ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

Toxicological study of *Balacaturbhadrika churna*

Mukeshkumar B. Nariya, Parag Parmar[1], Vinay J. Shukla[1], B. Ravishankar[1]

Department of Pharmacology, Ayurveda Contraceptive Drug Research Institute (Central Council for Research in Ayurveda and Siddha), Ahmedabad, [1]Pharmacology Laboratory, Institute of Postgraduate Teaching and Research, Gujarat Ayurved University, Jamnagar, India

ABSTRACT

Balacaturbhadrika churna has an important place in pediatric practice in Ayurveda. Millennia of use of this formulation bears testimony to its safety when used for prolonged duration in children. This prompted us to initiate a long-term, acute oral toxicity evaluation of *Balacaturbhadrika churna* in rats. The study was carried out by administering *Balacaturbhadrika churna* orally once only in a dose up to 2000 mg/kg. For long–term toxicity, *Balacaturbhadrika churna* was administered in doses of 450 and 900 mg/kg orally for 45 consecutive days. The effects of the drug on ponderal changes, hematological, biochemical and histological parameters were noted. The acute toxicity experiment showed that the drug did not produce any signs and symptoms of toxicity (or mortality) up to the dose of 2000 mg/kg. Long–term toxicity results showed that, even at higher dose of 900 mg/kg, *Balacaturbhadrika churna* did not affect the parameters studied, to a significant extent. The doses employed for these toxicity studies were several times higher than normal clinical doses of *Balacaturbhadrika churna*, hence the observed changes will probably not become apparent at therapeutic dose level.

Key words: Aconitum heterophyllum, acute toxicity, *Balacaturbhadrika churna*, long–term toxicity

INTRODUCTION

Ayurvedic medicines have been used in medical practice for thousands of years and have made great contributions to maintaining and improving human health. Even though its drugs have been used for millennia and are known not to produce toxic effects, and are presumed to be non-toxic, no objective verifiable data exists to support the many such claims. It is conceivable that some highly potent medicinal plant, if administered at a higher dose for a prolonged period could produce toxicity. The apparently uneventful use of these drugs is usually taken as evidence for their safety. Preclinical studies of herbal drugs provide a scientific justification for their traditional use and prove that they are safe and efficacious.[1]

Balacaturbhadrika churna is a popular formulation used in pediatric practice in Ayurveda specially in the treatment of vomiting, diarrhea, fever and respiratory disorders. The human clinical dose of *Balacaturbhadrika churna* is 1000 mg per day. It is prepared by mixing equal proportions of rhizome of *Cyperus rotundus* Linn. (Cyperaceae), fruit of *Piper longum* Linn. (Piperaceae), root of *Aconitum heterophyllum* Wall. ex. Royale. (Ranunculaceae) and gall of *Pistacia integerrima* Stew. Ex. Brandis. (Anacardiaceae).[2] Aconitum species are known for their toxic potential.[3] Their extensive use in children since more than a millennium without any report of untoward events can be considered as proof of their safety. However, experimental evaluation of their safety would provide proper proof of the same. Since reports of the toxicity evaluation of this classical preparation were not found in an extensive literature review it was decided to undertake a detailed toxicity evaluation.

Address for correspondence:
Dr. B. Ravishankar, Pharmacology Laboratory, Institute of Postgraduate Teaching and Research, Gujarat Ayurved University, Jamnagar – 361008. Gujarat, India.
E–mail: rx_mukesh@rediffmail.com

Received: 08-Oct-2010
Revised: 29-Mar-2011
Accepted: 06-Apr-2011

Access this article online

Quick Response Code:

Website: www.jaim.in

DOI: 10.4103/0975-9476.82526

MATERIALS AND METHODS

Plant materials and chemicals

The rhizome of *C. rotundus,* fruits of *P. longum,* roots of *A. heterophyllum* and gall of *P. integerrima* were collected from the forest of Dang and Barda hills, Gujarat, India. Pharmacognostical studies were carried out for the

authentication of the plant materials in the department of Pharmacognosy, IPGT and RA, Gujarat Ayurved University, Jamnagar and voucher specimens of each were deposited in the Institute. The drugs were shade dried, powdered in a micro–pulveriser (Shubh, Mumbai) and stored in an air–tight container. *Balacaturbhadrika churna* was prepared by mixing the powder of the above four ingredients in equal proportions.[2] All chemicals used in the study were of analytical grade.

Animals

Wistar albino rats of both sexes weighing between 150–200g were used for the study. The animals were maintained under ideal husbandry conditions and reared under standard conditions of temperature, humidity and exposed to 12–h light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (Pharmac/08) as per the guidelines of the *Committee for the Purpose of Control and Supervision on Experiments on Animals*, India.

Dose

The dose for the experimental study was calculated by extrapolating the clinical human dose of *Balacturbhadrika churna* (1000 mg per day) to an animal dose based on body surface area ratio by using conversion factor of 0.018.[4] Based on that the therapeutic dose of *Balacaturbhadrika churna* was calculated for rats i.e. 1000 mg x 0.018= 18 mg/200 g body weight of rat= 90 mg/kg body weight of rat.

Acute toxicity study

The study was carried out as per the World Health Organization (WHO) guidelines for acute toxicity test and modified as per experimental need.[1] Rats were randomized into five groups, each consisting of six animals. Group I formed the control group, received the vehicle as an aqueous suspension of 1% carboxymethyl cellulose in a dose of 10 ml/kg, orally. Groups II to V formed the drug–treated groups and received the *Balacaturbhadrika churna* orally in doses of 360, 720, 1440 and 2000 mg/kg respectively. The rats were observed closely for behavioral changes, signs and symptoms of toxicity and mortality continuously for the first four hours and thereafter periodically up to 14 days.

Long–term toxicity study

The study was carried out following WHO guidelines for long–term toxicity tests, modified according to experimental need.[1] The rats were randomized into three groups, each consisting of six animals. Group I formed the control group, and received a vehicle consisting of an aqueous suspension of 1% carboxymethyl cellulose in dose of 10 ml/kg, orally. Groups II and III were kept as drug–treated groups and received *Balacaturbhadrika churna* orally in the dose of 450 and 900 mg/kg respectively for 45 consecutive days. The administration period of the drug for the long–term toxicity study was determined from the WHO guidelines combined with the recommended period of clinical use of *Balacaturbhadrika churna* as stated in the literature.

The rats were carefully observed daily for any overt or apparent signs or symptoms of toxicity. The body weight change of individual rats was noted initially and thereafter weekly during the study period. At the end of the 45–day period, blood was drawn from the retro–orbital puncture under light ether anesthesia using a capillary tube. The body weight of each rat was noted on the last day when the rats were sacrificed.

Tests for Hematological parameters were carried out in blood samples collected from the rats viz. red blood cell (RBC), hematocrit (HCT), hemoglobin (HB), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), mean cell hemoglobin concentration (MCHC), white blood cell (WBC), lymphocyte percentage (%L), granulocyte percentage (%G), eosinophil percentage relative to other cells (MID%), platelet count (PLT), platelet crit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) by using an automatic hematological analyzer (MS–9 Veterinary Melet Schloesing Hematology Cell Counter, France).

The serum biochemical parameters' estimation was carried out using serum diagnostic kits in a Merck auto–analyzer. The parameters were alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein,[5,6] albumin and globulin,[7] urea,[8] bilirubin,[9] creatinine,[10] glucose,[11] total cholesterol, HDL–cholesterol, LDL–cholesterol,[12] triglyceride,[13] sodium and potassium.[14]

Bone marrow smears from the femur bone were prepared using the standard procedure. All the important internal organs were carefully dissected, namely the brain, liver, heart, thymus, spleen, kidney, testis, prostate, seminal vesicle, uterus, lung, adrenal gland, trachea, aorta, ovary and lymph node. After noting any signs of gross lesions or ponderal changes, the major organs were transferred to a 10% phosphate–buffered formalin solution for fixation, and later subjected to dehydrating, wax embedding, sectioning and staining with hematoxylin and eosin for histological evaluation by light microscopy.

Statistical analysis

The data are expressed as mean ± standard error of mean

for the six rats in each experimental group. One–way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnet's multiple *t*–test for unpaired data to determine significant between groups differences.

RESULTS AND DISCUSSION

Acute toxicity test

Acute toxicity test results showed that *Balacaturbhadrika churna* did not effect any behavioral changes or affect other parameters measured in the acute toxicity test. *Balacaturbhadrika churna* did not produce any signs or symptoms of toxicity or mortality up to a dose of 2000 mg/kg. This dose is more than 20 times the therapeutic equivalent dose in rats, clearly indicating that the formulation is unlikely to induce any drastic toxic effect in spite of containing *Aconitum heterophyllum* which is known for cardio–toxic potential.

Long–term toxicity test

The effect of *Balacaturbhadrika churna* on percentage change in body weight [Figure 1] showed that weight gain was similar in all three groups: the percentage body weight change pattern in the treated groups did not differ significantly from the changes observed in the control group. Body weight change is an important indicator of gross toxicity. Drastic toxicity or interference with absorption of nutrients will be reflected in body weight reduction. Since the body weight gain pattern in the test drug–treated groups did not differ significantly from the control group it can be inferred that the test drug formulation has no proclivity to produce drastic tissue destruction nor does it seem to interfere with absorption of the nutrients. Further, of the eight organs for which relative weight was recorded, a significant decrease was observed only in the kidney at a dose of 450 mg/kg of *Balacaturbhadrika churna* [Table 1]. If we consider the data together with the findings of the histopathological study, the kidney weight reduction does not seem to represent loss of tissue mass, because no pathological changes were observed in the histological study consistent with tissue mass. Furthermore, kidney weight decrease was not observed at the higher dose level.

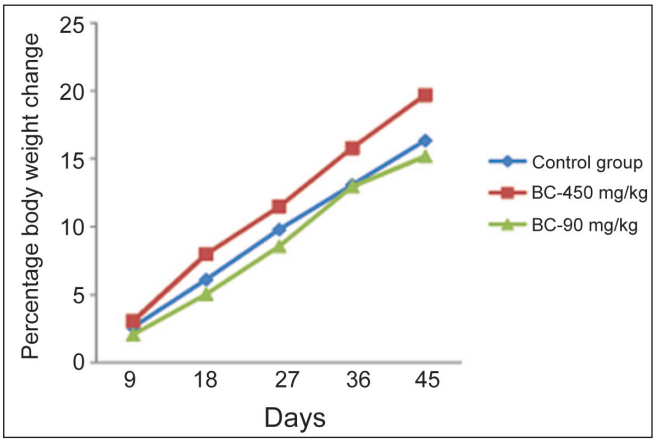

Figure 1: Percentage body weight changes observed in control and treated groups at different time intervals

Analysis of the effect of *Balacaturbhadrika churna* on the 15 hematological parameters [Table 2] found a single affected at each of the dose levels studied, as might be expected when so many parameters are measured. The observed changes were a significant decrease in the Hb level compared to the control group at 450 mg/kg dose and significant increase in WBC count at 900 mg/kg dose level. The decrease in hemoglobin content may be the result of a decrease in the number of circulating red cells or in the size of red cells, their concentration of Hb or any combination of these. In megaloblastic anemia the reduction in the number of red cells is the cause of anemia. In iron–deficiency anemia the reduction in the size of red cells (MCV) and MCH is the reason but reduction in cell number eventually contributes to the anemia. However, in both the treated groups the decrease observed in MCV and MCH was not significant. Hence it may be suggested that the decrease in Hb content is due to the decrease observed in the production of erythrocytes.[15] The decrease observed in the Hb level at a lower dose level was not evident at a higher dose level–this and the fact that even 450 mg/kg is five times more than the therapeutic dose rules out any serious toxicological implications during therapeutic use. Further, the observed values are within the normal range.[16]

The exact reason for the increase in the WBC count, especially at the higher dose level is not clear. Normally, leucocytosis occurs in association with acute inflammatory reactions, tissue necrosis, thrombosis, hemorrhage, acute lysis of red cells and sometimes due to neoplasia.[17] A mild to moderate increase occurs during strenuous exercise, severe mental stress and any factor leading to elevation of plasma level of glucocorticoids, corticotrophins or adrenalins. Since none of the organs studied during the study showed any significant change in cytoarchitecture suggestive of inflammatory reaction, and since the drug was administered over a long period, the increased count does not seem to be of inflammatory origin. Perhaps it reflect direct effects on blood cell–forming organs. Further, the observed values are within the normal range.[16] If the overall picture is taken into consideration, the data profile clearly indicates that the test formulation is not likely to produce any serious hematological changes.

The effects of *Balacaturbhadrika churna* on serum biochemical parameters are presented in Table 3. Out of 15 parameters studied a significant decrease was observed

Table 1: Effect of *Balacaturbhadrika churna* on the relative weight of organs recorded in rats

Body weight and relative weight of organs	Control group	*Balacaturbhadrika churna*	
		450 mg/kg	900 mg/kg
Liver (g/100 g)	3.484±0.13	3.512±0.13	3.357±0.12
Heart (mg/100 g)	317.05±10.95	291.12±14.72	299.12±5.05
Kidney (mg/100 g)	734.23±21.30	648.45±27.50[a]	724.40±11.43
Spleen (mg/100 g)	245.32±14.42	229.64±12.82	248 71±09.98
Thymus (mg/100 g)	332.50±17.25	295.02±10.95	321.42±16.25
Testis (g/100 g)	1.141±0.01	1.077±0.06	1.182±0.05
Prostate (mg/100 g)	90.23±0.23	105.51±6.49	116.33±14.25
Seminal vesicle (mg/100 g)	407.38±15.38	367.28±30.05	373.58±26.08

The values are expressed as mean ± SEM of six rats per group [a]$P < 0.02$ compared with control group

Table 2: Effect of *Balacaturbhadrika churna* on the hematological parameters recorded in rats

Hematological parameters	Control group	*Balacaturbhadrika churna*	
		450 mg/kg	900 mg/kg
RBC (10^6 /µl)	7.37±0.35	6.55±0.37	7.03±0.21
Hematocrit (%)	41.18±1.86	36.25±2.36	39.02±0.71
Hemoglobin (g/dl)	17.30±0.92	14.24±0.73[a]	16.65±1.35
MCV (µm³/red cell)	55.94±0.53	55.17±1.03	55.31±0.75
MCH (pg/red cell)	23.60±1.06	22.16±1.63	22.62±2.29
RDW (%)	5.957±0.11	5.90±0.10	5.79±0.05
MCHC (g/dl)	42.26±2.13	40.31±3.28	40.76±3.77
WBC (10^3 /µl)	3.72±0.49	4.98±0.63	7.45±1.35[b]
Lymphocyte (%)	74.24±5.11	82.06±2.16	75.75±3.27
Granulocyte (%)	12.10±1.27	9.90±1.28	15.12±2.31
MID (%)	8.66±0.15	8.04±1.48	9.26±1.25
PLT (10^3 /µl)	1077.0±97.0	931.0±133.0	833.0 ±81.0
PCT (%)	0.99±0.08	0.88 ±0.14	0.82 ±0.08
MPV (µm³)	8.27±0.294	8.44±0.146	8.85 ±0.62
PDW (µm³)	11.56±0.59	11.91±0.29	11.71 ±0.28

The values are expressed as mean ± SEM of six rats per group [a]$P < 0.05$, [b]$P < 0.01$ compared with control group

in urea and LDL–cholesterol and an increase observed in HDL–cholesterol at a dose level of 450 mg/kg of *Balacaturbhadrika churna*. At a dose level of 900 mg/kg of *Balacaturbhadrika churna*, significant decrease was observed in urea, creatinine and ALP and increase was observed in HDL–cholesterol and potassium.

Urea is the main product of protein metabolism in the body. Normally, increased level of urea in the blood has diagnostic value as a parameter indicating impairment in the functioning of the kidney.[17] Low urea level in the normal course has no diagnostic value. The observed decrease in both groups in the present study may be due to low turnover of nitrogenous materials, the reason for which remains to be determined. Alkaline phosphatase is found in most tissues. Serum ALP level increases in various liver diseases and it is a conventional indicator of liver injury. In the present study significant decrease in the level of this enzyme was observed at 900 mg/kg of *Balacaturbhadrika churna*. Since elevated levels are more important from a pathological point of view it can be suggested that the observed decrease does not indicate any serious toxic effects. Creatinine is normally produced from creatine in tissue containing high amounts of creatine phosphate. Its blood level depends on its production and excretion. In both the drug–treated groups the creatinine level was decreased. However, the decrease in creatinine was still within the normal range.[16] Thus, it can be suggested that these changes have no serious pathological implications, especially in clinical settings.

At both the dose levels, a significant increase in HDL–cholesterol level and a decrease in LDL–cholesterol level were observed. Total cholesterol level remained un–affected. This activity can have a good therapeutic application because elevation of HDL–cholesterol level with concomitant decrease in LDL–cholesterol level will be quite useful in patients with hypercholesterolemia conditions. Elucidation of the mechanism underlying this effect deserves attention and should be the subject of future investigations. Significant increase in serum potassium in the present study may be due to mobilization of potassium from intracellular sources and decreased secretion from the kidney, or could simply be an expected

Table 3: Effect of *Balacaturbhadrika churna* on biochemical parameters recorded in rats

Parameters	Control group	*Balacaturbhadrika churna*	
		450 mg/kg	900 mg/kg
Total protein (g/dl)	8.23±0.51	8.09±0.64	7.63±0.45
Albumin (g/dl)	4.22±0.16	4.08±0.13	4.27±0.14
Globulin (g/dl)	4.11±0.61	4.01±0.69	3.36±0.46
Urea (mg/dl)	69.30±3.83	42.83±3.57[d]	49.05±4.45[c]
Bilirubin (mg/dl)	0.673±0.089	0.94±0.16	0.92±0.15
Creatinine (mg/dl)	1.26±0.15	0.91±0.13	0.81±0.04[a]
Glucose (mg/dl)	109.00±05.57	93.64±06.81	88.57±10.28
ALP (µM phenol released/ mg protein/min)	25.27±1.19	27.32±3.18	17.66±2.16[a]
AST (µM pyruvate released/mg protein/min)	6.01±0.56	5.24±0.54	5.78±0.54
Total cholesterol (mg/dl)	70.47±2.56	63.06±3.86	60.40±4.31
HDL–cholesterol (mg/dl)	18.04±0.57	24.70±2.25[c]	22.50±1.31[a]
Triglyceride (mg/dl)	79.09±09.95	99.85±21.83	58.39±8.94
LDL–cholesterol (mg/dl)	38.57±3.16	19.39±5.69[b]	27.14±5.27
Sodium (meq/L)	43.63±0.85	39.88±2.64	42.57±2.66
Potassium (meq/L)	44.61±1.93	51.03±4.01	57.50±2.54[c]

The values are expressed as mean ± SEM of six rats per group [a]*P* <0.05, [b]*P* <0.02, [c]*P* <0.01, [d]*P* <0.001 compared with control group

Table 4: Effect of *Balacaturbhadrika churna* on bone marrow cellularity in albino rats

Parameters	Control	*Balacaturbhadrika churna*	
		450 mg/kg	900 mg/kg
Normoblast showing micronucleus*	4.00±0.91	2.33±0.33	3.00±0.91
Polychromatic normoblast (Pn)*	315.0±09.73	298.5±12.48	311.0±06.15
Erythrocytes (E)*	182.0±09.2	199.0±12.66	186.0±05.71
Pn/E Ratio	1.74±0.14	1.51±0.15	1.67±0.08

*Out of 500 cells counted, The values are expressed as mean ± SEM of six rats per group

statistical fluctuation from making 30 measurements.[17] Protein catabolism does not seem to be involved since there was no increase in the serum urea level, on the contrary a decrease in its level was observed.

The effect of *Balacaturbhadrika churna* was studied on bone marrow cellularity [Table 4]. The test drug did not affect the polychromatic normoblasts, erythrocytes and normoblast showing micronuclei at both the dose levels studied, in comparison to the control group. This suggests that it is not likely to have any mutagenicity potential.

The histopathological studies of 16 organs showed that *Balacaturbhadrika churna* at 450 mg/kg, increased the cellularity in the thymus and spleen. Other organs exhibited normal cytoarchitecture suggesting that the preparation is devoid of serious organ degenerative potential at this dose level. At the higher dose of 900 mg/kg changes were observed in the spleen, thymus, and testis. The white pulp (lymphatic tissue) of the spleen forms a sheath around the arteries. The stroma is a network of reticular fibers and phagocytic reticular cells or fixed macrophages. As in all lymphatic tissue, the meshes of the framework are filled with free lymphocytes of various size, distributed to form diffuse and nodular lymphatic tissue which vary continuously and reflect the reaction of lymphatic tissue to various generalized stimuli.[18] Increase in cellularity in white pulp at both the dose levels may be due to the increase in the lymphatic tissue and free lymphocytes in the spleen. The increase in white pulp and increase in cellularity in the thymus is indicative of increased immune activity. It may be due to increased formation of cytokines from the cells involved in immune mechanism.

Decrease in spermatogenesis was seen in the testis of one rat at 900 mg/kg of *Balacaturbhadrika churna*. However, it has to be noted that the above–mentioned changes did not occur in all the rats nor at the 450 mg/kg dose level. This indicated that these changes were not very severe.

Based on the above it can be inferred that even at a dose of 900 mg/kg, *Balacaturbhadrika churna* given for 45 days, the equivalent of which is not likely to be ever employed in clinical conditions, has only mild toxic potential. Since the doses employed are several fold higher than the doses normally employed in clinical settings, the observed changes may not become apparent at the therapeutic dose level.

ACKNOWLEDGMENTS

The authors wish to thank Dr. M. S. Baghel, Director, Institute of PG Teaching and Research in Ayurveda, Dr. Subrata De

and Dr. Malti Chauhan, Gujarat Ayurved University for their constant support.

REFERENCES

1. Research guidelines for evaluating the safety and efficacy of herbal medicines. Manila, Philippines: WHO, Regional Office, Western Pacific Region; 1993.
2. The Ayurvedic Formulary of India, Part–I. India: Govt. India, Ministry of Health and Family Planning, Department of Health; 1998. p. 92.
3. Gaillard Y, Pepin G. Poisoning by plant materials: Review of human cases and analytical determination of main toxins by high–performance liquid chromatography–(tandem) mass spectroscopy. J Chromatography B 1999;733:181–229.
4. Paget GE, Barnes JM. Toxicity tests. In: Laurance DR, Bacharach AL, editors. Evaluation of Drug Activities: Pharmacometrics, Vol. 1. New York: Academic Press; 1964. p. 135–65.
5. Thomas L. Clinical Laboratory Diagnostics. Frankfurt: TH books Verlagsgesellschaft; 1998. p. 55–65, 136–46 and 644–7.
6. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders and Company; 1999. p. 450, 617–721.
7. Doumas BT, Waston WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 1971;31:87–96.
8. Fawcett JK, Scott JE. A rapid and precise method for determination of urea. J Clin Pathol 1960;13:156–9.
9. *Jendrassik L*, Grof *P.* Colorimetric method of determination of bilirubin. *Biochem* J 1938;297:81–2.
10. Bartels H, Bohmer M. A micro–method for creatinine understanding. Clin Chim Acta 1971;32:81–5.
11. Burtis CA, Ashwood ER, editors. Carbohydrates. In: Teitz Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders and Company; 1996. p. 351–74.
12. Wybenga DR, Pillegi VJ. Direct manual determination of serum total cholesterol with single stable reagent. Clin Chem 1970;16:980–4.
13. Buccolo G, David M. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19:476–9.
14. Wooten ID, Freeman H. Microanalysis in Medical Biochemistry. Edinburgh: Churchill, Living stone; 1982. p. 53.
15. MacSween RN, Whaley K, editors. Muir's Textbook of Pathology. 13thed. London: ELBS Publication; 1992.
16. Gad SC. The rat: Pathology. In: Gad SC, Chengellis CP, editors. Animal Models in Toxicology. New York: CRC press, Boca Raton; 2007. p. 147–217.
17. Talwar GP, Srivastava LM, editors. Textbook of Biochemistry and Human Biology. 3rd ed. New Delhi: Prentice Hall of India Private Limited; 2006.
18. Fawcett DW, Editor. Bloom and Fawcett: A textbook of histology. 12th ed. New York: Chapman and Hall; 1994.

Source of Support: Nil, **Conflict of Interest:** None declared.