blood diseases. Nowadays, remedies through herbal medicines (alternative medicines) have been more demanding due to their natural source and less side effects. The results of this study have indicated that *A. vera* leaf extract significantly increase (<0.05) in MCH and



	1	2	3	4
day'0'	15.42	15.34	14.94	15.24
day'7th'	15.08	18.38	17.4	17.46
day'14th'	15.3	19.46	19.54	19.1
day'21st'	15.46	21.18	21.14	21.54

Figure 1. Mean corpuscular haemoglobin (MCH) on various days.

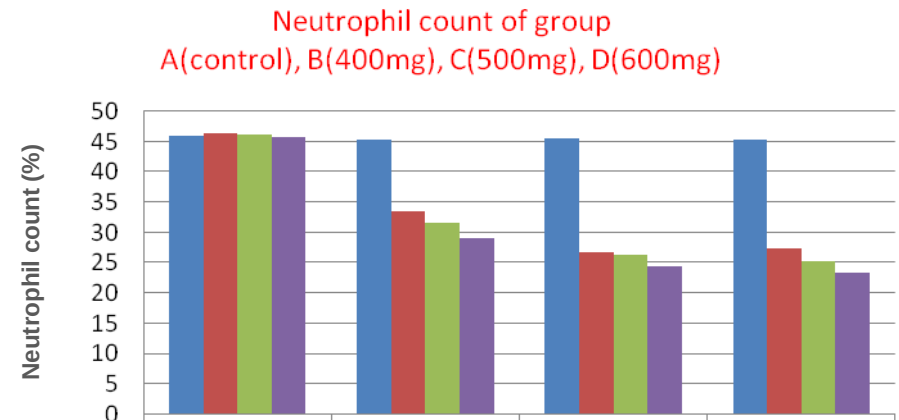


	1	2	3	4
day'0'	58.48	58.558	58.48	58.54
day'7'	58.26	62.2	60.86	59.52
day'14'	58.42	60.96	64	63.32
day'21'	58.56	65.72	65.04	65.66

Figure 2. Mean corpuscular volume (MCV) on various days.

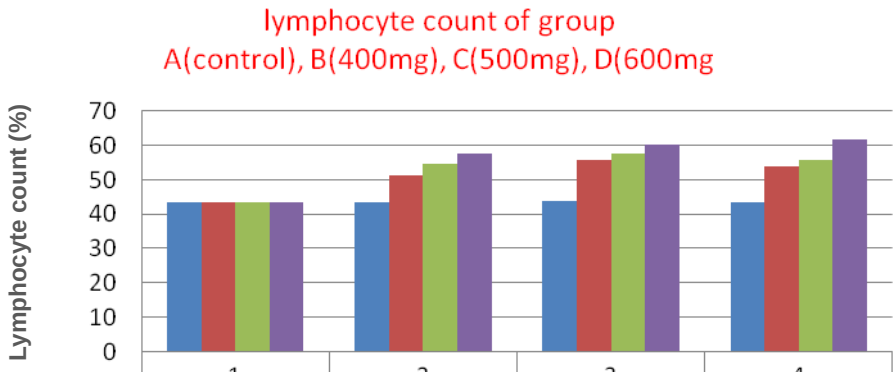
and MCV values. The erythropoietin effect of *A. vera* extract of hemopotetic cells of bone marrow have been reported by Iji et al. (2010). Similar findings have been reported by Hamman (2008), who has attributed the effect of increasing MCH to essential vitamins, e.g riboflavin, thiamine and folic acid; and essential and non essential amino acids in *A. vera*, that are required for synthesis of haemoglobin. Research has also been investigated that this increase in erythropoiesis is due to the presence of polysaccharides in *A. vera* leaf gel (Ni et al., 2004). It has been investigated that these hemo-poietic properties of *A. vera* is only due to the presence of thiamine that is responsible for formation of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase complex in Krebb's cycle (Oishi et al., 2002).

This enhancement in MCV also reveals aloe polysaccharide that can initiate the formation of cells. These results are contrary to Iji et al. (2010), who investigated no change occurs in MCV by chronic administration of *A. vera*. This also contradict the study of Taiwo et al. (2005) who had investigated that *A. vera* can cause tissue necrosis, heart, liver and kidney damage in fish In this study, it was found out that neutrophils count significantly decreases in rabbit blood treated with varying doses of *A. vera*. However, studies are being conducted to investigate the use of *A. vera* for treatment of cancer and blood disease associated with low white blood cell count, that is, leukaemia. It has been reported that veterinarians use *A. vera* for the treatment of cancer and feline leukaemia in animal patients. *In vitro* studies



	1	2	3	4
day'0'	45.94	45.46	45.5	45.38
day'7th'	46.36	33.64	26.78	27.48
day'14th'	46.1	31.62	26.36	25.24
day'21st'	45.76	29.18	24.52	23.38

Figure 3. Neutrohil count on various days.



	1	2	3	4
day'0'	43.52	43.66	43.86	43.68
day'7th'	43.64	51.54	55.7	54.16
day'14th'	43.56	54.86	57.6	56.02
day'21st'	43.68	57.84	60.32	61.74

Figure 4. Lymphocyte count on various days.

by British Cancer Research, UK has identified the presence of a compound in *A. vera*, named di 2-ethyl hexyl phthalate (DEHP), which stop the development of leukaemia cells in test tubes. Thus, the findings of these studies are very much important and the mechanisms involved in reducing neutrophil count should be further investigated.

A. vera has immunomodulatory effect that has been observed in the results of this study. Treatment of varying doses of *A. vera* extract to rabbits significantly increased ($P<0.05$) the total lymphocyte concentration. Lymphocytes are important blood cells that play a role in developing humoral and T cell immune response against foreign antigens, that is, bacterial, viral, etc. It is thought that a molecule in the aloe gel, known as acemannan, stimulates the body to produce disease-fighting white blood cells, particularly macrophages. Macrophages engulf and digest unwanted substances, such as bacteria and viruses.

It has been investigated that when any foreign protein

enter the body in response, some cytokines produced enhance cell mediated and antibody immune response (leung et al., 2004; Im et al., 2005). Research has investigated that *A. vera* has initiated phagocytic activity of recticuloendothellial system (Im et al., 2005). It has been investigated that it can enhance cellular as well as humoral immunity by proliferation of myeloid and erythrocyte colony forming cells, macrophage colony forming cells and pleuri-potent hemopoietic cells (Boudreau and Beland, 2006). It has been investigated that it significantly improves B lymphocyte that is responsible for formation of both immunoglobulin's M and G (Yates et al., 1992). It has also been investigated that *A. vera* can increase CD^4 and CD^8 receptor of T lymphocytes (Ghasem et al., 2011). Thus, increase in lymphocyte concentration might be due to the presence of low molecular weight proteins and glycoproteins that have mitogenic effect causing proliferation of immune cells, that is, lymphocytes.

Results of these studies also revealed that *A. vera* has dose dependent effects which is in accordance with Iji et al. (2010). It is observed that doses of 500 and 600 mg were slightly more beneficial than 400 mg at the level of $p<0.05$ (Figures 2 and 4).

Conclusion

The present research work investigated that the oral administration of *A. vera* extract showed significant increase in MCH and MCV level, therefore it was concluded that liquid extract of *A. vera* plant may be useful in the treatment of iron deficiency anaemia. It may be used as an immuno-stimulant. It is believed that further investigation will reveal more beneficial use of *A. vera*.

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Full Length Research Paper

Trichomonicidal activity of *Maytenus imbricata* (Celastraceae)

Carla R. Álvares Batista[1], Thaisa H. S. Fonseca[1], Vanessa G. Rodrigues[2], Grasiely F. de Sousa[2], Michelle O. Chacon[1], Sidney A. Vieira Filho[3], Lucienir P. Duarte[2] and Maria A. Gomes[1]*

[1]Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Belo Horizonte, MG, Brazil.
[2]Departamento de Química, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Belo Horizonte, MG, Brazil.
[3]Escola de Farmácia, Universidade Federal de Ouro Preto (UFOP), Rua Costa Sena,171, Ouro Preto, MG, Brazil.

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Trichomoniasis, most common non-viral sexually transmitted infection worldwide, is produced by protozoan *Trichomonas vaginalis*. The therapy of choice is metronidazole (MTZ). The drug has undesirable side effects, which may result in treatment discontinuation, leading to further spread of infection and emergence of resistant strains. This feature highlights the importance of studying new trichomonicidal substances. In this context, the importance of plants in relation to the research of new drugs is undeniable. The genus *Maytenus*, distributed throughout Brazil, is the largest of family Celastraceae, including about 80 recognized species with different biological activities. Therefore, the trichomonicidal activity of MTZ and extracts obtained from *Maytenus robusta* leaves and *Maytenus imbricata* roots on the JT strain of *T. vaginalis*, sensitive (JT) and resistant (JTR) to MTZ was investigated. Sample of *T. vaginalis* trophozoites were associated with extracts in 6 increasing concentrations ranging from 0.43 to 13.76 µg/ml. The solid that precipitated from the hexane/ethyl ether - 1:1 extract (SEH), obtained from *M. imbricata* roots proved to be active. This extract also impacted the viability of trophozoites of both strains, with IC_{50} value surprisingly low (1.09 µg/ml for JT and 1.57 µg/ml for JTR) signaling towards a promising candidate for phytotherapy or for isolation of substance with trichomonicidal activity.

Key words: *Maytenus* spp, metronidazole, *Trichomonas vaginalis*.

INTRODUCTION

Trichomoniasis, which etiologic agent is *Trichomonas vaginalis*, is the most common non-viral sexually transmitted infection worldwide (Johnston and Mabey, 2008). The parasite can be found throughout human genitourinary tract. Transmission occurs primarily through sexual intercourse (Bravo et al., 2010). Most infected men

*Corresponding author. E-mail: magomes@icb.ufmg.br. Tel: +55 31 3409 2846. Fax: +55 31 3409 2970.

are asymptomatic, but infection in women has been associated with problems in pregnancy, pelvic inflammatory disease and infertility (Maciel et al., 2004; Carli and Tasca, 2011). Importantly, there is a strong association of trichomoniasis with increased risk of acquisition and transmission of human immunodeficiency virus (Van der Pol et al., 2008). Despite trichomoniasis being a public health problem occurring worldwide, little attention has been given to its control (Burgess and Schwebke, 2004).

The choice treatment is metronidazole, a 5-nitroimidazole used to treat infections caused by protozoa and gram-negative anaerobic bacteria. The drug has some adverse side effects which may lead to poor compliance and treatment discontinuation (Cudmore et al., 2004). In addition, resistant cases of *G. lamblia* and *T. vaginalis* to metronidazole have been well documented over the past century (Upcroft and Upcroft, 1993; Voolmann and Boreham, 1993). Thus, the research of new substances with trichomonicidal activity aiming not only to overcome resistance but the side effects is a primary objective in the prevention of the disease (Miller and Clardy, 2009).

The importance of plants concerning the identification of bioactive substances is unquestionable (Li and Vederas, 2009). Studies with extracts or isolated compounds are frequent and can be used as herbal medicines or lead to models for the synthesis of more potent and selective similar substances. Recent studies showed isolation of constituents with antitrichomonal, amoebicidal and giardicidal activity from different species of *Maytenus* (Fahmy et al., 2014; Moo-Puc et al., 2014) and the literature reports the plant extracts and isolated secondary metabolites which can inhibit protozoan parasites, such as *Plasmodium*, *Trypanosoma*, *Leishmania*, *Trichomonas* and intestinal worms (Wink, 2012).

In this context, species of the family Celastraceae, mainly of genus *Maytenus*, stand out due to their various biological applications such as anti-inflammatory (Jorge et al., 2004), antibacterial (Lindsey et al., 2006; Santos et al., 2011), antiplasmodial, leishmanicidal (Alvarenga et al., 2008) and possible cytotoxic and antitumor activity (Morita et al., 2008). They are also used in folk medicine as antiseptic, anti-asthmatic, anti-tumor (Jeller et al., 2004; Nakagawa et al., 2004; Perestelo et al., 2010), antiviral (Hussein et al., 1999), in treatment of gastric problems (Baggio et al., 2007; Cipriani et al., 2009) and anti-inflammatory (Sosa et al., 2007).

Maytenus robusta has adapted very well to the conditions of the South and Central regions of Brazil and is commonly known as “espinheira santa” or “cancerosa”. This species is used in popular medicine to treat stomach ulcers (Niero et al., 2011). The antiulcerogenic, anti-nociceptive and acetylcholinesterase inhibitory activities of *M. robusta* were previously investigated (Niero et al., 2006; De Andrade et al., 2008; Sousa et al., 2012) but the trichomonicidal activity of extracts from the species of *Maytenus* has not been tested up to the present moment.

The main aim of this study is to further investigate the extracts activity of *M. robusta* and *M. imbricata* about *T. vaginalis* JT strains, sensitive and resistant to MTZ as an alternative therapy to trichomoniasis.

MATERIALS AND METHODS

Parasites and culture conditions

T. vaginalis trophozoites of JT strains were obtained from a symptomatic female patient. Metronidazole resistant JTR derived from JT was maintained in 8 µM metronidazole. Trophozoites were axenically grown in YI-S-32 medium (Diamond et al., 1995) at 37°C with thrice-weekly sub-cultures, assuring their use in the log phase of growth. To determine the best inoculum for association with drugs for 48 h, amounts of trophozoites ranging from 4×10^4 to 8×10^4 were evaluated.

Collection and identification of plant material

M. robusta branches were collected in 2010 in the Itacolomi State Park, Ouro Preto, Minas Gerais, Brazil. A voucher specimem (Exsicata number OUPR 25559) was deposited in the Herbarium Prof. José Badini of the Universidade Federal de Ouro Preto. *M. imbricata* roots were collected at “Morro Santana”, Ouro Preto, Minas Gerais. A voucher specimem (Exsicata number 27780) was deposited in the Herbarium at the Federal University of Viçosa.

Obtaining solids and crude extracts

After grinding in a hammer mill, 864.4 g of *M. robusta* leaves and 1.5 kg of *M. imbricata* roots were obtained. *M. robusta* extracts were obtained by cold extraction in exhaustive extractions with hexane (Hex), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH). During the evaporation of the solvents in a rotatory evaporator there occurred precipitation of solids, which were filtered from Hex (SEH, 4.51 g) and $CHCl_3$ (SCE, 1.78 g) extracts. After the complete solvent removal, Hex extract (HE, 31.43 g), $CHCl_3$ extract (CE, 18.49 g), EtOAc extract (EE, 5.74 g) and MeOH extract (ME, 130.27 g) were obtained. *M. imbricata* roots were submitted to exhaustive extraction in Soxhlet apparatus (heating) with solvents in increasing order of polarity: Hex-Et_2O (1:1), AcOEt and MeOH. After filtration and solvent removal by distillation under reduced pressure, the respective solids and extracts were obtained: SEH (solid that precipitated from the hexane/ethyl ether - 1:1 extract), FSEH (hexane/ethyl ether - 1:1 extract), SEAT (solid that precipitated from the ethyl acetate extract), FSEAT (ethyl acetate extract) and SEM (methanol extract). The amounts obtained from plant extracts and solids used in the screening, ranged from 5 to 12 mg.

Drug susceptibility assays

A screening-type preliminary test of all crude extracts was initially performed in order to select those with activity against both strains, sensitive (JT) and resistant (JTR). The crude extract (16.5 mg) was dissolved into 1.o ml dimethylsulfoxide (DMSO) and 100.0 ml aliquots of this solution were diluted in 5 ml culture medium, resulting in stock solution at concentration of 0.33 µg/ml. These solutions were filtered through nitrocellulose membrane 0.22 µm

and added to glass tubes containing trophozoites to achieve final concentration of 34 µg/ml. After 48 h of incubation at 37°C, each tube was evaluated using an inverted microscope (Nikon TS100F). Each assay was performed in triplicate and repeated at least twice using negative control (only trophozoites) and DMSO control (0.2%). Extracts showing activity were later associated to the parasite in increasing concentrations to determine the IC_{50}.

Determination of IC_{50}

T. vaginalis trophozoites were distributed in glass tubes (Pyrex® 13 × 100 mm) containing culture medium to a final volume of 6 ml. The extract previously active in the screening was added to the culture to obtain test concentrations ranging from 0.43 to 13.69 µg/ml. Trophozoites associated with drugs were grown at 37°C for 48 h. After incubation, the viability was qualitatively verified by observing the mobility and adhesion of trophozoites using inverted microscope and quantitatively for the determination of IC_{50} of JT and JTR strains. MTZ was dissolved in DMSO and an aliquot was added to 5 ml of culture. After filtration in sterilizing nitrocellulose membrane (0,22 µm), aliquots of all solutions were added separately in sterile glass tubes containing trophozoites to test in increasing concentrations ranging from 0.1 to 3.2 µM. These tubes were incubated at 37°C in intervals of 48 h to determine the IC_{50}. All tubes were quantified in hemocytometer. The experiments were performed in triplicate and repeated twice using negative control and DMSO control (0.2 %).

Statistical analysis

Simple linear regression analysis (Werkema and Aguiar, 1996) was used to estimate the relationship between the concentration and the percentage of inhibition. The dependent variable was the percentage of inhibition and the independent variable was the concentration. Data on the inhibition percentage for each concentration of test substance were compared by analysis of variance (ANOVA). For validation and subsequent use of the proposed regression equation was held residue analysis and outliers. The adjustment of the model was evaluated by the determination coefficient. From the relationship obtained, the IC_{50} was estimated beyond the confidence interval using the method of inverse regression. To determine the error rate the confidence intervals was used and estimated for the IC_{50} which was obtained using the technique of reverse regression, given a confidence level of 95 and 90%. The Minitab 15 was the statistical software used.

RESULTS

The trophozoites inoculum used was $6.0x10^4$/ml, since after 48 h of incubation at 37°C, the culture showed formation of monolayer of elongated trophozoites, good vitality and no precipitation (data not shown). In order to investigate for new active drugs against *T. vaginalis* resistant to MTZ treatment, we attempted to achieve *in vitro* resistant trophozoites to this drug for use during the tests with the extracts. The strain of *T. vaginalis* JT was maintained in axenic culture in the presence of increasing concentrations of metronidazole that started with 1 µM, reaching a final concentration of 8 µM. In the initial screening, MTZ and all extracts were evaluated as for the *in vitro* trichomonicidal activity. None of the extracts obtained from *M. robusta* leaves showed inhibitory effect up to concentration of 34 µg/ml. Among the extracts obtained from *M. imbricata* roots, only SEH proved to be active. Therefore, new studies on the activity in this extract were carried out in order to determine its IC_{50} after an incubation period of 48 h for both JT and JTR strains.

The IC_{50} value for SEH on the JT strain was 1.09 µg/ml, ranging from 0.59 to 2.1 µg/ml (confidence interval of 95%). The IC_{50} value for SEH on the JTR was 1.57 µg/ml, ranging from 0.93 to 2.65 µg/ml (confidence interval of 95%). Thus, it was observed that this extract also impacted the viability of trophozoites of JT strain showing high trichomonicidal activity, including MTZ resistant strain, JTR (Figure 1).

DISCUSSION

Reports of resistance to metronidazole, limited options of drugs for treatment and the occurrence of adverse effects strengthen the need for the development of new trichomonicidal drugs. In this context, natural products obtained from plants are a promising area for the discovery of innovative bioactive substances (Miller and Clardy, 2009). Species of the Celastraceae family stand out due to their various biological applications. The study of extracts and compounds isolated from leaves, roots and branches of some species of *Maytenus* presented antioxidant activity. *M. rigida* stands out in treating infections and inflammations, *M. truncata* showed analgesic and antiulcerogenic activity and *M. senegalensis* antiplasmodial activity (Fonseca et al., 2007; Estevam et al., 2009; Malebo et al., 2009).

A pentacyclic triterpene, 3,4-seco-friedelan-3-oic acid, isolated from leaves of *M*. *imbricata*, has inhibitory activity in the synthesis of adenosine triphospahte (ATP), which may be used in developing natural herbicides (Silva et al., 2007). The extracts (hexane/ethyl ether - 1:1, ethyl acetate and methanol) of *M. imbricata* roots and the isolated triterpenes 11α-hydroxylup-20(29)-en-3-one, tingenone and 6-oxo-tingenol, showed antimicrobial properties were submitted to in vitro assays concerning the bacteria *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and the fungus *Candida albicans* (Rodrigues et al., 2012). It was also observed that tingenone, compound isolated from the roots of M. imbricata, present antinociceptive activity when tested on mice (Veloso et al., 2014). Therefore, the trichomonicidal activity of extracts and solids obtained from *M. imbricata* roots and *M. robusta* leaves was herein investigated.

Initially, the best inoculum to obtain cultures at exponential growth phase after 48 h of incubation was evaluated. This phase is crucial for obtaining replicas of different concentrations of the test substances associated

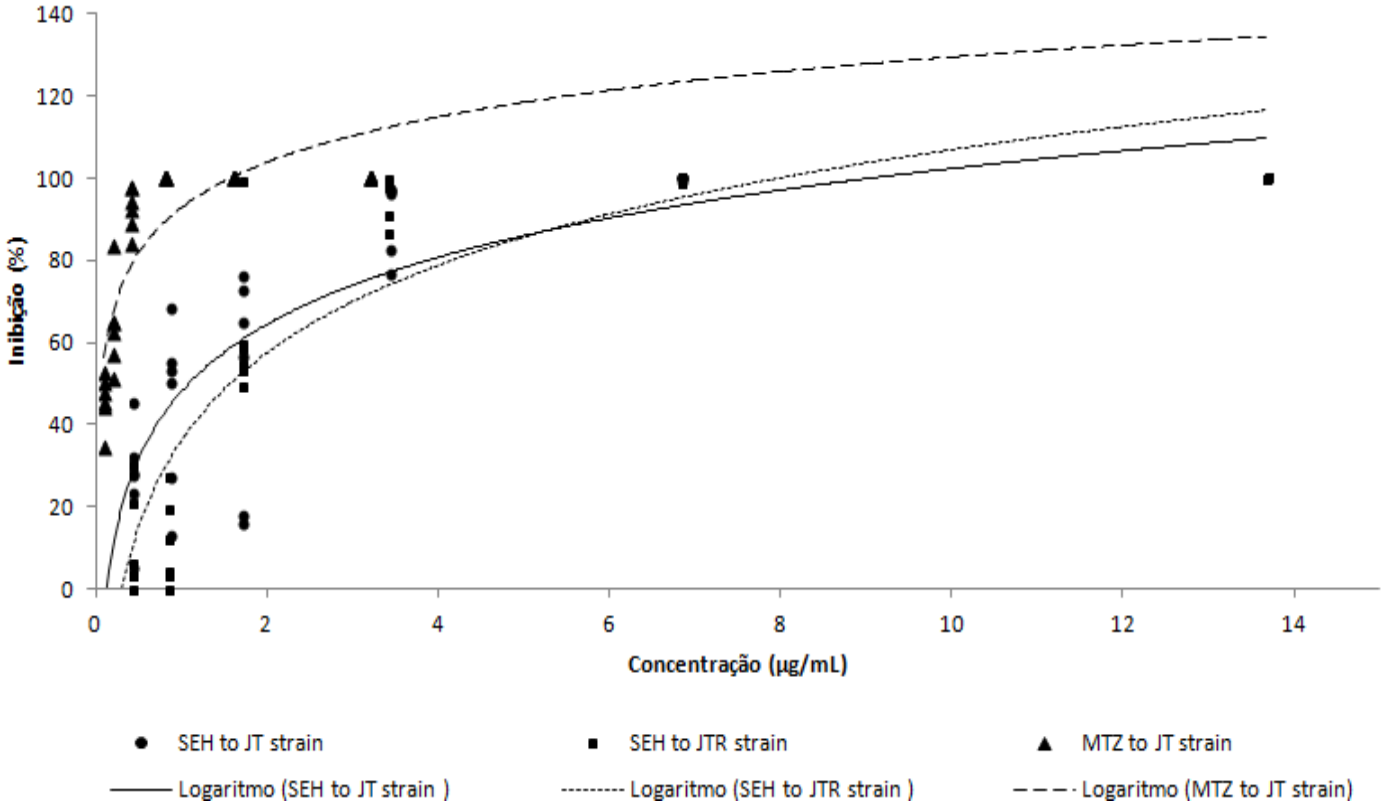

Figure 1. SHE and MTZ dose - response curves to JT and JTR strains.

with trophozoites. The inoculum of 6 × 10^4 trophozoites/ml was used because it showed the formation of monolayer of elongated trophozoites with good vitality and lack of precipitation, considered ideal for identifying the action of extracts on strains. In the screening performed with JT strain sensitive and resistant to metronidazole, only the solid of hexane/ethyl ether - 1:1 extract from *M. imbricata* roots (SEH) showed inhibitory effect. So, other studies were carried out to determine its IC_{50} value against sensitive and resistant *T. vaginalis* strains. The values found were evaluated by descriptive analysis techniques and scatter plots, which showed the trend line of the relationship between extract concentration and the growth inhibition percentage of *T. vaginalis* under study. These results were supported by the simple linear regression analysis method. There were no outliers and the residuals were independent and normally distributed, indicating the adequacy of the chosen model.

The results corroborate those of other studies, showing antimicrobial (Rodrigues et al., 2012), antimalarial, giardicidal (Mena-Rejón et al., 2007; Lhinhatrakool et al., 2011), trypanocidal (Godjiman et al., 1985), HIV, antitumoral (Ravelo et al., 2004) and cytotoxicity activities (Rodrigues et al., 2012), among others for genus *Maytenus* (Lindsey et al., 2006; de Andrade et al., 2007; Santos et al., 2011; Niero et al., 2011; de Araújo Júnior et al., 2013). The potential of natural products for the treatment of diseases is evident in traditional medicine. Compared to other protozoa, investigations of natural products with trichomonicidal activity are rarely found in literature (Frasson et al., 2012). The IC_{50} value of the SEH fraction for JT and JTR strains was surprisingly low, signaling a new candidate for trichomonicidal herbal medicines, as well as for isolation of substance with trichomonicidal activity.

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Collection and marketing of high value pharmaceutically and therapeutically important plants from the Swat District, Pakistan

Hassan Sher[1]* and Saeeda Yousaf[2]

[1]Centre of Botany and Biodiversity Conservation, University of Swat, Pakistan.
[2]Department of Environmental Sciences, University of Peshawar, Pakistan.

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In 2012, existing practices in trading high value minor crops (such as medicinal and aromatic plants) from the Swat district, Pakistan, were analyzed. The goals of the study were to: (1) examine the current status of the high value medicinal plant trade in Pakistan and (2) investigate the linkages in the market chain from collectors in the Swat district to final consumers. Within the Swat District, the focus was on the collection pattern of medicinal plants as an economic activity and its likely destinations in national or international markets. Herbal markets in major cities of Pakistan were surveyed for current market trends, source of material, import and export of herbal material, price patterns and market requirements. Collectors are mostly women and children of Middle Hill tribes and whose earnings are generally done for supplementary income through this activity. Pakistan exports of high value plants generate over US $10.5 million annually, with over 70% of the supply coming from Swat District. However, the market share of the high value plants from the Swat District due to the unreliable and often poor quality of the material supplied the length of the supply chain and poor marketing strategies. These problems could be addressed by improving the knowledge of those at the start of the supply chain, improving linkages among all steps in the chain and developing sustainable harvesting practices.

Key words: Trade pattern, medicinal plants, import, export, economic growth.

INTRODUCTION

The phrase “High value minor crops” refers to plants that contribute relatively little to a country’s agricultural output. They fall into two major groups: (1) herbs and spices and (2) medicinal and aromatic plants. Although individually small contributors to output, the global importance of medicinal and aromatic plants (MAPs) in total is evident

*Corresponding author. E-mail: hassan.botany@gmail.com.

from the fact that in 2006, global trade of MAPs reached $60 billion (Adhikari, 2001; Hamilton and Hamilton, 2006). Europe alone annually imports about US $1 billion in MAPs from Africa and Asia (Ghimire et al., 2004; Sher and Hussain, 2009). Such trade is expected to expand substantially by the year 2050 (Lang, 2008) because of the increasing popularity of herbal medicines and expanding use of herbs for flavoring a wide diversity of foods (Al-Quran, 2008; Khan et al., 2011).

MAPs have been cultivated or collected for centuries in many parts of Pakistan, including the Swat District. About 50 to 60% of the rural households in the Swat District collect MAPs (Sher and Hussain, 2009). In all cases, such activities constitute only a small part of the household's farming operation. However, because much of the farming in the mountains of the Swat District is near subsistence levels, collection of MAPs could become a more important source of supplementary income for these rural farmers (Sher and Hussain, 2009; Khan et al., 2011). Although the plants collected have a high value in the market place, the collectors usually have no knowledge of marketing (Shinwari and Qaiser, 2011). This study is a first step in examining a means of increasing the value and expanding the benefit of MAPs production for people in the Swat District. A consequent aim is to examine various steps in the supply chain, from collector/cultivator to final domestic market or exporter, suggesting possible ways in which the market value of both raw and processed MAPs could be improved.

Value-added activities currently carried out by the collectors and dealers in the area include product cleaning, drying, cutting and in some cases, washing of the plants or plant parts to be sold. However, these activities are usually conducted only to the extent needed to meet the minimum quality standard required by the local market (Annonymous , 2000) and without regard to modern management techniques. This suggests that collectors would benefit from training in how to better meet market needs, as well as means of adding value to their products which could impact demand and sale value.

There exist other opportunities to improve collector profit margins as well. This includes improving the collectors' skills and abilities to produce marketable surpluses, introduction of modern management practices, improved market linkages (including the implementation of secure trade contracts) and the ability to store product (for example, a species collected in the present day may bring about a higher price six months later). In this way, collectors may begin to market products more effectively, which could transfer a portion of the margin from middlemen to collectors. Final MAP markets are split primarily into three segments: Domestic industrial, export and local hakims (traditional healers). Collectors do not typically have linkages with these markets and therefore must rely on local traders to sell their products within the existing value chain. Collectors are therefore, often isolated from the final consumers of MAP products and do not always have a good understanding of market needs beyond the traders themselves. To enhance incomes from MAPs, collectors need a better understanding of the needs of individual markets. This is critically important and especially regarding quality specifications and their impact on post-harvest management and proper product handling.

The study for District Swat was therefore, initiated to identify constraints such as a fragmented information base, training and educational deficiencies, an uncoordinated approach to collection and marketing of MAP species and the need to clearly identify traders and markets. This paper concentrates on describing the levels of collections and marketing channels and prices at each level for the MAPs originating in Swat. It provides baseline analysis for a larger study sponsored by US Agency for International Development's Bureau - the International Food Policy Research Institute (USAID–IFPRI) under the Pakistan Strategic Support Program which is implemented in conjunction with the Planning Commission of Pakistan. This specific study was launched in Swat District to respond to the afore-mentioned challenges and to support a new framework for economic growth of the country.

METHODOLOGY

A market study of trade patterns for high value MAP species was conducted during summer 2012 in many parts of Pakistan with the particular reference to plants from the Swat District. The areas surveyed can be broadly classified as source and markets. In studying the Swat District as a source, we examined the collection pattern for MAPs as an economic activity, as well as likely MAPs destinations in National or International markets. Information on various aspects of collection, such as collection method, time and marketing of each species were gathered from local collectors and farmers through interviews and discussions. A total of 120 collectors and farmers were interviewed for this survey. In the study of markets, a survey was conducted on how and from whom plant material was obtained and when it was sold. Herbal markets in major cities of Pakistan (Karachi, Lahore and Peshawar) were surveyed to determine current market trends, sources of material, state of the import and export market for herbal material, price patterns and market requirements. Hakims (practitioners of Eastern medicine) as well as representatives of large herbal manufacturing industries were also interviewed in these areas for their raw material preferences and quality requirements. Respondents were asked about their annual income earned from the sale of targeted species and returns to the work invested. Interviews were conducted with a total of 120 collectors and farmers participants in the marketing chains.

RESULTS

Between them, the 120 collectors surveyed harvested 80 MAP species for commercial use. Most of the harvesting activity took place in the summer but three of the 80 species

(*Morchella esculenta*, *Viola serpense* and *Colchicum luteum*) were gathered from March to May. Most of the species were sold to wholesalers via middlemen. Of the 80 species, 24 were high value MAPs that are exported to both National and International markets (Table 1). There were 80 medicinal species collected, 24 of which (Table 1) had a high market value and were collected in large amounts for sale.

Marketing of MAPs in District Swat was conducted by some collectors and a few local shopkeepers. There were several regular collectors among the residents. They supplied domestic trading centers such as local bazaars and bazaars in Mingora, Peshawar, Islamabad, Lahore and Karachi, as well as some foreign trading centers.

Collection and trade of MAPs was highly uncoordinated. To obtain plant materials from District Swat, dealers from national markets send representatives to local dealers in Mingora and placed their orders. The local dealers then passed the orders on to their agents, i.e. local shopkeepers. In turn, these agents pass the orders to smaller shopkeepers and collectors. In some instances a local agent may employ a daily wage laborer to collect plants for Pakistan Rupees (Rs) 200 to 300 per day (approximately US $2 to 3), but this was unusual. The collectors are generally illiterate and usually do not negotiate a sales price when selling their plant materials to an agent. Agents sell the plant material to dealers in Mingora who then pass it up the supply chain which may include up to seven steps (Badakhshan to Karachi) in total.

Market channels

Figure 1 displays the marketing channels identified in the interviews of MAP market participants. It was noted that the recordings of the present study in Swat District show that Mingora is the main trade center for many high value plants. Mingora supplies considerable quantities of plants to various national trading centers of Pakistan including Peshawar, Islamabad, Lahore and Karachi and also abroad. Figure 1 shows the direct linkages in the market channels between the various herbal markets in Swat District and the national and international levels. Mingora receives material from various hilly areas, while the Lahore herbal market acts as the major center of trade in the country receiving imported material from abroad and from the countries sources.

In district Swat, the trade and collection of high value plants is highly uncoordinated and vary that is, from area to area and species to species. For obtaining plants from Swat, dealers from the national market send representatives to local dealers in Mingora to put up their demand. The local dealers pass the message to their agents that is, the local shopkeepers of the valley. These agents inform small shopkeepers and collectors. The collectors collectors gather the species for the local shopkeepers and agents. The collectors are illiterate and do not negotiate for the price of the plant materials that is, they gather sizeable quantities but do not receive reasonable returns. The dealers of Mingora in turn receive the material from the agents and in this way, the plant material gathered pass through three or four sections of the supply chain (Figure 1).

As shown in Table 1, at each step up the marketing chain, the purchase price increases. The greatest increase in both the national and international price (numbers are how many times higher the national and international price, respectively was a multiple of the purchase price from collectors in District Swat) was for *Aconitum heterophyllum* (10, 15), *Asparagus adscendens* (6.4, 10), *Adiantum capillus-veneris* (5, 12.5) and *Acorus calamus* (4.5, 10). As shown by the two sets of data for *Viola serpense*, the value of the plant material is determined by the plant parts constituting the sample sold that is, flowers of *V. serpense* sell for 500 Rs/kg, where a mixture of leaves and flowers has a retail price of only 200 Rs/kg. For these products, the international price is 3 to 4 times higher than the price received by collectors. The price of each species varies from year to year, at least partly due to changes in demand and supply. The MAPs species that brought collectors the highest price were *Morchella esculenta* (10,000 Rs/kg), *V. serpens* (flowers only, 500 Rs/kg), *Bunium persicum* (400 Rs/kg) but the MAPs that were the best income source for the collectors because of their combination of price and quantity were *Morchella esculenta, Diospyros lotus*, *Plantago major* and *V. serpense* (flowers only or flowers and leaves). The price for *Bunium persicum* was high but the amount sold was low.

It was generally observed in the interviews with local collectors, farmers and dealers that in the surveyed valley, the local agent (middleman) received handsome returns. The local shopkeepers or other persons acting as agents of the traders of the regional herbal markets are the major buyers from the local collectors. It is also observed that the local wholesalers control price information to the collectors which has enabled them to maintain high profits. The price differentials for other species can similarly be traced in Table 1. The export of crude herbal items of the MAP species to different countries is largely through individual and local exporters in JoddiaBazzar, Karachi and AkbariMandi, Lahore. The species concerned were being exported to Germany, Japan, France, Switzerland, Middle East, India and South Africa.

Extent of activity and income of collectors in Swat

The MAPs that sold best in 2011 to 2012 were *D. lotus* (90t), *B. amplexicaule* (12t), *P. major* (8t) and *V. serpens* (flowers and leaves mixed; 7t). The interviews with local

Table 1. High value plants of district Swat origin with their incremental values at different stages of the trade chain.

Botanical name	Part Sold	Qty. (Mound)	Price/Kg					Increase
			Purchase	Retail	Wholesale	National Market	Intern. Market	
Geranium wallichianum	Rh.	2000	190	250	300	500	1000	5.26
Aconitum heterophyllum	Rh.	1000	20	40	160	200	300	15.00
Acoruscalamus	Rh.	5000	20	25	50	90	200	12.50
Adiantumcapillus-veneris	W.P.	4000	20	30	50	100	250	10.00
Trachyspermumammi	Fr	5000	150	200	250	400	550	3.67
Asparagus adscendens	Rh.	2000	50	100	160	320	500	10.00
Berberis vulgaris	B.	4000	200	250	300	350	450	2.25
Bergenia ciliate	Rh.	3000	100	150	200	250	300	3.00
Colchicum luteum	C.	3000	100	190	230	350	500	5.00
Plantago major	Fr.	8000	350	400	450	500	800	2.29
Dioscoreadeltoidea	Rh.	3000	100	220	250	300	400	4.00
Diospyrus lotus	Fr.	90,000	50	90	150	200	300	6.00
Geranium wallichianum	Rh.	2000	50	70	90	150	300	6.00
Morchella esculenta	W.P.	5000	10,000	12,000	15,000	20,000	30,000	3.00
Paeonia emodi	Rh.	5000	50	70	100	150	250	5.00
Bistorta amplexicaule	Rh.	12,000	60	100	150	200	300	5.00
Pistacia integrimmea	Pod.	1000	200	350	400	600	1000	5.00
Podophyllumhexandrum	Rh.	2000	70	150	200	300	500	7.14
Polygonatummultiflorum	Rh.	5000	50	80	100	200	350	7.00
Buniumpersicum	Fr.	1000	400	430	450	600	1000	2.50
Valerianawallichii	Rh.	2,500	90	100	200	300	400	4.44
Viola serpens	Fl..	4000	500	550	600	1000	1500	3.00
Viola serpens	L+Fl	7000	200	250	300	500	800	4.00
Commiphoramukul	Fl.	5,000	100	150	220	350	650	6.50

farmers suggested a larger amount of these species could have been sold. The amount collectors in distrcit Swat earned from MAPs in 2011 to 2012 was about Rs 0.7 million. The money generated by selling these MAPs outside the district was about Rs 20 million. The amount of MAPs collected per household was 12 to 150 kg. Collection of MAPs was considered an important activity by about 315 households that is, 78.75% of the households surveyed. Such households collected an average of over 100 kg per year. Assuming that in total 35 days were involved in the harvesting, collection and portage of MAPs and that about 1.7 persons were involved per household, the total employment generated by the activity comes to about 15,500 person days per year. The present endeavor also estimated that the total beneficiaries and the income distribution from the MAPs trade in the study area were about 405 households. In terms of per household benefits, the village trader appeared as the largest beneficiary. However, trading was a full time activity for much of the year. Also the agent (middlemen) received a higher proportion of the income after marketing the produce directly.

Incremental value and price fluctuations of MAPs in the trade chain

The price of the high value MAPs increases at each step in the supply chain (Table 1). Partly, this increase is because of the incremental transportation and labour costs and partly because at each step which some profit must be generated to support the individuals involved. Another factor contributing to the price increase is that some plant material is lost at each level from such processing activities as cleaning, processing, grading and packing etc. This weight loss varies from species to species and the modes of processing for sale. Other factors affecting the increase in price from the collector to the final point of sale in Pakistan are the collectors' lack of knowledge concerning appropriate procedures for preparing the plant material in such a way that it maintains the maximum possible value as well as their general ignorance concerning prevailing prices and demand. Plants were often dried in humid, dark areas where they are subject to infestation by microrganisms and consumption by insects. From the interviews we

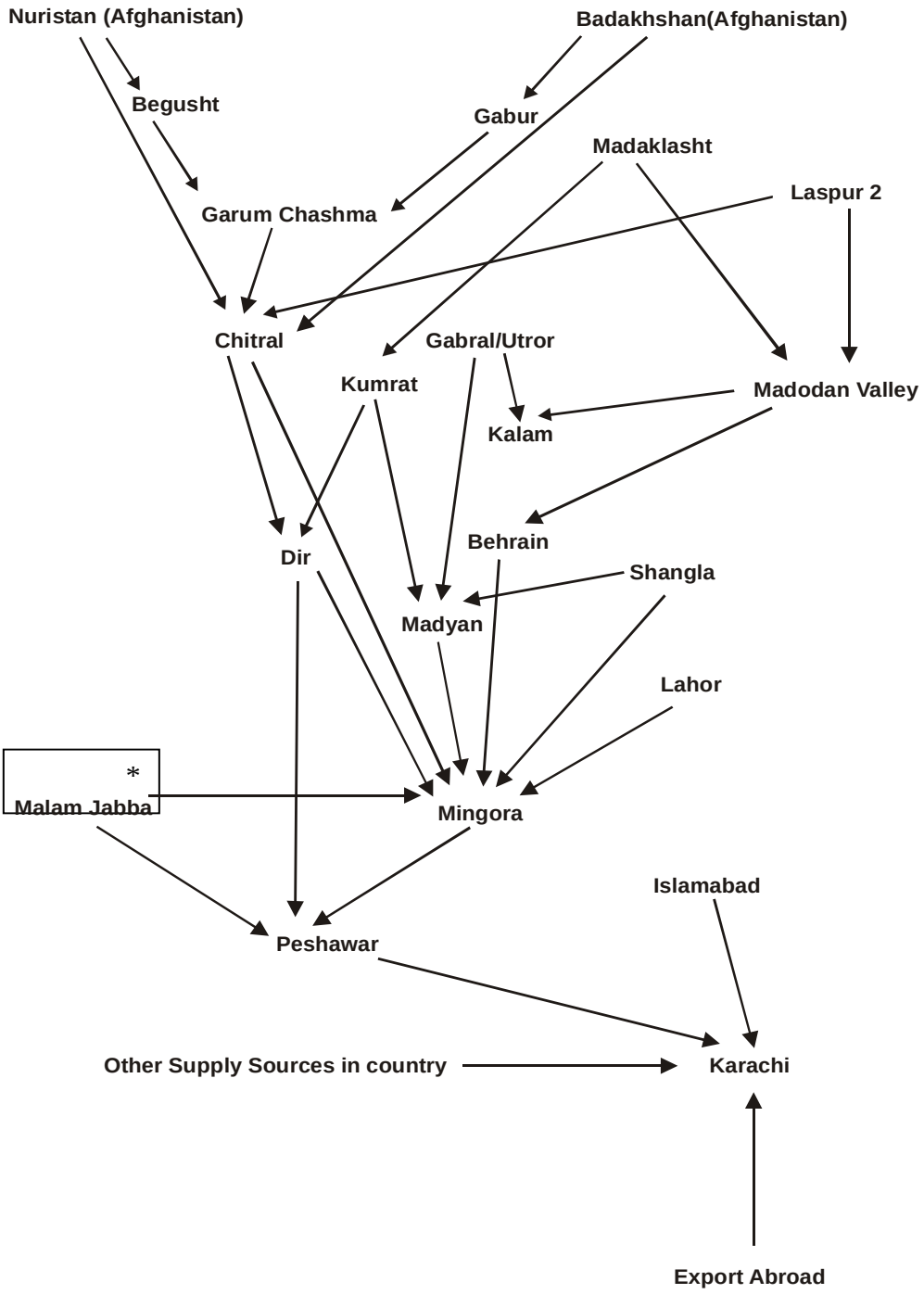

Figure 1. Supply routes from collection sites and other areas to local, national and international markets.

estimate that 90% of the collected MAPs material was being sold without any value added care being given to them. Another problem identified in the market chain interviews, one that will probably become more severe in future, is that many of the MAPs currently being collected in District Swat are now being produced and exported from other countries, including India and China. In 2011 to 2012, plant material from these areas was competitive in price and often of better quality. The difference in quality resulted in buyers showing less interest in plant

material from Swat and/or offering lower prices for it.

DISCUSSION

The geographical location of District Swat provides an ideal physical environment for the growth and nourishment of many high value medicinal and aromatic plants. These crops can make a contribution to economic development of the area, in particular, and the country in general. The study on the trade pattern of MAPs in District Swat revealed that various MAPs are sold in large quantities both in national and international markets, indicating their importance as a source of income for the inhabitants of the mountain communities. The study generally observed that District Swat is the collection and trading center for many high value minor crops including MAPs, having a well-established market which supplies various trading centers in Pakistan and abroad. Rashid et al. (2011) also reported that the majority of marketable medicinal plants are collected from Northern areas of Pakistan including District Swat.

The MAPs trade in Pakistan, including District Swat, operates with minimal state intervention and documentation. This is also true for many European and Asian countries. Because of this poor documentation (Hamilton and Hamilton, 2006), decision makers are usually unaware of the significance of the trade in MAPs and of the negative impacts that unsustainable harvesting of these plants may have on the environment and on people's welfare. A vibrant private market is a desirable outcome as long as none of the participants is able to exploit those at a lower level in the marketing chain and as long as the natural environment is not deteriorated by the over-harvesting of the collectors. These concerns can justify educational efforts, collective marketing activities by collectors and regulations of harvesting as government policies.

Pakistan as a whole exports medicinal plants worth over US $10.5 million (Shinwari and Gilani, 2003). The herbal markets of Karachi (Jodia Bazar) and Lahore (Akbari Mandi) are the primary centers for this export trade. The destination of the exports includes many parts of the world, including Europe, North America and the Middle East. The Swat District's share of this market is about 70% (Shinwari, 2010). Generally, plants which are either indigenous to Pakistan or found in abundant quantities are exported abroad. Pakistan not only exports MAPs but also imports over US $130 million annual of herbal material from other countries, primarily from India, Thailand, China, Indonesia, Tanzania, Iran, and Afghanistan (FAO, 1995; Annonymous, 1999). The herbal market of Lahore (AkbariMandi) is a primary hub for this trade, receiving large quantities of imported herbs from India and recently, from China. The increasing import market is largely attributed to a combination of increasing market demand and the inferior quality of indigenous plant material. Collection of plant material in Swat District appears to be on a "first-come-first-served" basis. There is no management structure involved. This is a cause for concern because as Saganuwan (2010) reported, the lack of any check, even on the collection of rare or threatened species, endangers this important source of income. Similarly, Larsen and Smith (2004) who examined stakeholder perspectives on commercial medicinal plant collection in Nepal noticed that most of the commercially important MAPs are becoming rare and sparse, due to the combination of unregulated collection and overgrazing.

In the interviews, very few of the collectors knew about the existence of a major market for MAPs. Olsen and Larsen (2003) also found that the trade and collection of plant materials is mostly handled by unskilled persons. As a result, valuable medicinal plants are lose value on their way to their final market while over-extraction, destructive harvesting techniques and habitat loss are severe threats to the long term viability of this important source of income (Mardis, 2008). This suggests that there is a dire need to develop practical and economically sound strategies for the efficient utilization of Pakistan's natural resources such as the MAPs in order to improve the condition of marginalized communities, a process that should eventually lead the country towards economic stability and peace.

The livelihood of many people in district Swat is based in part on the collection and trade of MAPs. Collection from the wild is so far the only source of MAPs in the region. Many of the MAPs collected in Swat find their way to the herbal markets of large cities and from there to the international markets. There is need for a program that focuses on assisting collectors and local dealers in providing consistently high quality, well preserved material to purchasers, combined with a sharing of information as to why this is important. This program should also address the issue of sustainable harvesting and the possibility of cultivating MAPs that are currently collected from the wild. This paper provides a baseline analysis of the marketing channels and price differences that underscore these needs. There are, as is to be expected, price increases as the product moves through the supply chain but these are exacerbated by the lack of knowledge among collectors and local dealers concerning the demand of the plants they are asked to collect. MAPs markets split primarily into three segments: Domestic industrial, export and local hakims (traditional healers). Collectors usually have no direct knowledge of the markets for their products and therefore, must rely on local traders to sell their products within the existing value chain. To enhance their income from MAPs, collectors and local dealers need a better understanding of the markets they are supplying. This means understanding quality expectations and their impact on post-harvest

management and handling as well as the extent and seasonality of the demand. There exist other opportunities to increase the value of MAPs trade for collectors and local dealers. These include improving the collectors' skills and abilities to produce marketable surpluses, improved market linkages (including the implementation of secure trade contracts) and developing the ability to store product until it can obtain a higher price. In this way, collectors may begin to market products more effectively which could transfer a portion of the margin from middlemen to collectors. Household incomes can also be enhanced if communities acquire a better understanding of the economic importance of high-demand natural plant resources, the interests and respective roles of the key stakeholders involved (including pharmaceutical and exporter buyers). This must include understanding of the importance of sustainable collection and cultivation practices to the long term livelihood of the community.

Moreover, the household incomes can be enhanced if communities acquire a better understanding of the economic importance of this high-demand natural plant resources, the interests and respective roles of the key stakeholders involved (including pharmaceutical and exporter buyers).The purpose of this study was to examine the current status of the high value medicinal plant trade in Pakistan and investigate the linkages in the market chain from collectors in District Swat to final consumers. During the course of the study, a range of possible interventions were identified, such as training of collectors/farmers, improving marketing linkages and improving understanding of the needs of the national and international markets, which would enable MAPs to become the prime 'engines of growth' for the local economy. Indeed, simply conducting the study helped inform local collectors/farmers about the value-added products derived from MAPs. It is reasonable to expect that improving market linkages between producers and buyers will result in increased economic benefit for local collectors, farmers and dealers, enabling their communities to become hubs of significant economic activities with a multi-dimensional impact on the economic development of district Swat. Such developments are also essential if Pakistan is to maintain or improve its position as an international supplier of MAPs.

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