Therapeutic properties of processed aqueous extract of *Asteracantha longifolia* in the human

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Treatment of disease with herbal extracts is common in both Ayurveda and herbal systems of medicine. We have identified that therapeutic activity of *Asteracantha longifolia* is associated with the dialysable portion of the extract. The extract was partially purified by a method using alcohol precipitation. This partially purified *A. longifolia* extract (PALE) was studied for its therapeutic activity in the human when administered orally. Oral administration of 0.1 ml of PALE (equivalent to 5 ml of original extract) was found to increase haemoglobin and lymphocytes and reduce neutrophils in the blood. The effect was not short term as this profile persisted for as long as 6–8 months, indicating that PALE is an excellent herbal medicament for improving the quality of blood in the human.

Keywords: *A. longifolia*, bone marrow, herbal restorative, lymphocytes.

For evolution nature has provided human with abundant energy from sunlight and innumerable materials in the form of water, air and plants to take care of his bodily needs for survival and procreation. In order to enjoy life one needs to have good health defined as 'a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity[1,2]. It is here that nature has put a shadow of sickness (discomfort) in the human for the privilege of 'free will' bestowed. Nature has offered humanity an abundance of hidden secrets to cure sickness. As evolution progressed, humans started understanding these secrets by observation over centuries and used material substances from plants for relief from discomfort. About 5000 years ago this knowledge was systematized in India and this science of curing sickness is called by the name Ayurveda, still practised in India[3]. As materials science, especially chemistry, progressed in Europe extracts of plant materials used for relief of discomforts were chosen and the therapeutic property of these plant extracts was identified with individual chemical entity present in the plant[4]. Such chemical entities could be synthesized and were used to treat sickness and this order of medicine is called Allopathic system of medicine. With the development of synthetic organic chemistry medicines could be prepared in bulk to take care of the need of increased population and hence became popular all over the world[5].

Allopathy and Ayurveda systems are used routinely in clinical practice in India. The advantage of allopathic system of medicine is that it can cater to an enormous number of sick people. But one disadvantage is the resistance developed to these medicines, resulting in chronic diseases and poor quality of life[6]. Ayurvedic system has the advantage of curing chronic diseases with herbal/plant/animal extracts. However, the use of Ayurvedic system is limited by the lack of availability of quality products and lack of R&D that needs to go with times to suit the existing lifestyles. Here we report on partial purification of the extract of *Asteracantha longifolia* useful as a palliative for pain in advanced chronic diseases.

Modification of the seed assay

For tracking the anti-mitotic activity of *A. longifolia* extracts reported earlier[7], a suitable modification was incorporated. The assay was made convenient and more accurate by taking about 5 seeds in a petri dish (3.5 cm diameter) and adding samples to be tested in a total volume of 2.5 ml, covered with a lid and allowed to grow at 25–28°C. A photograph of the samples after the growth of seeds is shown in Figure 1. This modification was found to be very convenient for handling samples, with qualitative result obtained overnight.

Materials and methods

Preparation of partially purified A. longifolia extract for therapeutic use

The methodology adopted for purification of *A. longifolia* extract is based on the observation that the *in vitro* assay showed all the activity in water soluble low molecular weight components[7]. Tannins and lignins from the small molecular weight phytochemicals were separated by

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solvent precipitation procedure (Prov. patent App. No. 5872/CHE/2015). One kilogram of dry material gave 150 ml of aqueous extract (360 mg/ml) and when processed with solvent precipitation method using alcohol gave 120 ml of partially purified active component (60 mg/ml), which was stored in the refrigerator until use. This is termed PALE (partially purified *A. longifolia* Extract) with which the therapeutic activities were studied (Table 1).

Safety and toxicity studies on PALE

The studies were done as follows. It was found that as much as 1 ml/rat IP did not cause any adverse reaction for as long as 15 days.

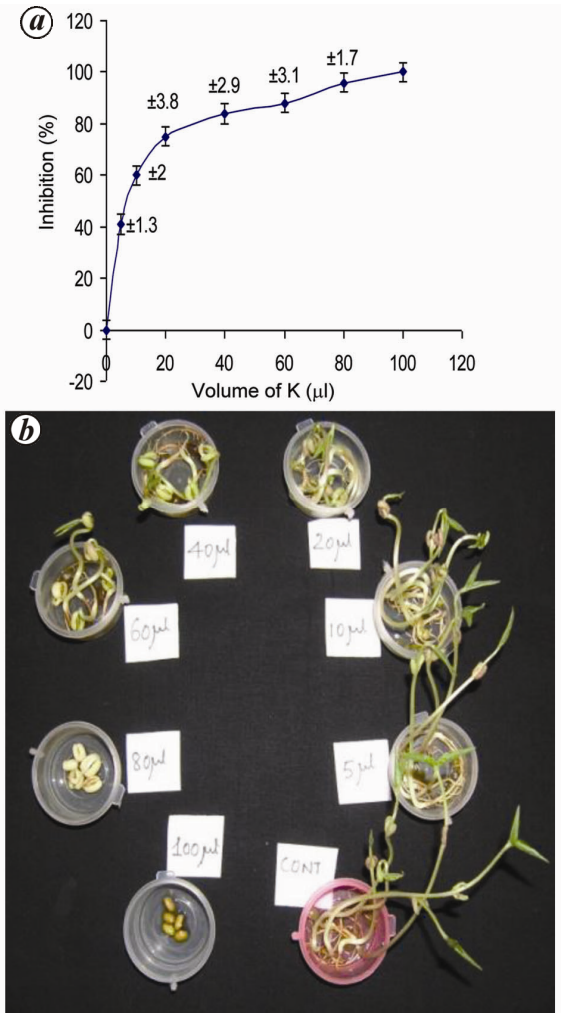

Figure 1. Assay method for quantification of antimitotic activity. Each petri dish had 5 seeds per dish (approximately 100 mg/seed) and was incubated with water (2.5 ml) containing different volumes of the sample (0–100 µl/ml). Time of incubation was 48 h at around 30°C in a humid chamber. ***a***, Graph shows the changes in weight of the seedlings (normalized to 500 mg of dry weight of seeds ± SD); ***b***, Photograph shows the growth of seedlings at different concentrations of the sample. A unit is defined as the concentration of the sample required to inhibit the growth by 50%.

Experimental animals: Wistar albino rats procured from Indian Institute of Science, Bengaluru, were used in the present study. The animals were kept separately in cages and were allowed to acclimatize to experimental conditions for one week before the actual studies commenced under standard hygienic conditions and provided with Amruth pellet feed (supplied by Sai Durga Feeds and Foods, Bengaluru) and water *ad libitum*. The feed was tested for the presence of mycotoxins and confirmed that it was free of aflatoxins. The animals were maintained as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals standard guidelines (CPCSEA) and approval obtained from Institutional Animal Ethics Committee (IAEC) (reference no. 32/LPM/IAEC/2009) for laboratory animals.

The rats were observed keenly for all clinical signs, body weight and general behaviour, feed, water intake, etc. Blood sample was collected three times, i.e. on day 0, 7 and 14th day. The biochemical findings like aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen and serum creatinine (Crt) were studied and later the animals were humanely euthanized and the organs were subjected to histopathology (aorta, heart, liver, kidney, spleen) for any changes.

Serum biochemical parameters

The serum biochemical parameters were estimated by using clinical chemistry analyzer (Microlab 300, Vitalab Scientific, The Netherlands). The following parameters were estimated using commercially available diagnostic kits from Merck (Ecoline®, Merck Specialties, Ambernath) following the manufacturer instructions furnished in the leaflet supplied along with the diagnostic kit.

Histopathological studies

Tissue pieces of liver, kidney and aorta were collected from rats that were sacrificed and were processed for histopathology by routine paraffin embedding technique. Sections of five microns thickness were cut and stained with haematoxylin and eosin[1]. The stained sections were examined under microscope and photomicrographs were taken.

Statistical analysis

The data obtained were subjected to statistical analysis. The data were analysed by applying two-way ANOVA, Bonferroni post-test. Mean values and standard error of mean were calculated and all the values are expressed as mean ± SE (GraphPad Prism, 2007).

Table 1. Purification table

Step	Volume Ml	Activity (μ/ml)	Total activity (μ/ml)	Dry weight (mg/ml)	Specific activity (U/mg)	Yield (%)	Purification factor
1	150	68	10,200	360	4.72		1
2	120	68	8,160	90	15.10	70	3.5

Table 2. Experimental design

Group	Groups ($n = 6$)	Details	Dosage of sample
Group 1	Control	Rat offered normal feed and water	1.5 ml saline
Group 2	TEST group	Rats on normal feed and water	1.5 ml PALE (ip)

Administration of PALE: The volunteers were educated, fully aware of the objectives of the project and willing to participate in the study. The administration was through oral route. At a time 3–5 drops were added to a spoonful of water and taken orally. As suggested by the Ayurvedic physician, the first dose was given in small quantity (equivalent to 1/10th of a drop in 10 drops of water) and waited for 5 min for any possible reactions. The dose was increased to 1 drop in a spoon of water and then increased to 5 drops in 2 spoons of water. No volunteer reported adverse reaction. Subsequently, the dosage was maintained to 2 drops of the PALE in a spoon of water taken two times a day.

Analysis of serum samples: Serum samples from volunteers were collected at the pathology laboratory and analysed for haematology and biochemical parameters by the standard protocol adopted at the pathology laboratory of Krishnadevaraya hospital, located at MVIT campus. Serum samples were drawn from the volunteers at 10–11 a.m. for studies.

Results

Toxicity studies

Serum biochemistry: The values obtained for ALT (U/L), AST, BUN and CRT for the control group and treated group are shown in Table 3, which were quantified at 0, 7 and 14th day.

There were no significant ($P > 0.05$) changes in estimated values of all the four parameters. Histopathological studies conducted in all the animals were compared with that of the control group and test group.

Liver: Sections showed mild fatty change, perivascular infiltration of inflammatory cells, congestion, haepatocyte degeneration and necrosis and loss of architecture. This was of reversible in nature.

Kidney: Tubular epithelial cells showed mild fatty change with vacuolar cytoplasm, eosinophilic homogenous material in the tubular lumen and degeneration and necrosis with desquamation into the lumen by the end of HFD gavaging. This was reversible in nature.

Spleen: There was no change in architecture of the spleen in histopathology.

Intestine: Section of intestine showed excessive goblet cell activity and necrosis, damage to the epithelium.

Heart: There was no change in the architecture of the heart in histopathology.

Lung: There was no change in the architecture of the lung in histopathology. The test substance was non-toxic for the dose and duration of administration. Even though it induced mild pathological changes in kidney and liver, they were reversible.

Clinical effects of PALE administration

Table 4 shows the values of clinical parameters done on two successive days for serum samples of subject 1. The changes in all parameters were mild, indicating that a change of over 20% can be considered as significant. Volunteer 1 had no clinical problems but reported a better sense of well being after taking the PALE. He had tooth complaints for which he routinely used dental floss. He also had a history of fever twice in 6 years which needed antibiotics, and had an episode of typhoid in 2007. Since 2011 he has not gone to any physician for health problems.

Volunteer 2 at the start of the experiment had some allergy and apparently had no other problem with nearly 1–2 episodes/year of sickness requiring conventional medication (allopathic) for the last several years. He had allergic cold, and had early morning sneezing, pimples on the face, and hardness of the skin at the elbow.

Tables 4 and 5 show the hematological and serum biochemical analysis before and after administration of PALE in two volunteers. The dosage was 3 drops/dose, two doses a day (morning and evening). Striking changes were seen in the values of haemoglobin, the percentage of lymphocytes and nutrophiles. In the first subject after 15 days of PALE, haemoglobin increased from 13% to 14.2% and after discontinuation of PALE it was reduced

to 13.6%. In the case of the second volunteer, the duration of administration of the PALE was 45 days. Haemoglobin increased progressively from 13.2% to 14.6% in 100 days. Lymphocytes increased from 25% to 30% to almost 40% to 48% in both cases and neutrophiles reduced from 60% to 70% to less than 50%, which also lasted for 1–2 months. When the values were normalized (no./cmm), increase in lymphocytes was from 2100 to 4200 after 15 days of PALE and reduced marginally in the next 50 days to 3612, still far higher than the initial counts (2100) on day 1 in the first subject. Reduction in neutrophiles was 5900/ml to around 4300/ml on days 15 and 65. In the second subject lymphocytes increased from 2400 to 3337 and maintained around 3100 all through till 110 days. Neutrophiles reduced from 5141 to 3479 (day 45), 3400 (day 75) and 3096 (day 110). The changes are very significant. In subject 2 eosinophiles reduced from 8% to 4% and associated with reduction of the allergic reactions. In the 1st subject eosinophile counts increased from 2% to 3%. Both the volunteers started to improve within 3 days after PALE.

For volunteer 1 the pain at the heels disappeared within 15 days after the administration of PALE. Volunteer 2 had remarkable improvement in his health, felt within a week. After 30 days of administration he obtained complete relief from cold as well as sneezing. His eosinophiles had reduced from 8% to 4%. Subjectively he reported enhanced feeling of well being. In addition to the above subjective symptoms, he showed the following objective clinical improvements: (a) clearance of lymph node lump in neck region; (b) improvement in bowel movement (he had bowel movement once in 2 days and it became daily in 15 days PALE); (c) disappearance of skin rashes at elbow and (d) complete relief from neck and joint pains. In the last 3 years both subjects did not have a single episode of sickness requiring conventional medication.

Table 3. Clinical parameters (mean ± SE value)

Days	0	7	14
ALT (U/L) (mg/dl)			
Control	56.66 ± 0.80	55.50 ± 1.47	56.01 ± 1.06
Test	54.83 ± 1.57	54.83 ± 1.36	57.85 ± 0.55
AST (U/L)			
Control	122.16 ± 4.86	124.33 ± 4.50	122.33 ± 2.8
Test	128.16 ± 3.77	130.33 ± 1.49	123.66 ± 1.2
CRT (mg/dl)			
Control	0.425 ± 0.02	0.428 ± 0.020	0.366 ± 0.016
Test	0.403 ± 0.01	0.383 ± 0.015	0.411 ± 0.014
BUN			
Control	18.00 ± 1.36	17.56 ± 0.80	18.40 ± 0.66
Test	20.45 ± 1.02	19.66 ± 1.02	18.16 ± 0.59

Effect of long-term administration of PALE on serum profile: Volunteer 2 was continued with PALE administration with the same dosage at identified days of administration as shown. Blood was drawn periodically and results are tabulated in Table 6. Only parameters which showed a difference were selected for tabulation. WBC, ESR and platelet counts did not show significant changes related to PALE administration.

Table 6 shows parameters where significant changes were observed during the experiment (18 months), including the time and duration of PALE administration. In the first cycle lymphocytes increased markedly, retained for 30 days when PALE was not used, but additional 30 days did not increase the lymphocytes. During 5 months lymphocytes had remained at around 3100/ml, indicating that PALE is balancing the lymphocyte concentration. During the succeeding period of 257 days (158–415) lymphocyte concentration remained at 3300 without a single dose of PALE. Hb gradually increased until 158 days to 14.6%. On the other hand nutrophile decreased dramatically to 3500 and increased gradually to 5100 by 158 days. This increase is probably associated with increased WBC counts. On the lipid profile cholesterol, triglyceride, HDL, LDL and VLDL did not show marked changes throughout the period of 1–158 days.

During days 400–420, volunteer 2 had cold for some time. During this period he went through PALE medication (405–420 days) and the results are discussed below. The results pertain to values during days 158–475. Lymphocyte counts reduced from 3695 to 1548 in a period of 1 year (430–445 days). Subsequently it increased to 2650, clearly demonstrating that PALE increases lymphocyte production in the system. Neutrophiles are not altered significantly and are on the higher side. Hb increased to 14% within 15 days of PALE medication.

Therapeutic activities of PALE

PALE showed many apparently unconnected therapeutic activities in volunteers. It restored immunity as seen by lymphocyte concentration and increased Hb in blood. Skin rashes disappeared and skin texture restored. Allergy was cured. Pains at heel, neck and wrist (tennis elbow) were completely relieved. Bowel movement was normalized. In volunteer 1 the muscle growth in the tooth cavity resulted in stronger gums (did not need the dental floss which was used before). The interesting activity was relief from pain at the right arm pit in a patient who underwent surgery for breast cancer 6 years ago, and was considered to be probable relapse by the physician. Similarly a volunteer with slow growing tumour experienced complete relief from pain on use of PALE within 7 days.

Table 4. Haematological studies (volunteer 1)

	Start	PALE Day 1	D1–15 (with PALE)	D16–65 (without PALE)
Hb	13.2 g	13.6 g	14 g	13.6 g
WBC	8300	8200	8800	8400
DC: poly (N)	70% (5810)	72% (5904)	47% (4136)	54% (4536)
Eosinophil	2%	2%	5%	3%
Lymphocytes	27% (2241)	25% (2050)	48% (4224)	43% (3612)
Monocyte	1%	1%	0%	0%
Basophile	0%	0%	0%	0%
Platelets	2.6 lakhs	2.7 lakhs	2.9 lakhs	2.6 lakhs
Urea	30	30 mg	29 gm	30mg
Creatinine	1.0	0.9 mg	0.8 mg	0.9 mg
Uric acid	3.5	3.2 mg	3.8 mg	3.8 mg
Total bilirubin	0.7	0.7 mg	0.7 mg	0.8 mg
Direct bilirubin	0.2	0.2 mg	0.2 mg	0.2 mg
SGOT	26	28 U/L	29 U/L	30 U/L
SGPT	24	26 U/L	26 U/L	26 U/L

Table 5. Haematological studies (volunteer 2)

Particulars	PALE D1	D45 (P45)	D75 (P45 + 30)*	D110 (P45 + 30 + P20)
Hb (14–18 g/dl)	13.2 g%	13.90 g%	14.2 g%	14.6 g%
WBC (4000–11,000 cell/mm)	8300	7100 (–15%)	6800 (–18.1%)	7200 (–13%)
DC: poly (N) 40–70%	62% (5146)	49% (–21%) (3479)	50% (–19.3%) (3400)	53% (–14.5%) (3816)
Eosinophils 01–05%	8% (664)	4% (–50%) (284)	4% (–50%) (272)	4% (–50%) (288)
Lymphocytes 20–40%	29% (2407)	47% (+62%) (3337)	46% (+58%) (3128)	43% (+48%) (3096)
Monocytes (01–05%)	1%	0%	0%	0%
Basophils (00–01%)	0%	0%	0%	0%
ESR (00–20 mm/h)	02 mm/h	02 mm/h	03 mm/h	03 mm/h
Platelets (1.5–4.0 lakhs)	2.9 lakh	2.6 lakh (–10%)	2.4 lakh (–17%)	2.6 lakh (10%)
Total bilirubin (0.1–1.2 mg/dl)	0.7 mg	0.6 mg (–16%)	0.6 mg (–16%)	0.7 mg (0 %)
Direct bilirubin (0.0–0.4 mg/dl)	0.2 mg	0.2 mg	0.2 mg	0.2 mg
SGOT 23 IU/L	25 U/L	14.60 U/L (–42%)	24.2 U/L (–3%)	33 U/L (+24%)
SGPT 25 IU/L	22 U/L	16.70 U/L (–25%)	20.2 U/L (–8%)	27 U/L (+23%)
Alkaline phosphatase 25–100 IU/L	92 U/L	82 U/L (–11%)	96 U/L (+4%)	71 U/L (–23%)
Total cholesterol (up to 200 mg%)	174 mg	171 mg	175 mg	160 (–8% mg)
Triglycerides (up to 180 mg%)	64 mg	53 mg (–17%)	62 mg (–3%)	60 mg (–6.25%)
HDL cholesterol (30–70 mg%)	34 mg	33 mg (–3%)	33 mg(–3%)	36 mg (+6%)
LDL cholesterol (up to 130 mg%)	127 mg	128 mg (–1%)	130 mg (+2%)	112 mg (–12%)
VLDL (up to 40 mg%)	13 mg	10 mg (–23%)	12mg (–8%)	12 mg (–8%)

**P* represents the duration of administration of PALE: Days without *P* represent number of days without PALE.

Discussion

Use of herbal extracts as neutraceutical is popular, but they do not have a documented data about their specificity and/or efficacy. Since they contain many compounds conventional allopathic method of study cannot be undertaken until the active factor is identified, purified to >99% purity, and pass through toxicity studies in three species of animals before it is considered fit for human trials. And yet we know from tradition that herbal extracts are the major class of medicaments used in herbal medicine and Ayurveda, practised even today specially for the cure of chronic diseases, like rheumatism, arthritis, gout, etc. The prescription of an Ayurvedic medicament is filled by the physician on the basis of subjective symptoms. Objective analyses are rarely employed in the practice, as it had not developed at that time. Therefore a need for reassessing therapeutic activity in herbal extracts using modern objective methodology is lacking. Contemporary methods try to isolate an active herbal component/s and develop synthetic analogues for use as a synthetic substitute for herbal extract, like in taxol, vincristine, vimblastine, etc.[8,9]. The concept of single drug and single therapeutic activity has become a dogma to such an extent that ignorance of alternate approaches is considered as unimportant or/and irrelevant.

Table 6. Effects of using pale for long duration on the clinical parameters of blood

Days from start	Lymphocyte per ml	Neutrophil per ml	Hb%	WBC	ESR	Platelets (in lakhs)
Day (01)	2407	5146	13.2	8300	2	2.9
45* (45)	3337	3472	13.9	7100	2	2.6
(75)	3128	3400	14.20	6800	3	2.4
20* (110)	3090	3816	14.60	7200	3	2.6
15* (144)	3078	4860	14.6	8100	3	2.8
(158)	3182	5160	14.40	8600	3	2.8
(370)	3348	2670	13	6200	2	2.4
(415)	3692	3195	13.4	7100	2	2.6
15* (445)	1584	4884	14.00	6600	4	2.4
(475)	2652	4836	13.2	7800	3	2.8

*Indicates the number of days PALE was taken before the sample was drawn for analysis; ( ) Values in brackets indicate the days after the start of PALE administration.

Unlike synthetic drugs, herbal extracts will have many phytochemicals which together act as a medicament[10,11]. In Ayurveda it is clearly stated that the therapeutic activity essentially consists of bringing balance of the three doshas, namely vata, pitta and kapha in such a way that other components present in the extract will neutralize the imbalance that could be created by the principal therapeutic component[11]. Likewise in Chinese system it is stated 'The beauty of nature is that plants contain not only the active ingredients to produce a positive clinical effect on a particular disease process, but also compounds to counteract any possible ill effects. In contrast, when pharmacologists successfully isolate or synthesize a naturally occurring compound for use in a drug they lose the yin that balances the yang action of the substance resulting in potentially deadly side effects[12].' Thus isolation of a single component from herbals for its potential therapeutic activity is not advised, and the whole mixture itself should be considered as a Herbal drug. Hence there is a need to investigate the therapeutic effects of such herbal extracts using objective methodology. As of now these herbals have never been investigated as therapeutic drugs[13], even though they have unquestioned therapeutic activity. In this study we have investigated the effect of herbal extract (PALE) on changes in blood constituents, and provide an acceptable objective assessment of its therapeutic activity in the human.

Aqueous extract of *A. longifolia* exhibits therapeutic activity, but cannot be called as a drug because it is a mixture of many components. Removal of high molecular components by the method described above has made PALE a better quality herbal extract because many inert large molecular components which can irritate the stomach were removed. PALE retained its therapeutic activity, yet it is not a therapeutic drug. In this article we have assessed its therapeutic activity by purely clinical (subjective and objective) and finally purely objective parameters through blood analysis. This is done in two volunteers. Reports on both volunteers are similar in all the three parameters (Tables 4–6). Rapid increase in lymphocytes which stays stable for a long period of time, increase in haemoglobin and rapid decrease in neutrophiles were a feature of blood analysis in both. This probably indicated that the primary action of PALE is in balancing the bioactivity at the bone marrow level. Since the constituents of the blood are quantitatively altered for better, blood is healed. Effect of PALE has been curative as there is no relapse in the status of blood parameters. Multiple therapeutic effect of PALE indicates multiple therapeutic components in it. Increased levels of Hb and lymphocytes recorded after PALE (Tables 4 and 5) did not reduce (after 50 days without PALE) which show that PALE has restored the functions of the bone marrow. Similarly increased values of HB and lymphocytes did not decrease after 200 days without PALE (Table 6) (days 158–370). Thus PALE is re-establishing equilibrium in the synthesis at the bone marrow, indicated by serum Hb and lymphocytes. This conclusion is corroborated by clinical observation that the therapeutic effect for unrelated disorders like allergy, constipation, analgesic effect, anaemia, skin disorders, immunomodulation, etc. were noticed. The only common feature among all is blood, and PALE having many components has been able to restore the equilibrium of the biochemical functioning of the bone marrow.

With only two volunteers the therapeutic activity is established and its site of action is objectively proved in the case of PALE. Considering the fact that drug development is looking for plant-based drugs these days, PALE can be classified as herbal restorative, because it has restored (the relief from discomfort lasted without further medication) bone marrow function. More importantly, the development of herbal extracts with known therapeutic activity into herbal restoratives can be undertaken easily using the blood picture as a highly objective analytical tool. Few more examples of herbal extracts investigated as a possible herbal restorative may make this approach acceptable and make herbal extracts to be used as herbal restoratives in India, making them the first choice of medical treatment for efficacy and economy.

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