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A single blind randomised placebo controlled clinical trial of a classical Ayurvedic formulation Ashokarista in the treatment of menorrhagia and dysmenorrhoea

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SUMMARY

A well known Ayurvedic formulation Ashokarista, used for menstrual disorders has been studied in a single blind randomised placebo controlled clinical trial for the treatment of menorrhagia and dysmenorrhoea. Dysmenorrhoea and menorrhagia patients who were taking Ashokarista (20 ml twice daily) for 10 menstrual cycles had an increase in haemoglobin level. Menorrhagia treated group has shown to reduce the erythrocyte sedimentation rate level that has been increased in the menorrhagia control group. The platelet count, total count and differential count were observed unchanged in the study. The Ashokarista did not affect the SGPT and SGOT level, which signify its lack of toxicity in hepatic function. The treated menorrhagic patients showed an increase in serum albumin content and decrease in blood clotting time, whereas the serum protein content was observed unchanged. There was a significant increase in both serum cholesterol and triglyceride level, which usually associated with the use of oral contraceptives. No major side effects were observed by the clinicians during the study.

Key words: Ashokarista; Menorrhagia; Dysmenorrhoea; *Saraca asoca*; Clinical trial; Ayurvedic

INTRODUCTION

Dysmenorrhoea or painful menstruations are an abdominal pain stemming from uterine cramps during a menstrual period and a common gynaecologic complaint (Lewis and Elvin-Lewis, 2003). It has been reported that dysmenorrhoea affects 40-95% of menstruating women (Jones, 2004). Excessive prostaglandin production and the consequent prostaglandin induced myometrial hyperactivity are the basis for painful menstruation (Ylikokala, 1978). Menorrhagia is manifested by excessive bleeding (> 80 ml/cycle) and is due to impaired progesterone production because of failure of the corpus luteum to develop. It is estimated that approximately 30% of women complain of menorrhagia (Bowman, 1980; Oehler and Rees, 2003). Thus dysmenorrhoea and menorrhagia are an important health care problem for women. Common treatment for dysmenorrhoea is NSAIDs or oral contraceptive pills which both work by reducing myometrial activity. The combined oral contraceptive pills, prostaglandin

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inhibitors and tranexamic acid are first line of therapy for menorrhagia. The efficacy of conventional treatments is considerable; however the failure rate is still often 20 - 25%. Many consumers are now seeking alternatives to conventional medicines (Wilson and Murphy, 2001; Tapanainen, 2004).

Several plants have shown as a source of human hormone synthesis and have been used in menstrual disorders (Lewis and Elvin-Lewis, 2003). Ayurvedic medical treatment involves with complex combinations of several herbs. In some cases, the medicinal value of the preparation may be due entirely to the combination of constituents and cannot be reproduced by one or two so called active principles alone. This medical benefit was observed because of the mix of constituents that have synergistic effects and act upon different molecular targets (Williamson, 2001; Wagner, 2004). Ashokarista is a well known Ayurvedic medicine consists of 13 medicinal plants (Table 1), traditionally used in India, Bangladesh and Sri Lanka for the treatment of menorrhagia and dysmenorrhoea,. It controls the excessive bleeding and cures the pain in uterine area (Middelkoop and Labadie, 1985; Anonymus, 1992). We have previously reported the neuropharmacological activity of Ashokarista (Akhtar *et al*., 2001). The aim of the present study was to investigate the clinical effectiveness of Ashokarista for the treatment of dysmenorrhoea and menorrhagia.

MATERIALS AND METHODS

Preparation of the drug

The Ashokarista (ASK) was purchased from the Shakti Aushadhalaya a licensed Ayurvedic manufacturer of Bangladesh and was prepared according to the Bangladesh National Formulary of Ayurvedic Medicine (Anonymus, 1992). The test medication was manufactured in liquid form according to Good Manufacturing Practice (GMP). The plants were authenticated by the Bangladesh National Herbarium, Dhaka, where voucher specimens were deposited and in-process and quality control for the preparation was strictly controlled and monitored by the experienced officials of Shakti Aushadhalaya.

Study design

A single blind placebo controlled randomized clinical trial of Ashokarista was undertaken in 12 patients of dysmenorrhoea, 12 patients of menorrhagia

Table 1. Composition of the Ayurvedic formulation Ashokarista

Name of the components	Family	Parts used	Amount
Saraca asoca (Roxb.) De Wilde.	Fabaceae	Bark	10 kg
Woodfordia fruticosa (L.) Kurz.	Lythraceae	Flower	1.6 kg
Nigella sativa L.	Ranunculaceae	Seed	100 g
Cyperus rotundus L.	Cyperaceae	Rhizome	100 g
Zingiber officinale Roscoe	Zingiberaceae	Rhizome	100 g
Berberis aristata DC.	Berberidaceae	Wood	100 g
Nymphaea lotus L.	Nymphaeaceae	Root/Flower	100 g
Terminalia chebula Retz.	Combretaceae	Fruit rind	100 g
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	Fruit rind	100 g
Phyllanthus emblica L.	Euphorbiaceae	Fruit rind	100 g
Mangifera indica L.	Anacardiaceae	Seed kernel	100 g
Adhatoda vasica Nees	Acanthaceae	Bark	100 g
Pterocarpus santalinus L.f.	Fabaceae	Wood	100 g
Molasses	-	-	20 kg
Water	-	-	100 l

and 6 normal healthy female volunteers to represent the control group. The patients were taken from the heath workers of Gonoshashthaya Medical Centre, Dhamrai, Dhaka. They were all informed of the study and were fully willing to participate in this study. Written informed consent was taken from the patients. The ethical clearance was obtained from the Gonoshashthaya Hospital, Savar, Dhaka.

During the clinical study the patients were randomized (n = 6) in the following five groups, dysmenorrhoea control (DC), dysmenorrhoea treated with Ashokarista (DT), menorrhagia control (MC), menorrhagia treated with Ashokarista (MT), and control group (CG). Ashokarista was given in two doses 20 ml each, taken daily after meal for ten menstrual cycles to the treated groups. Rest of the groups was given placebo in a similar bottle and could not be distinguished one from another.

Inclusion criteria were unmarried, aged between 18 - 24 years, suffered from either dysmenorrhoea or menorrhagia. Exclusion criteria were chronic or major diseases, medicated with any other drugs, pregnancy/lactation, smoking. The patients were asked to record any symptoms and other indicators of ill health as contained in their prescribed form. Patients were assessed clinically during their monthly visits to the clinic.

Biochemical procedure

Blood was taken from each patient pre trial and at the end of the trial. Blood was immediately transferred into EDTA tubes and blood smears were prepared at the time of blood collection. The blood was tested for hemoglobin (Hb), erythrocyte sedimentation rate (ESR), blood clotting time, platelet count, and total count and differential count (WBC, lymphocyte, monocyte, eosinophil, neutrophil). Hematologic evaluation was performed within 24 h of sample collection. Hb concentration was determined by a cyanmethemoglobin method following centrifugation of the lysate. WBC counts were performed using the modified Natt-Herrick's technique. Liver enzymes SGOT, SGPT, serum creatinine and blood urea nitrogen (BUN) were measured by colorimetric method. Nutritional and metabolic status was assessed by the concentrations of serum cholesterol, triglyceride, total protein, albumin and were measured by colorimetric method at the Gonoshashthaya Medical Centre, Dhamrai, Dhaka.

Statistical analysis

Statistical analyses were performed by SPSS 7.5 for Windows. Independent samples *t*-test and paired samples t-test were done as the test of significance where applicable. Values were considered significantly different if $P < 0.05$. Data were expressed as mean ± S.E.M.

RESULTS

The MC group patients have showed a significant decrease ($P < 0.01$) in their Hb level compared to pre treatment. The Hb level significantly improved in the Ashokarista treated group (MT) compared with the pre treatment group ($P < 0.05$). It also significantly increased when compared with the MC ($P < 0.05$) and CG ($P < 0.05$). The ESR found to be increased in the MC group compared to their pre treatment ($P < 0.05$) and post treatment CG ($P < 0.05$). The MT group had a reduced ESR than MC group ($P < 0.05$). The blood clotting time in post treatment MT group was found significantly lowered than pre treatment MT group ($P < 0.01$), and post treatment MC ($P < 0.05$), CG ($P < 0.01$) group. There was no change observed on the platelet count (Table 2). The total count and differential count of blood (WBC, lymphocyte, monocyte, eosinophil, neutrophil) had no significant changes between their pre treatment, post treatment or control group (Table 3).

In the liver function evaluated as SGOT and SGPT showed no significant changes between the groups (Table 4). The renal function evaluated as

Table 2. Effect of Ashokarista on Hb, ESR, blood clotting time and platelet count

Group (n = 6)	Hb (g/dl)		ESR (mm/h)		Blood clotting time (min)		Platelet count ($\times 10^3$/mm^3)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CG	10.11 ± 0.45	9.50 ± 0.60	10.16 ± 2.27	10.87 ± 1.58	9.0 ± 0.25	9.66 ± 0.56	418.16 ± 17.69	420.16 ± 18.75
DC	9.65 ± 0.20	9.76 ± 0.33	16.50 ± 2.81	15.33 ± 2.47	8.71 ± 1.08	8.50 ± 0.99	376.66 ± 19.14	392.33 ± 19.28
DT	10.46 ± 0.42	10.17 ± 0.31	8.33 ± 1.68	1017 ± 2.60	7.33 ± 1.35	7.00 ± 1.09	410.33 ± 18.74	405.83 ± 16.43
MC	9.88 ± 0.30	8.50 ± 0.22[d]	10.00 ± 4.42	17.08 ± 3.41[b,e]	7.55 ± 1.03	8.66 ± 1.02	399.16 ± 10.19	386.33 ± 27.79
MT	10.26 ± 0.55	12.50 ± 0.92[b,c,e]	13.33 ± 5.76	10.50 ± 2.63[c]	8.66 ± 1.22	5.83 ± 0.70[a,c,d]	386.66 ± 19.15	427.16 ± 7.97

[a]$P < 0.01$ post treatment compared with control, [b]$P < 0.05$ post treatment compared with control, [c]$P < 0.05$ MC compared with MT, [d]$P < 0.01$ pre treatment compared with post treatment, [e]$P < 0.05$ pre treatment compared with post treatment.

Table 3. Effect of Ashokarista on WBC, lymphocyte, monocyte, eosinophil and neutrophil count

Group (n = 6)	WBC (Œ10^3/mm^3)		Lymphocyte (%)		Monocyte (%)		Eosinophil (%)		Neutrophil (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CG	5.64 ± 1.26	5.95 ± 0.57	30.66 ± 1.83	36.67 ± 2.91	1.50 ± 0.34	2.08 ± 0.61	3.16 ± 0.75	3.33 ± 0.56	69.00 ± 1.61	67.83 ± 3.26
DC	6.49 ± 1.14	6.52 ± 0.94	34.16 ± 2.19	36.17 ± 1.74	3.83 ± 1.19	3.17 ± 0.79	2.00 ± 0.68	2.00 ± 0.63	64.66 ± 1.60	65.66 ± 2.36
DT	7.21 ± 1.13	6.86 ± 0.73	35.66 ± 2.02	36.17 ± 1.78	1.83 ± 0.16	2.17 ± 0.61	1.66 ± 0.49	1.67 ± 0.42	61.16 ± 2.72	60.67 ± 2.28
MC	7.00 ± 0.70	7.31 ± 0.96	34.16 ± 3.59	37.33 ± 3.23	2.00 ± 0.52	2.00 ± 0.36	2.33 ± 0.62	2.00 ± 0.52	62.66 ± 4.1	63.17 ± 4.69
MT	5.15 ± 5.11	5.85 ± 0.47	38.66 ± 2.17	38.33 ± 1.93	2.66 ± 0.76	2.33 ± 0.71	2.00 ± 0.71	2.33 ± 0.62	59.33 ± 1.92	61.00 ± 3.53

Table 4. Effect of Ashokarista on liver function

Group (n = 6)	SGOT (IU/l)		SGPT (IU/l)	
	Pre	Post	Pre	Post
CG	40.83 ± 4.90	36.00 ± 4.07	41.66 ± 8.36	40.17 ± 8.03
DC	36.17 ± 4.58	35.83 ± 5.47	41.16 ± 6.88	40.00 ± 7.92
DT	39.00 ± 5.17	38.17 ± 4.78	35.16 ± 3.92	32.00 ± 5.92
MC	35.00 ± 2.58	33.50 ± 2.58	33.83 ± 2.33	32.00 ± 1.98
MT	40.83 ± 4.90	36.00 ± 4.07	41.66 ± 8.36	40.17 ± 8.03

Table 5. Effect of Ashokarista on renal function

Group (n = 6)	Creatinine (mg/dl)		BUN (mg/dl)	
	Pre	Post	Pre	Post
CG	0.66 ± 0.06	0.62 ± 0.06	16.56 ± 2.24	18.60 ± 1.97
DC	0.87 ± 0.06	0.82 ± 0.05[a]	13.75 ± 0.93	15.38 ± 1.55
DT	0.64 ± 0.08	0.75 ± 0.08[a,d]	17.50 ± 0.79	26.58 ± 2.75[b,c]
MC	0.80 ± 0.10	0.73 ± 0.12[b]	18.75 ± 3.84	25.52 ± 4.74
MT	0.89 ± 0.09	0.88 ± 0.04[a]	15.62 ± 2.14	22.39 ± 2.09

[a]$P < 0.01$ post treatment compared with control, [b]$P < 0.05$ post treatment compared with control, [c]$P < 0.01$ DC compared with DT, [d]$P < 0.05$ pre treatment compared with post treatment.

serum creatinine showed a significant ($P < 0.01$) increase in the post treatment group of the dysmenorrhoea (DC and DT) and menorrhagia group (MC and MT) compared to the CG (Table 5). There was an increasing trend in blood urea nitrogen in the post treatment groups of the study than the pre treatment groups. This was statistically significant only in the DT group ($P < 0.01$).

The post serum cholesterol level was observed to significantly increase both in the DT and MT

Table 6. Effect of Ashokarista on nutritional and metabolic function

Group (n = 6)	Cholesterol (mg/dl)		Triglyceride (mg/dl)		Protein (g/dl)		Albumin (g/dl)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CG	128.04 ± 9.25	135.50 ± 6.51	82.49 ± 19.61	87.5 ± 17.26	7.84 ± 0.31	6.43 ± 0.48	4.88 ± 0.19	4.73 ± 0.16
DC	110.97 ± 10.47	116.66 ± 4.59[a]	88.88 ± 17.09	91.17 ± 15.86	7.94 ± 0.30	7.17 ± 0.23	4.18 ± 0.24	3.88 ± 0.13
DT	103.87 ± 15.63	170.33 ± 11.16[a,c,e]	73.88 ± 17.68	140.0 ± 16.93[b,f]	7.42 ± 0.34	7.50 ± 0.23	4.07 ± 0.16	3.80 ± 0.12
MC	130.89 ± 13.03	135.00 ± 9.48	85.55 ± 21.18	87.58 ± 14.18	8.20 ± 01.20	7.16 ± 0.27	4.24 ± 0.15	4.01 ± 0.41
MT	94.33 ± 17.19	158.33 ± 4.94[a,d,f]	57.22 ± 19.06	108.33 ± 11.81[e]	7.33 ± 0.23	8.01 ± 0.36	3.88 ± 0.17	4.89 ± 0.21[b,e]

[a]$P < 0.01$ post treatment compared with control, [b]$P < 0.05$ post treatment compared with control, [c]$P < 0.01$ DC compared with DT, [d]$P < 0.05$ MC compared with MT, [e]$P < 0.01$ pre treatment compared with post treatment, [f]$P < 0.05$ pre treatment compared with post treatment.

group compared to the CG ($P < 0.01$), and also with their pre treatment and respective control group ($P < 0.01$ - 0.05). The triglyceride level was observed higher in the DT ($P < 0.05$) and MT ($P < 0.01$) compared to their pre treatment and CG group. The protein level was statistically unchanged in the experiment. The albumin level was significantly higher in the post treatment group of MT compared to pre treatment MT ($P < 0.01$) and post treatment CG ($P < 0.05$) group (Table 6).

DISCUSSION

Dysmenorrhoea and menorrhagia patients who were taking Ashokarista for 10 menstrual cycles had an increase in haemoglobin level compared to control and pre treatment group, thus it has a beneficial effect in anaemia to the patients with menstrual disorders. MT group have shown to reduce the ESR level that has been increased in the MC group, suggesting Ashokarista may have some effect in the prevention of infection process. But the platelet count, total count and differential count was observed unchanged in the study. The Ashokarista did not affect the SGPT and SGOT levels indicate that Ashokarista might not exert any toxic effect on the hepatic system. The increased serum creatinine and blood urea nitrogen level in this study was considered not related to renal function, as the level was unchanged compared to their pre treatment group. Excessive tissue protein catabolism may be responsible here for this activity and similar results were also reported during estrogen administration (Sonnnenwirth, 1982). There was a significant increase in both serum cholesterol and triglyceride level. This hyperlipidaemia is not new and usually associated with the use of oral contraceptives (Wynn, 1979). No major side effects were observed by the clinicians during the study.

It has been reported that the main ingredient of Ashokarista, *Saraca asoca* inhibited the PGH_2 synthetase which has been responsible for the menstrual problems, and may explain the mode of action in menorrhagia (Middelkoop and Labadie, 1985). In India, a decoction of the dried bark of *Saraca indica* (Fabaceae) is taken for various ailments of uterus (Lewis and Elvin-Lewis, 2003). This genus *Saraca* found to contain anthocyanins and phytosterols which have potential benefit to the uterine problems. Another major plant of the formulation *Woodfordia fruticosa* also reported to contain anthocyanins (Lakshmi and Chauhan, 1976; Behari *et. al*., 1977; Banerjee and De, 2001). There was an evolutionary kinship with anthocyanins and steroid hormones, which has a common ancestor with enzymes important in their synthesis and regulation (Baker, 1995). The volatile oil of *Nigella sativa* seed proved to have a relaxant effect on uterine smooth muscle. Thymoquinone, the main active constituent of the volatile oil of *Nigella sativa* seed reduced spontaneous contraction of isolated rat uterus (Taha, 2004). *Cyperus rotundus* is an ingredient of an herbal composition for

treating gynaecological disorders (Clavey, 2004) and sesquiterpene of *Cyperus rotundus* showed inhibition of prostaglandin biosynthesis (Kiuchi *et al*., 1983). It has been found that á-cyperone was one of effective components of *Cyperus rotundus* rhizomes for therapy of menorrhagia (Wen *et al*., 2003). The crude extracts of fresh rhizomes of *Zingiber officinale* showed potent inhibitory activity against prostaglandinbiosynthesizing enzyme (cyclo-oxygenase and PG synthetase) in an in vitro bioassay (Kiuchi *et al*., 1992); Venkateshwarlu, 1997). Thus it was considered that the major herbs and some minor herbs of the formulation Ashokarista have the beneficial effects for the treatment of menorrhagia and dysmenorrhoea. Although most of the herbal drugs of the formulation and the chemical constituents and their pharmacology is known, but recent research has shown that in some cases fixed combinations of plant extracts show greater than expected medicinal benefit. (Narimanian *et al*., 2005). Synergistic effects of mix constituents of Ashokarista and their action upon different molecular targets may play a role in the beneficial effect of Ashokarista and justify the uses of various individual herbs. Thus the present study provided a basis for Ashokarista for its treatment in menorrhagia and dysmenorrhoea.

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