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EFFICACY STUDIES OF A POLYHERBAL VITAMIN SUPPLEMENT IN EXPERIMENTAL ANIMALS

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ABSTRACT

Acute oral toxicity and efficacy study of poly herbal drug was carried out in albino rats and rabbits respectively to assess its effects on gross behavior, body weight, biochemical and hematological parameters. Animals of the Test group were fed with the test drug, animals of positive control group were fed with standard multivitamin tablet "Unicap" and animals of negative control group received distilled water i.e. vehicle only. Autopsy findings showed no gross changes in vital organs and mortality during 30 days of study. Significant increase in animal's weight was noted in test drug treated groups. Biochemical analysis indicated the presence of all essential minerals and trace elements in herbal preparation. The hemoglobin level was enhanced in the test drug treated animals as compared to the groups fed with standard drug and vehicle clotting time, WBC and RBC count indicated that drug didn't produce any uncertain reaction in blood parameters. The sign and symptoms exhibited by chronic use were almost alike and confirm that the test drug is a safe drug and have significant nutritional supplement value.

Key words: Herbal multivitamin, Toxicity, Efficacy, Biochemical analysis

INTRODUCTION

It is reported that all over the world that medicinal plants are used as a major source of drug for the treatment of various diseases and aliments. Pakistan abounds in herbs presenting a challenge to the triumvirate of hakims, doctors and scientists (Said, 1993). Due to unwanted effects produced by the use of synthetic drugs, efforts are focused to explore and to utilize the indigenous resources as a safe curative agent for various ailments.

The daily intake of multivitamin supplements not only improves general body functions but also enhances mental and physical conditions. The test drug used in this study, presented in the form of tablet, claimed to be a unique natural poly herbal energy tonic, which improves general health by providing energy through nutritional supplements, vitamins and minerals by removing mental and physical stress. The test drug is based on the aqueous extracts of various medicinal plants i.e. *Medicago sativa***,** *Centella asiatica*,*Winter cherry, Avena sativa, Emblica officinalis, Centaurea behen, Trigonella foenum-graecum* and *Cinnamon zelenicum* whose efficacy have been authenticated in the treatment of general weakness, mental stress, physical exhaustion, nutritional deficiency during pregnancy, lactation and convalescence.

These medicinal plants contain pharmacologically active compounds like glycosides, flavonoids, alkaloids, resin, tannin, phyto-sterols, saturated & unsaturated fatty acids, essential oils, mucilage, fat, starch, sugar, vitamin E, vitamin C, albumin (soluble & insoluble), cellulose and minerals like iron, calcium, zinc etc.

Brief account of above mention herbs is as under:

Emblica officinalis **(**Euphorbiacae)

Many studies reported that *Emblica officinalis* fruit is a rich source of vitamin C almost twenty times richer as compared to orange juice. (Jain *et al*., 2004; Cai *et al*., 2004; Naryan and Kumar 2003).

Withania somnifera **(**Solanaceae)

Withania somnifera is commonly known as winter cherry. It acts as tonic, astringent, nervine sedative, bitter astrid, thermogenic stimulant and diuretic. (Naryan and Kumar 2003, Nandkarni, 1954)

Avena sativa **(***Poaceae)*

Avena sativa or oat improves stamina and acts as tonic. (Naryan and Kumar, 2003, Bown,1995) as it gently restores vigor after debilitating illnesses, helps to lower the cholesterol levels in the blood. It also act as antidepressant by raising energy levels and supporting an over-stressed nervous system. (Chevallier, 1996).The seed also act as antispasmodic, cardiac stimulant, diuretic, emollient and nervine stimulant (Grieve, 1984; Chiej, 1984).

Cinnamomum zeylanicum **(**Lauraceae)

Cinnamomum zeylanicum or Darchini is used in dyspepsia, dirrohea, vomiting, toothache, and headache. It also act as antipyretic, antiseptic, astringent, carminative, diaphoretic, fungicidal, stimulant, stomachic and also used for preventing and treating asthma and chronic obstructive pulmonary disease (Narayan and Kumar 2003; Nadkarni, 1954).

Trigonella foenum-graecum **(**Fabaceae.)

Many medicinal properties of *Trigonella foenum-graecum* are reported as it is used in inflammatory conditions, in skin irritations, promote hair growth and prevent falling of hairs (Narayan and Kumar 2003; Nadkarni, 1954; Keller and Frey, 2006), diabetes mellitus and cardio protection (Tilak, 2006).

Medicago sativa (Leguminocea)

Medicago sativa or alfalfa is used for treating anemia, diabetes, as a diuretic for increased urination, to lower blood cholestrol level, to stimulate appetite and contribute towards weight gain, as an estrogen replacement in order to increase breast milk and to mitigate premenstrual syndrome (Foster, 2006).

Centella asiatica (Apiaceae)

Centella asiatica or Gotu Kola enhances the healing process, decrease capillary fragility and stimulate lipids and proteins synthesis required for healthy skin. It is also mildly antibacterial, anti-viral, anti-ulcerogenic, anti-inflammatory, anti-oxidant, anxiolytic, cerebral tonic, circulatory stimulant, diuretic, and nervine tonic (Winston, 2007 ; Bredwejn *et al*, 2000).

Centaurea behen (Asteraceae)

It is an annual or perennial herb, native to Iran and considered as source of the drug Safed Behman which acts as anti-flatulent, aphrodisiac, Cardiac Tonic, exhilarant, fattening and nutritive (www.druginfosys.com)

MATERIAL AND METHODS

Analysis of test drug

The test material was collected from the local market of Karachi (manufactured by Real Herbal Laboratories, Karachi), in packet of 30 tablets in strip packing. Each tablet weighed 880mg weight and was reported to contain *Medicago sativa* 30 mg *Centella asiatica* 30 mg *Withania somnifera* 60 mg *Avena sativa* 60 mg Blood vened safe 30 mg *Emblica officinalis* 30 mg *Centaurea behen* 30 mg *Trigonella foenum-graecum* 30 mg and *Cinnamomum zeylanicum* 05 mg. The tablets were analyzed for protein, iron, zinc, calcium and vitamin E content.

Preparation of sample

Twenty tablets were powdered and weighed in different doses according to body weight of animals and mixed in a fixed quantity (5g) of bread with some water and given to each animal separately at fix time with great care.

Selection of animals

Albino rats of Wistar strain of either sex weighing from 150-180gm reared at animal house of PCSIR Labs Complex Karachi, were selected and grouped accordingly while rabbits of either sex weighing between 1.5-2kg were purchased from local market. All these animals were housed separately under strict observation for the period of two weeks before start of experiment with allowing free access to food and water.

TOXICITY STUDY

Acute toxicity study of vitamin supplement tablet was carried out in albino rats (Wistar strain). Twenty four (24) albino rats of either sex were selected and divided into four groups, (n = 06). Animals of group I, II & III received the test drug in a dose of 150, 300, 600mg/kg body wt. respectively while the animals of group IV i.e. control group received bread only in the same quantity according to standard method of acute oral toxicity test (Loomis, 1978). The cages of animals were marked with their respective doses and were observed strictly for the first six hours and then for the period of 72hours. Daily observation on general health, growth, gross physical and behavioral activities and also the mortality if any was noted and recorded.

EFFICACY STUDIES

For efficacy study 24 rabbits of either sex were selected and divided into three groups (n = 08). Group I was treated as test group and received the test drug in a dose of 200mg/kg body weight of animal twice daily, group II received the test drug in a dose of 400mg/kg body weight of animal twice daily, while group 3 was treated as control group received normal rabbit feed only (Gram, bread, Cucumber, Carrot etc).The following parameters were used for efficacy evaluation.

Effects of the test drug on body weight

For this study, rabbits of both sexes were given drug @200 and 400 mg/kg of body weights given twice daily for 30 days and observation of the effect of the test drugs on body weight and health conditions of animals (both male and female) were recorded. The weight of each animal was recorded initially before starting the experiment and at 15 days interval throughout the course of study and the groups mean body weight was calculated and compared with the control group.

Hematological studies

Blood samples were collected from marginal ear vein of animals from each group at beginning and after 30 days of treatment and analyzed for blood parameters such as estimation of hemoglobin by Sahli method (Stannely,1976), clotting time, RBC count, MCH, MCV, and MCHC in two replicates (Gradwohl, 1956).

Biochemical studies

The study to assess any toxic effect of the drug on the function and the structure of liver were also carried out by analyzing blood samples of animals of each group at 0 and 30 days of study. The parameters investigated included serum enzymes glutamic oxaloacetate (SGOT) and glutamic pyruvate transaminase (SGPT) were measured by the calorimetric determination of transaminases Kit (TGO) of biometrix, using Boehringer Mannhein Photometer 4010. Total bilirubun, alkaline phosphates, glucose random, total protein, albumin, and globulin were determined by the standard methods.

STATISTICAL ANALYSIS

Results were expressed as Mean ± S.E. The data were analyzed by student *t*-test and least significant level at $p \leq 0.5$.

RESULTS AND DISCUSSION

The aim of present study was to evaluate a vitamin supplement herbal drug (Subvit) available in local market of Karachi. The test drug was analyzed for its acute oral toxicity, efficacy and safe use in human being in therapeutic doses on scientific line in comparison with standard synthetic multivitamin tablets available in local market. The food and nutrient content on per tablet basis presented in Table 1.

The effects of the test drug on bodyweight / mortality in albino rats and body weight of rabbits presented in Table 2 and 3 respectively. The data of hematological and biochemical parameters studied are outlined in Table 4 and 5.

The data clearly indicated that the test drug is a health protecting agent. It produces beneficial effects on health in terms of growth, erythropoiesis, body weight and other physiological functions of the body. The use of the test drug did not produce any unusual change in behavior or in locomotors activity, no ataxia and no sign of intoxication were observed during 30 days study period. The food consumption of male and female rats of all groups was similar indicating that the feed intake and the utilization were not affected. All the animals (each of 5 groups) were quite normal, alert and active in response and no mortality was observed during the observation period.

A number of biologically active amines, bioactive polysaccharides, trace elements, minerals, vitamins and other ingredients present in the plants used for the formulation of The test drug should have helped to maintain the proper nourishment of the tissues, it was found to effect the body weight of animals both in males and females as compared to standard and control groups. The effect of test tablet on body weight was found to be dose dependent (Table 2, 3). Animals of test group were found to be more active energetic with improved diet intake. It also acts as a mood stabilizer which also helps to support healthy immune system function. The test drug improves the body's ability to maintain physical effort and helps the body adapt to various types of stress.

There was an increase in Hemoglobin concentration, RBC count, PCV and normal clotting time which gave a clear cut picture that the feeding of test drug for long time has good effect on liver function.

Table I. Food and Nutrient Contents of Subvit Tablet.

Sr.#	Nutritional Ingredients	Quantity per Tablet
1	Iron	0.023 mg
2	Protein (N×6.25)	4.57 mg
3	Calcium	1.7 mg
4	Zinc	0.007 mg
5	Vitamin E	0.38 mg

Table 2. Acute Oral Toxicity of Test Drug in Albino Rats.

Sr.#	Observation	GP 1	GP 2	G3	G4
1	Oral dose of drug mg/kg/body weight	150mg/kg	300mg/kg	300mg/kg	0mg/kg
2	No. of animals	06	06	06	06
3	Avg. body weight of animals (g) before treatment	150 ± 0.78	165 ± 0.19	180 ± 0.22	170 ± 0.36
4	Avg. body weight of animals (g) before treatment	150 ± 0.92	168 ± 0.03	183 ± 0.05	168 ± 0.52
5	No. of days of drug administration	03	03	03	03
6	Mortality	Nil	Nil	Nil	Nil
7	No. of animals survived	06	06	06	06
8	Behavior observed	Quiet normal and active through out studies	Quiet normal and active through out studies	Quiet normal and active through out studies	Quiet normal and active through out studies

Table 3. Average Body Weight of Rabbits in efficacy study.

Sr.#	Observation	Test Group		Control Group	
		GP 1	GP 2	G3	G4
1	Oral dose of drug	400mg/kg	200mg/kg	Uni cap	Only placebo
2	No. of animals	06	06	06	06
3	Avg. weight of animals before treatment (kg)	1.52± 0.05	1.75±0.12	1.55±0.05	1.7±0.24
4	Avg. weight of animals after 30 days (kg)	2.2 ± 0.31	2.3 ± 0.31	2.0 ± 0.17	2.1± 0.36
5	Difference in weight after 30 days(kg)	0.67	0.5	0.47	0.4
6	% Increase in weight	44.736%	31.429%	29.03%	23.53%

Table 4. Average values of hematological parameters.

Sr. No	Observation	Test Group				Standard Group		Control Group	
		G1 (200mg/kg)		G2 (400mg/kg)		G3 (400mg/kg)		G4 (0mg/kg)	
	Day	Initial	After 30 Days	Initial	After 30 Days	Initial	After 30 Days	Initial	After 30 Days
1.	Clotting Time (min)	*2.4±0.03	*2.5±0.03	2.1±0.1	3.6±0.02	1.6±0.01	2±0.23	1.5±0.01	2.4±0.03
2.	Prothrombin Time (min)	2.4± 0.01	3.1± 0.2190	*3.10±0.02	*3.19±0.01	2.52±0.01	2.5± 0.02	2.3± 0.03	3.0±0.01
3.	Erythrocyte sedimentation rate/hour	*0.8± 0.16	*1.0± 0.1788	0.65± 0.0141	0.80± 0.1673	0.50± 0.0752	0.62± 0.0748	0.07± 0.0752	1.0± 0.0894
4.	Blood density	**1.08± 0.01	**1.08± 0.01	*1.07± 0.01	*1.07±0.010	1.06±0.01	1.06± 0.01	1.08± 0.01	1.08± 0.01
5.	Hemoglobin %	*94.8±1.5	*96.7±2.13	*84.6±1.06	*87.8±1.25	80.1±0.07	82.05±0.63	79.5± 1.76	88.46±1.6
6.	RBC%	*88.33± 1.31	*91.88± 1.05	*82.55± 1.37	*84.33± 1.48	71.00±1.7995	73.11± 1.377	72.22± 1.9878	73.22±2.231
7.	WBC/Count	*7950± 270.18	*9350± 89.442	*7650± 151.65	*8150± 141.42	6450±126.49	6750± 70.710	5350± 176.06	6400± 44.721
8.	Hematocrit %	*46±1.2	*47±1.0	*44±2.0	*46±2.0	44±1.78	46± 1.09	42± 1.26	46± 1.78
9.	Mean corpuscular volume (μ^3) MCV	*56.83± 0.91	*57.87± 1.11	*59.22± 0.96	*60.61± 1.60	60.79± 1.3114	62.65± 2.0875	55.77± 1.5085	66.76± 1.9511
10.	Mean hemoglobin% concentration (MHC)	*18.25± 1.25	*18.61± 0.90	17.65± 0.5985	18.79± 0.6978	19.45± 0.4697	19.56± 0.4788	18.32± 0.4896	19.23± 0.4112
11.	Mean Corpuscular Hemoglobin Conc (MCHC)	**32.1± 1.30	**32.1± 1.04	*30.00± 0.72	*31.17± 0.77	31.77± 0.8351	32.00± 0.8444	30.48± 0.8619	32.85± 0.6542
12.	Volume index	**1.04± 0.02	**1.06± 0.03	*1.02± 0.0141	*1.04± 0.0236	1.11± 0.0489	1.29± 0.0576	0.96± 0.0428	1.09± 0.0460
13.	Color index	*1.05± 0.0219	*1.07± 0.0303	*1.02± 0.0167	*1.09± 0.0303	1.12± 0.04	1.13± 0.0303	1.05± 0.03	1.10± 0.06
14.	Saturation Index	*1.0±0.01	*1.0±0.01	*1.0± 0.01	*1.0± 0.01	0.87± 0.05	1.0± 0.03	1.02± 0.01	1.09± 0.02

*Significant at $p \leq 0.5$ ** Significant at $p \leq 0.1$

It inhibits accumulation of fat in the body by lowering serum cholesterol level and improves cardiac activity. It improves the blood circulation of vital organs and thus has beneficial, energizer aphrodisiac and stimulant effects on the human body.

Most of the plants used in the formulation of the drug possessed antioxidant activity. The antioxidants are considered to be important in maintaining long term good health and to protect the body from the effect of disease and aging. The principal antioxidants are Vitamin C, E and B-Carotene & others are flavonoids, polyphenols, and minerals like selenium, zinc, iron, and copper. The antioxidants protect the body from the damaging effects of free radicals (Dasgupta and Bratati, 2006).

Literature survey, as presented above (see introduction), supports the view that the test drug as an energetic tonic associated with many other benefits in protecting and maintaining better life.

Table 5. Average values of biochemical analysis of rabbit's blood.

SR. NO	TEST	Test Group-G1			Test Group-G2			Standard Group- G3			Control Group-G4		
		Time in Days			Time in Days			Time in Days			Time in Days		
		0	15	30	0	15	30	0	15	30	0	15	30
1	Total Bilirubin (mg/dl)	0.6± 0.014	0.59± 0.028	0.5± 0.012	*0.65± 0.024	*0.63± 0.008	*0.7± 0.007	0.65± 0.023	0.64± 0.007	0.6± 0.023	0.6± 0.021	0.61± 0.035	0.58± 0.021
2	Direct Bilirubin (mg/dl)	0.16± 0.021	0.13± 0.015	0.15± 0.023	0.16± 0.023	0.19± 0.007	0.2± 0.018	0.17± 0.018	0.17± 0.014	0.16± 0.021	0.16± 0.007	0.16± 0.021	0.15± 0.017
3	SGPT (IU/lit)	*83± 0.660	*85± 0.927	*110± 0.632	*70± 4.049	*61± 1.788	*48± 2.366	185± 6.752	110± 1.264	108± 1.673	98± 2.097	97± 1.549	92± 1.897
4	Alkaline Phosphatase (IU/lit)	135± 2.915	140± 2.236	156± 1.732	*42± 5.522	*54± 3.535	*91± 1.224	320± 5.431	310± 2.738	300± 3.361	114± 1.870	111± 3.000	118± 1.870
5	Glucose Random (mg/dl)	*86± 2.121	*85± 2.345	*99± 3.535	*96± 1.732	*92± 1.870	*90± 1.732	47± 2.236	68± 2.345	93± 2.549	134± 1.224	130± 1.224	110± 2.738
6	Total Protein (g/dl)	*6.3± 0.212	*6.9± 0.406	*7.3± 0.300	5.3± 0.412	5.9± 0.187	7.0± 0.173	6.7± 0.331	6.5± 0.122	7.0± 0.070	6.2± 0.234	6.0± 0.187	6.1± 0.173
7	Albumin (g/dl)	*4.7± 0.1732	*3.9± 0.2345	*3.3± 0.0707	*3.5± 0.0700	*3.8± 0.1581	*4.8± 0.463	5.0± 0.070 7	4.7± 0.0707	4.6± 0.187	4.1± 0.100	4.3± 0.291	4.3± 0.254
8	Globuline (g/dl)	*1.6± 0.367	*3.0± 0.353	*2.4± 0.346	*1.8± 0.0708	*2.1± 0.273	*2.9± 0.187	1.7± 0.406	1.8± 0.122	2± 0.1224	2.1± 0.2345	1.9± 0.0463	1.8± 0.0216
9	Albumin/ Globulin ratio	2.9	1.3	1.3	1.94	1.80	1.10	2.9	2.6	2.15	1.95	2.26	2.3

*Significant at $p \leq 0.5$ ** Significant at $p \leq 0.1$

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