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Research Article

TOXICITY STUDIES OF THE ACTIVE FRACTION OF *STEREOSPERMUM TETRAGONAM* DC.

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ABSTRACT

Objective: The present study was to evaluate the oral toxicity of acute, sub acute and histopathological studies of the active fraction of *Stereospermum tetragonum* DC using albino mice.

Methods: In acute toxicity studies the active fraction of *Stereospermum tetragonum* DC was administered orally at doses ranging from 500-2000 mg/kg p.o the animals were observed for mortality and any toxic symptoms up to 14 days. In sub acute toxicity studies the active fraction was administered daily for 28 days with doses ranging from 100-400 mg/kg. The animals were observed for signs of toxicity, morbidity and mortality for 28 days. The animals were subjected to bio-chemical and hematological parameters on the 29th day and the animals were sacrificed for histopathological study.

Results: The results of 14 days acute toxicity studies up to a dose of 2000mg/kg of the active fraction neither produced mortality nor show any symptoms of behavior or any physiological changes in body weight, food and water intake. The 28 days sub acute studies repeated doses of oral toxicity did not show any toxic signs or any mortality when three doses 100, 200 and 400 mg/kg of the active fraction administered. No significant changes were absorbed in Biochemical and hematological parameters when compared with the control group. The two higher doses slightly reduced serum glucose concentrations. The relative weights of animals were found to be comparable with control. The vital organs treated with the active fraction of the drug for 28 days did not show any gross abnormalities, histopathological changes or pathological lesions.

Conclusion: From the results it is concluded that the dose at 400mg/kg is safe for long term treatment in diabetic conditions.

Keywords: *Stereospermum tetragonam*; Active fraction; Acute Toxicity; Sub acute toxicity; Histopathology.

INTRODUCTION

The use of herbal drugs for the management of certain ailments continues unabated in most developing communities due to easy access and for economic reasons. Plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in the traditional medicine for treating a number of diseases [1]. The traditional indigenous system of the traditional medicine contains active organic compounds and is employed in the treatment of diseases of diverse origins. Traditional medicines are used by about 60% of the world population both in the developing countries and developed countries where modern medicines are predominantly used [2]. The World Health Organisation survey indicated that about 70-80% of the world's population rely on non conventional medicine, mainly of herbal source in the primary health care [3]. Experimental screening method is, therefore, important to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies [4].

To determine the safety of the plant products for human use, toxicological evaluation such as hepatotoxicity, CNS toxicity and renal toxicity to be carried out in various experimental animals to predict the toxicity and to provide guidelines for selecting a 'safe' dose in humans [5]. It is quite difficult to ascertain certain adverse effects in animals such as headache, abdominal pain, dizziness and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. Nevertheless, the evaluation of adverse effects of sub-chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. Acute toxicity studies with a range of doses have to be conducted first to select proper dose(s) for chronic and sub-chronic studies; the doses selected for chronic and sub-chronic toxicity studies should be at and above the suggested human dose [6].

Stereospermum tetragonam is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in Northern India and other parts of Tamil nadu. It is used in folk medical practices to treat DM in certain remote villages of Thirunelveli district of Tamilnadu. In ethno-medical practices, the plant is also used as diuretic, treat antiulcer, anti-pyritic etc. Phytochemical analysis showed the presence of tannins, phenol, glycosides, terpinoids, coumarins, in the active fraction [7]. Preliminary studies have showed promising anti-hyperglycemic activity of the roots of *Stereospermum tetragonam*. Other studies showed the active fraction showed presence of anti-diabetes mellitus activity in type-1 and type-2 diabetic rats. Two active principles were isolated and characterized by spectral data. One of them was identified as an iridoid type glycoside and the other one was a lapachol like compound [8, 9]

Since the active fraction obtained from the extract showed remarkable antidiabetic activity, there is a need to determine its safety. Although the plant root is used in folklore medicine without any known and/or recorded adverse effects, when active fraction is used, safety evaluation is required.

The present study was carried out to assess the toxicity of the active fraction in root parts of *Stereospermum tetragonam*. Hence, the active fraction was subjected to acute and subchronic toxicity studies to further confirm these activities using animals. Furthermore, *S. tetragonum* root was not subjected to any kind of toxicity evaluation in light of modern science. Therefore, acute and sub-acute toxicity, if any, of the active fraction obtained from the water extract was determined in mice.

MATERIALS AND METHODS

Collection of plant materials

S. tetragonum root (family: Bignoniaceae) was collected from Tirunelveli and Chengalpatu districts of Tamil Nadu, India and identified by the taxonomist of TBGRI and a voucher specimen (TBGRI 8282) has been deposited in the institute herbarium.

Animals

Inbred albino mice (20-25 g weight), reared in TBGRI animal house were used. Animals were caged in uniform hygienic conditions and

fed with standard pellet diet (Lipton Indian Ltd, Bangalore) and water *ad libitum* as per the guide lines of Institute Animal Ethics Committee (IAEC). IAEC is controlled by Committee for the Purpose of Control and Supervision of Experiments on Animals [Approval No: B-31/03/2010/PPD-7 dated 31-03-2010].

Isolation of an active fraction (AF)

The water extract of *S. tetragonum* root powder was precipitated with absolute ethanol (1:1 v/v) and separated in to precipitate and soluble fractions. The activity was found in the soluble fraction. This active fraction (AF) was used for the toxicity studies.

Toxicity evaluation in mice

Acute oral Toxicity

Overnight fasted albino mice were used for the studies and provided only water, four groups of mice each containing three male mice (20-25 g body weight) was used. The therapeutic dose is 25 mg/kg. One group was kept as control and groups 2, 3, and 4 received 500, 1000, and 2000 mg/kg of active fraction (single dose). The animals were then observed for 14 days and maintained with normal food. Toxic symptoms for which the animals were observed for 72 hrs included behavioral changes, locomotion, convulsions and mortality. Cage side observations included changes in skin, fur, eyes, mucous membranes, respiratory, autonomic, central nervous systems, somato-motor activity and behavior pattern. Special attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Body weight, food and water intake were recorded [10].

Sub Acute Toxicity

To evaluate sub-acute (short term) toxicity, four groups of mice, each containing six male mice (20-25 g body weight) were used. Group one was kept as control and groups 2, 3, and 4 received 100, 200, and 400 mg/kg of active fraction, respectively, for 28 days (p.o.). Control group received the vehicle in an identical manner. The behavior of the animals was observed daily for 1h for 28 days. Initial and final body weights, water and food intake, state of stool and body temperature were also observed. The animals were sacrificed on the 29th day [11,12].

Estimation of serum biochemical parameters, and hematological parameters

Serum glucose was estimated spectrophotometrically using a commercial assay kit (Monozyme, India,Ltd). Liver with comparison standards. Glutamate pyruvate transaminase (GPT) and Glutamate oxaloacetate transaminase (GOT) [13] and alkaline phosphatase [14] were measured glycogen was estimated by the method [15]. Hemoglobin was measured using hemoglobinometer following standard methods. Urea, cholesterol, total lipids and protein were determined by conventional methods [16].

Histopathological studies

For histopathological study, sample of the organs (heart, kidney, spleen and liver) of the two groups were collected (control group and higher dose group) and fixed with 4% formaldehyde, dehydrated with ascending grades of alcohol and then embedded in paraffin (melting point 58-60°). From these blocks, 0.5 µm transverse sections were cut on rotary microtome and the sections were stained by haematoxylin and observed under microscope on different magnifications [17,18]. The histopathological studies were carried out with due permission from the ethical committee for research animal experimentation.

Statistical analysis

Statistical comparisons were done using one-way analysis of variance (ANOVA) followed by Dunnetts' test. $P < 0.05$ was considered significant.

RESULTS

There was no mortality or morbidity observed in animals through the 14-days period following single oral administration at all selected dose levels of the active fraction. The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose were appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal. Body weight as well as food and water intake were not significantly changed by the herbal drug administration (Table 1). In short term toxicity evaluation, administration of the active fraction (100, 200 and 400 mg/kg) daily for 28 days (p.o.) did not show any significant effect on body weight, food intake and water intake. (Table 2). Further, administration of the active fraction (100, 200 and 400 mg/kg) daily for 28 days (p.o.) did not show any significant effect on serum concentrations of glutamate pyruvate transaminase, glutamate oxalate transaminase, alkaline phosphatase, urea, cholesterol, HDL, LDL, triglycerides, creatinine, uric acid, total protein, albumin, globulin and total bilirubin compared with control values. The two higher doses slightly reduced serum glucose concentrations (Table 3). Moreover, the extract, even up to a dose of 400 mg/kg (16 times higher than the therapeutic dose) had no effect on red and white cell count, differential white cell count and blood haemoglobin (Table 4). The general behavior of the animals were also not altered by the treatment. Histopathological studies of heart, kidney, spleen and liver were performed after 28 days of continuous drug treatment (400 mg/kg), and in untreated animals. Figure 1 (a,b,c,d) shows the images of the different organs of the albino mice, normal control and the mice treated with active fraction(400 mg/kg). No significant changes were observed in the histology as well as normal anatomy of the skin, heart, kidney, spleen and liver treated and untreated groups of animals, Thus, histopathological studies show that the active fraction is non-toxic (400 mg/kg).

Table 1: Effect of single oral dose of the active fraction of *S. tetragonum* on body weight, food intake and water intake in mice

Treatment	Body weight			Food intake		Water intake	
	Day 1	Day 3	Day 13	Day 3	Day 13	Day 3	Day 13
Control	25.5 ± 0.8	24.5 ±1.1	27.5 ± 1.3	9.1± 0.9	9.0± 0.8	6.5 ± 0.9	6.3 ± 0.8
AF 500 mg/kg	25.8 ± 0.9	26.0 ± 0.6	30.2 ± 1.1	9.0± 0.7	9.4± 1.0	6.2 ± 0.9	6.6 ± 0.7
AF 1000 mg/kg	28.5 ±1.1	28.8 ± 0.7	31.6 ± 0.7	8.8± 0.9	9.1± 0.9	6.2 ± 0.9	6.1 ± 0.8
AF 2000 mg/kg	27.1 ± 0.8	27.6 ±1.4	31.3 ± 0.9	8.5± 0.8	8.9± 0.8	6.8 ± 0.9	6.6 ± 0.7

Values are mean ± S.D.; n=3; Values are not significantly different from each other.

Table 2: Effect of the active fraction of *S. tetragonum* on body weight, food intake and water intake in short term (28 days) toxicity study

Treatment groups	Initialbody weight (g)	Finalbody weight (g)	Food in take (g/mouse/day)	Water in take (ml/mouse/day)
Control	26.3 ± 0.6	30.1 ± 0.9	9.8 ± 0.2	6.2 ± 0.8
100 mg/kg AF	26.0 ± 0.8	29.7 ± 2.1	9.9 ± 0.4	6.9 ± 0.6
200 mg/kg AF	25.6 ± 1.1	29.8 ± 0.8	9.8 ± 0.2	6.4 ± 0.9
400 mg/kg AF	26.6 ± 0.7	30.7 ± 0.7	9.9 ± 0.5	6.2 ± 0.6

Values are mean ± S.D.; n = 6 in each group; Values are not significant compared to control values (P ≥ 0.5); Food and water intake were measured on 28th and 29th days (values are given per day)

Table 3: Effect of the active fraction of *S. tetragonum* on serum biochemical parameters in short term (28days) toxicity studies

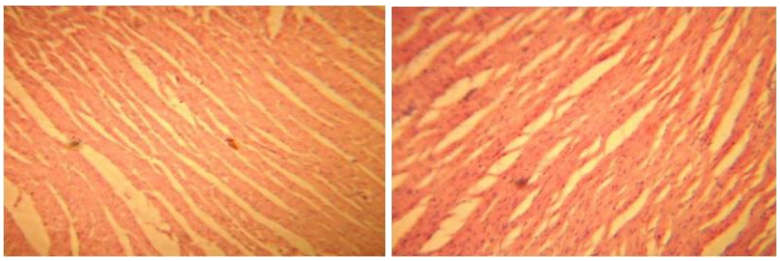
Parameters	Active fraction (mg/kg)			
	Control (0)	100	200	400
SGPT (IU/L)	10.3 ± 0.9	10.0 ± 1.2	11.0 ± 1.0	12.0 ± 2.0
SGOT (IU/L)	18.7 ± 1.7	18.3 ± 0.7	18.5 ±1.5	19.0 ± 1.9
ALP (IU/L)	67.6 ± 2.8	70.0 ± 2.1	66.5 ± 2.5	70.0 ± 2.3
Urea (mmol/L)	9.1 ± 0.9	9.2 ± 0.8	9.4 ± 1.0	9.3 ± 1.1
Glucose (mmol/L)	5.6 ± 0.3	5.4 ± 0.3	5.1 ± 0.3*	5.0 ± 0.3**
Cholesterol (mmol/L)	4.1 ± 0.2	4.1 ± 0.3	4.2 ± 0.2	4.1 ± 0.3
HDL (mg/dl)	47.6 ± 1.9	50.0 ± 1.2	48.5 ± 2.5	50.0 ± 2.3
LDL (mg/dl)	97.1 ± 0.9	96.1 ± 0.6	96.1 ± 1.0	96.0 ± 0.4
Triglycerides (mmol/dl)	6.1 ± 0.2	6.2 ± 0.3	6.1 ± 0.3	6.3 ± 0.2
Creatinine (µmol/L)	88.3 ± 4.1	85.3 ± 5.1	91.1 ± 5.3	93.3 ± 5.9
Total protein (g/L)	73.2 ± 1.9	72.1 ± 2.1	74.0 ± 2.5	69. 5 ± 4.3
Albumin (g/L)	47.3 ± 4.4	46.4 ± 2.4	48. 8 ± 3.4	49.5 ± 5.1
Globulin (g/L)	35.0 ± 2.2	36.1 ± 2.3	37.0 ± 2.2	37.1 ± 5.0
Direct bilurubin (µmol/L)	2.22 ± 0.13	2.21 ± 0.14	2.30 ± 0.15	2.32 ± 0.13
In direct bilurubin (µmol/L)	3.26 ± 0.23	3.23 ± 0.25	3.27 ± 0.25	3.76 ± 0.22*

SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxalate transaminase; ALP, alkaline phosphatase; LDL, low density lipoprotein; HDL, high density lipoprotein; Values are mean ± S.D.; n=6, * P<0.05, ** P<0.01 (compared to control).

Table 4: Effect of the active fraction of *S. tetragonum* on hematological parameters in short term (28 days) toxicity studies

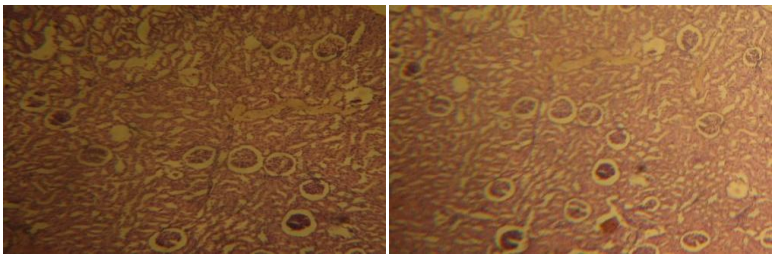
Parameters	Active fraction (mg/kg)			
	Control (0)	100	200	400
Hb (gm%)	14.73 ± 0.4	14.60 ± 0.2	14.80 ± 0.3	14.90 ± 0.5
WBC (cells/cumm)	5900 ± 278	5925 ± 325	5975 ± 425	6100 ± 50
RBC (m3/ml)	4.75 ± 0.15	4.76 ± 0.14	4.85 ± 0.10	5.00 ± 0.10
PCV (%)	45.00 ± 1.30	45.80 ± 1.50	45.95 ± 1.65	45.50 ± 0.95
MCV (fl)	92.59 ± 5.41	94.35 ± 4.65	94.75 ± 4.42	94.15 ± 1.35
MCHC (g/dl)	34.00 ± 4.50	36.36 ± 2.78	36.61 ± 1.31	37.80 ± 0.78
Differential count				
Neutrophil	52.33 ± 0.88	52.00 ± 0.57	53.00 ± 1.00	53..33 ± 2.40
Lymphocyte	36.33 ± 1.73	37.66 ± 1.45	35.70 ± 2.00	37.50 ± 1.45
Eosinophil	4.66 ± 0.88	4.33 ± 2.18	4..50 ± 1.50	4.9.3 ± 1.33
Monocyte	5.73 ± 0.43	5.60 ± 0.25	5.80 ± 0.44	5.90 ± 0.50

PCV, Packed cell volume; MCV, Mean corpuscular volume; MCHC, Mean corpuscular hemoglobin concentration; Values are mean ± S.D.; n=6 (compared to control, values are not significant)



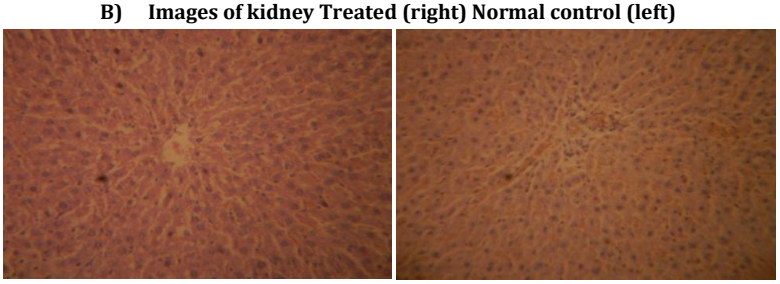
Normal control **Treated with drug**

A) Images of Heart Treated (right) Normal control (left)



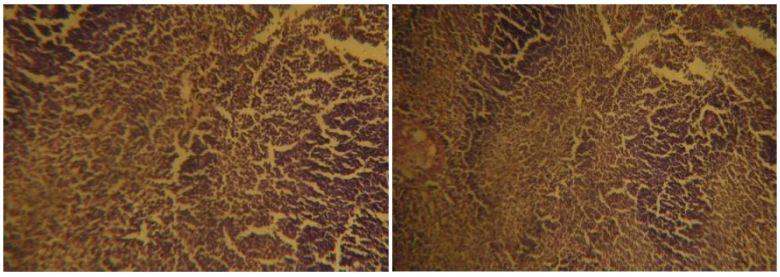
Normal control **Treated with drug**

B) Images of kidney Treated (right) Normal control (left)



Normal control **Treated with drug**

C) Images of Liver Treated (right) Normal control (left)



Normal control **Treated with drug**

D) Images of Spleen Treated (right) Normal control (left)

Fig. 1: Images of the organs of albino mice showing no toxic effect

DISCUSSION AND CONCLUSION

The study reports for the first time that the active fraction obtained from the water extract is safe as judged from the limited acute and short term toxicity evaluation in mice. The highest dose studied in sub-acute toxicity is 16 times higher than therapeutic dose. Even at this relatively high dose the herbal drug appears to be very safe. However, detailed toxicity studies using different species remains to be studied. Reproductive toxicity, genotoxicity, long term toxicity, if any, remains to be studied. Since the plant root is used in ethno-medical studies from ancient time on wards without any recorded or known adverse effects, the active fraction is likely to be devoid of any toxic effects. Although there are considerable numbers of drugs to treat type-2 diabetes in the market, in long term treatment, immune response dependent or other toxicity develop; the drug becomes in effective in some cases. In this context, the herbal drug is likely to be useful as an alternative medicine. The toxicity study report strongly suggests that the herbal drug is safe. Therefore, further follow up studies are warranted to develop phytomedicines for DM from this plant. Since *S. tetragonum* root is used in traditional medicine mortality is not expected even at relatively very high doses. Therefore, attempts were not made to determine the dose required for 50 % mortality.

The present findings suggest that active fraction of stereospermum tetragonam is non toxic, no marked changes in hematological, biochemical, and histopathological parameters were observed. Thus the active fraction is considered to be safe for long term treatment of diabetes.

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REFERENCE

1. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr. J. Biotechnol 2008; 7(6): 701-705.
2. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J. Health Sci. 2007; 53(4): 351-358.
3. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J, Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.) Roxb (Ceasalpiniaceae) Afr. J. Biotechnol 2006; Vol. 5(3): 283-289.
4. Tedong L, Paul Desire Djomeni Dzeufiet, Theophile Dimo, Emmanuel Acha Asongalem, Selestin Ndogmo Sokeng , Jean-François Flejou, Patrice Callard, Pierre Kamtchouing. Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr. J. Tradit. Complement. 2007; 4 (2): 140-147.
5. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. J Ethnopharmacol 2007; 112: 138-144.
6. Rhiouani H, El-Hilaly J, Zafar H Israili, Badiaa Lyoussi. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. J Ethnopharmacol 2008; 118: 378-386.
7. Wagner H, Bladt S, Zgainski EM. Springer-Verlag, Berlin Heidelberg, New York, Tokyo. 1984; 197.
8. Bino Kingsley R, Ajikumaran Nair S, Anil John J, Mishra M, Brindha P, Subramoniam A. Anti-diabetes mellitus activity of *Stereospermum tetragonum DC*. in alloxan diabetic rats J Pharmacol Pharmacother 2012; 3: 191-193.
9. Renjit Bino Kingsley, Manisha Mishra, Pemaiah Brindha, Appian Subramoniam. Anti-diabetic activity of active fractions of *Stereospermum tetragonum* DC and isolation of active principles J Young Pharmacists. 2013; 3: 7-12.
10. Shajeela PS, Kalpanadevi. V, Mohan VR. Potential antidiabetic, hypolipidaemic and antioxidant effects of *Xanthosoma sagittifolium* extract in alloxan induced diabetic rats Int J Pharm Pharm Sci. 2013; 5 suppl 1: 27-31.
11. Ajikumaran NS, Shylesh BS, Gopakumar B, Subramoniam A. Anti-diabetes and hypoglycemic properties of *Hemionitis arifolia* in rats. J Ethnopharmacol. 2006; 106: 192-197.
12. Yee KM, Noraisyah Z, Vandana KC, Yogendra KC. Acute and Sub-acute oral toxicity of *Polygonum minus* aqueous extract in wistar rats. Int J Pharm Pharm Sci. 2013; 5:120-124.
13. Reitman S, Frankel S. Colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. Am. J. Cilin. Pathol. 1957; 28: 56-63.
14. Kind PRN, King GJ. Estimation of plasma alkaline phosphatase by determination of hydrolysed phenol with antipyrine. J. Clin. Pathol. 1954; 70: 322-330.
15. Caroll NV, Longly RW, Joseph HR. Determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol. Chem. 1956; 220: 583-593.
16. Jam NC. Schalm's veterinary haematology. Philadelphia: Lea and Fabiger; 1986. pp.43-48.
17. Millard PR. Essential Histopathology, London, Blackwell Scientific Publications, 1990.