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Original Article

Acute and subacute toxicity study of 'Peptoshis', a digestive disorder protective polyherbal formulation in Wistar albino rat

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ABSTRACT

Present study focused the toxicity screening of 'Peptoshis', a polyherbal formulation composed of thirteen medicinal plants for the management of digestive disorders i.e. indigestion, constipation, loss of appetite, dyspepsia etc. As oxidative stress is one of the consequences of digestive disorders so the activities of hepatic and pancreatic antioxidant enzymes along with lipid peroxidation levels were evaluated. For acute toxicity evaluation of 'Peptoshis', it was administered orally at the dose ranging from 25, 50, 100, 200, 400, 800, 1600, 3200 mg/kg body weight. Insignificant variations were noted in antioxidant enzymes (i.e. catalase, glutathione-S-transferase, super oxide dismutase) activities along with the lipid peroxidation level in between vehicle control (Group-I) and Peptoshis treated control (Group-II) animals. Treatment of 'Peptoshis' to the control animals (Group-II) did not reflect any significant variation in the levels of hematological and liver function test (LFT) biosensors in respect to the vehicle control animals (Group-I). Acute toxicity study illustrate, there was no toxic symptom up to the dose level of 3200 mg/kg. From these results, it may be stated that 'Peptoshis' at the dose of 50 mg/kg is safe for long term treatment of digestive disorders management.

Key words*:* Peptoshis, digestive disorders, antioxidant enzymes, lipid peroxidation, Wistar rat

INTRODUCTION

The gastrointestinal (GI) tract is a long muscular tube that functions as the food processor for the human body. The GI tract is not a passive system it has the capability to sense and react to materials passed through it. For a healthy digestive system, every person requires different food selections that match their GI tract capacity. It is estimated that some form of digestive disorder affects more than 100 million people in America [1, 2]. For some people, digestive disorders are a source of irritation and discomfort that may cause them to drastically limit their lifestyles and frequently miss work. For others, the disorders may be extremely crippling and even fatal. Aging also causes many people to experience problems with digestion. It is estimated that after age 40 there is an approximate decrease of 20-30% in the body's ability to produce enzymes [3]. The use of specific enzymes can help improve the efficiency of digestion. Enzymes can be used to enhance the proper breakdown of foods in order to more properly digest, absorb, and utilize nutrients. The pancreas and liver are digestive organs that produce most of the body's digestive enzymes [4, 5]. The remainder should come from uncooked foods, such as fresh fruits and vegetables, raw sprouted grains, seeds and nuts, unpasteurized dairy products, and enzyme supplements. The function of the liver is to control the food supply for the rest of the body by further processing the food molecules absorbed through the intestines. The liver does this by dispensing those food molecules in a controlled manner and filtering out toxins that may have passed through the GI tract wall [6].

The importance of medicinal plants in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation is now well recognized. However, information on the uses for plants for medicine is lacking from many interior areas of our country [7]. In India, more than 43% of the total flowering plants are reported to be of medicinal importance. Herbal formulations, which have attained widespread acceptability as therapeutic agents in India, include antidiabetics, hepatoprotective agents, digestive

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disorders protective and lipid-lowering agents [8]. The pharmacological effects of many plants have been studied in various laboratories, where as there are many limitations regarding the safety and efficacy of these preparations [9]. 'Peptoshis' is a digestive disorders management polyherbal formulation containing thirteen medicinal plant parts; it is used in traditional medicine to treat digestive disorders. In our state (West Bengal, India), 'Peptoshis' is used in Ayurvedic medicine for the management of digestive disorders. Its constituents are *Emblica officinalis, Terminalia chebula, Spondias pinnata, Zingiber officinalie, Syzygium aromaticum, Myristica fragrans, Foeniculum vulgare, Carum copticum, Elettaria cardamomum, Operculina turpethum, Piper nigrum, Piper longum and Glycyrrhiza glabra* (**Table-1**). Inspite of the past reputation of 'Peptoshis' as a medicine at the local level, the scientific basis of its mode of action on digestive disorders management and toxicity profile have not been investigated. We investigated the toxicity and digestive disorders protective effects of 'Peptoshis' to assess its safety and tolerability profile for long term treatment.

MATERIALS AND METHODS

Preparation of polyherbal formulation 'Peptoshis'

Above mentioned thirteen medicinal plants which are used for the preparation of polyherbal formulation 'Peptoshis', have been provided by Pharmaceutical Division of Southern Health Improvement Samity (SHIS), 24 -Parganas (S), West Bengal, India. The plants were taxonomically identified by Prof. R. K. Bhakat, Department of Botany and Forestry, Vidyasagar University, West Bengal, India. Herbal specimens in the form of dried samples are preserved in the Departmental Herbarium Museum.

Desired parts of thirteen medicinal plants (**Table-1**) were dried in an incubator for 24 hours at 37 degrees Celsius, crushed separately in an electrical grinder and then pulverized. Powder forms of uses parts of medicinal plants were mixed in fixed ratio as per Table-1 and named as 'Peptoshis'. Polyherbal formulation of 'Peptoshis' has been prepared on the basis of an Ayurvedic digestive disorders protective formulation proposed by some workers [10].

Table 1: Composition of ingredient(s) present in polyherbal formulation, 'Peptoshis'

Botanical Name	Family	Common Name	Part(s) Used	Ingredients of 'Peptoshis'(stock sample)*
Emblica officinalis	Phyllanthaceae	Amloki	Fruit	40 mg
Terminalia chebula	Combretaceae	Haritaki	Fruit	40 mg
Spondias pinnata	*Anacardiaceae*	Amada	Roots	40 mg
Zingiber officinalie	Zingiberaceae	Adrakam	Rhizome	20 mg
Syzygium aromaticum	Myrtaceae	Lavang	flower	10 mg
Myristica fragrans	Myristicaceae	nutmeg	Seeds	10 mg
Foeniculum vulgare	Apiaceae	Mouri	Seeds	10 mg
Carum copticum	Apiaceae	Ajwain	Seeds	25 mg
Elettaria cardamomum	Zingiberaceae	Elaich	Seeds	10 mg
Operculina turpethum	Convolvulaceae	Krishnatribit	Leaves	20 mg
Piper nigrum	Piperaceae	Marich	Seeds	20 mg
Piper longum	Piperaceae	Pipul	Seeds	25 mg
Glycyrrhiza glabra	Fabaceae	Yastimadhu	Seeds	30 mg

***** This stock sample was used as per dose mentioned in the experiment.

Experimental design

Acute toxicity study of Peptoshis: The acute toxicity studies (LD_{50} determination) were carried out by the method described by Mythilpriya et al. [11], in which healthy Wistar albino male rats weighing about 90 ± 10 g were randomly distributed to 8 different groups with six animals in each group. Animals were housed in Polyvinyl Chloride (PVC) cages at an ambient temperature of 25 ± 2 ^{0}C with 12 h light: 12 h dark cycle. Rats have free access to standard food and water *ad libitum*. Permission from Animal Ethical Committee (AEC) was obtained for the conduction of this experiment. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by our Institutional Ethical Committee (IEC) was followed regarding the treatment of animals. The animals were fasted overnight and the 'Peptoshis' was administered orally at the dose levels of 25, 50, 100, 200, 400, 800, 1600, 3200 mg/kg body weight. After the treatment of 'Peptoshis', the animals in each group were observed for awareness, interactivity, posture, tremors, salivation, diarrhea, lethargy, sleep, coma and death as well as continuous observation for the first four hours up to 14 days to find out the mortality if any [12].

Sub-acute toxicity study of Peptoshis

Sub-acute toxicity study was conducted as per method described by Ghosh [13]. Wistar albino male rats weighing about 210 ± 10 g were divided into following two equal groups (n=6) and were housed under the same conditions as described above.

Group I (Vehicle control) received 0.5 ml of distilled water/100 gm body weight/day/rat for 29 days by gavage forcefully.

Group II (Peptoshis treated control) control rats were forcefully feed by gavage of polyherbal formulation i.e. 'Peptoshis' at a dose of 5 mg/0.5 ml of distilled water/100 gm body weight/rat/day at early in the morning and fasting condition for 29 days.

[This dose was selected from our pilot study using doses starting from 2 mg up to 20 mg/100 gm body weight where the said dose (5 mg/100 gm body weight) was noted as threshold dose. In traditional medicine, the dose of 'Peptoshis' given to the human as the dose of 20 mg-30 mg/ kg body weight (2-3 mg/ 100 gm body weight).]

All animals were observed daily for toxic manifestation, mortality, body weight, food intake and water intake etc. On the 30th day of experiment all the animals were sacrificed by light ether anaesthesia followed by decapitation after recording the final body weight. About 6 ml of blood were collected from dorsal aorta of each rat. Out of 6 ml, 2 ml was dispended into ethylene diamine tetra acetic acid (EDTA) for hematological study and remaining 4 ml into heparin for biochemical analysis. Liver, kidney testis and cerebrum were excised and weights were determined. After that some small pieces of liver and pancreas tissues were stored in Bouin's fixative for histological study. Remaining parts of said tissues (liver and pancreas) were stored at -20 ^{0}C for the assessment of antioxidant enzymes activities according to the standard protocol i.e. catalase (CAT), peroxidase (Px), glutathione-S-transferase (GST) and super oxide dismutase (SOD), as well as quantification of thiobarbituric acid reactive substances (TBARS) respectively [14-18].

Liver function test (LFT) biosensors: For liver function test (LFT), we were determined the levels of serum total billirubin (TB), and activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP) by using standard kits [19-21].

Hematological markers: Hemoglobin (Hb), red blood cell (RBC) count, total leukocyte count (TC), packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) were determined by standard techniques [22].

Statistical analysis: Analysis of Variance (ANOVA) followed by two-tail 't' test was used for statistical analysis of collected data [23]. Differences were considered significant at the level of $p<0.05$. All the values were indicated in the tables and figures by Mean ± SEM.

RESULTS

Acute toxicity study of 'Peptoshis': In the acute toxicity study, 'Peptoshis' up to the dose level of 3200 mg/kg of body weight did not exhibit any lethality or toxic symptoms. There was no mortality or morbidity observed in animals through 14 -day period following single oral administration at all selected dose levels of 'Peptoshis'. No tremors, salivation, diarrhoea, sleep, coma, death or unusual behaviors such as self-walking backward, reactivity to handling were all normal. The LD_{50} value for oral administration of 'Peptoshis' is larger than 3200 mg/kg body weight. As 50 mg/kg body weight was well tolerated by the animals without any behavioral changes during long term treatment, further sub-acute toxicity study was also carried out with 50 mg/kg of body weight (**Table 2**).

Sub-acute toxicity study of 'Peptoshis'

Body weight and organo-somatic indices: 'Peptoshis' treatment for 29 days resulted no significant ($p>0.05$) changes in the organo-somatic indices i.e., hepato-somatic, reno-somatic, testiculo-somatic and cerebro-somatic indices along with the body weight in respect to the vehicle control group (**Table 3**).

Antioxidant enzymes activities and lipid per oxidation levels: The hepatic peroxidase (Px) enzyme activity was decreased significantly ($p<0.05$) in 'Peptoshis' treated control animals (Gr II) in respect to vehicle control animals (Gr I). However, insignificant variations were noted in the activities of antioxidant enzymes i.e. catalase (CAT), super oxide dismutase (SOD), glutathione-S-transferase (GST) along with the lipid per oxidation level i.e. thiobarbituric acid reactive substances (TBARS) in the liver and pancreatic tissues in between 'Peptoshis' treated control and vehicle control animals (**Table 4 and 5**).

Liver function test (LFT) biosensors: Activity of serum alkaline phophatase (ALP) was decreased significantly in 'Peptoshis' treated control animals (Gr II) in respect to vehicle control animals (Gr I). Treatment of 'Peptoshis' to the control animals resulted an insignificant variation were noted in the

levels total billirubin (TB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) in between vehicle control and 'Peptoshis' treated control animals (**Table 6**).

Hematological parameters: Significant diminution was noted in the erythrocyte sedimentation rate (ESR) after treatment of 'Peptoshis' to the control animals (Gr II) when comparison was made with the vehicle control animals (Gr I). However, insignificant deviation were noted in hemoglobin (Hb) level, red blood cell (RBC) count, total leukocyte count (TC), packed cell volume (PCV) in between vehicle control (Gr I) and 'Peptoshis' treated control animals (Gr II) (**Table 7**).

Histopathological evaluation of liver and pancreas tissues: Histological observation of liver of the vehicle control animals shows the normal architecture of prominent central vein and irradiated hepatic cells. Treatment of 'Peptoshis' to the control animals for 29 days resulted no significant changes in the architecture of central vein and irradiated hepatic cells in respect to vehicle control animals (**Fig. 1**). Vehicle control group also showed normal histoarchitecture of pancreatic islet of Langerhans and acinar cells. No significant changes in the histoarchitecture of pancreatic islet and acinar cells in 'Peptoshis' treated control group in respect to the vehicle control group (**Fig. 2**).

DISCUSSION

An Ayurvedic polyherbal formulation, 'Peptoshis', is used for the treatment of digestive disorders (indigestion, constipation, loss of appetite, dyspepsia etc.) as folk medicine but toxicological profiles was not known to us. For such assessment we have studied organo-somatic indices, LFT biosensors and serum toxicity marker enzymes along with hematological parameters. Moreover, we have also assessed the oxidative status and lipid peroxidation level in liver and pancreas tissues of experimental animals as metabolic disorders has a strong association with oxidative injury [24]. Usually acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the 'Peptoshis', as there was no previous report on toxicity of 'Peptoshis'. Here eight dose levels starting at 25, 50, 100, 200, 400, 800, 1600, 3200 mg/kg were selected for acute toxicity study. No mortality was observed in polyherbal formulation treated groups. Animals in all groups did not exhibit any sign of adverse effect. According to Organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe.

In sub-acute toxicity study, the polyherbal formulation was given orally at the dose of 50 mg/kg body weight. There was no change in animal behaviour, organo-somatic indices and changes in body weight were not significantly different in between vehicle control and 'Peptoshis' treated control animals. Since, the changes in body weight have been used as an indicator of adverse effect of drugs and chemicals [25, 26], the present results suggest that at the dose levels administered, 'Peptoshis' is non-toxic digestive disorders management polyherbal formulation. The CAT, SOD, Px and GST antioxidant enzymes are mutually supportive team of defence against reactive oxygen species (ROS) [27]. Decrease in the activities of above mentioned antioxidant enzymes this would cause an increased accumulation of highly- reactive free radicals (i.e. peroxy, super oxide, hydroxyl etc.), leading to harmful effects such as loss of integrity and function of cell membranes. Treatment of 'Peptoshis' to the control animals resulted no significant alteration in the antioxidant enzymes activities along with the lipid peroxidation level in respect to the vehicle control animals. These results suggested that 'Peptoshis' was not adverse effect to the antioxidant defense mechanism, not interfere the free radicals production. Treatment of 'Peptoshis', to the control animals for 29 days resulted there was no significant changes in the hematological parameters i.e. hemoglobin, RBC, TC, PCV, and ESR when comparison was made with the vehicle control animals. These results indicated that 'Peptoshis' was not toxic to the circulating red cells, not interfere with their production. Hematopoiesis and leucopoiesis were also not effected even though the haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals [28]. Therefore, it possible to assume that 'Peptoshis' is not hematotoxic. 'Peptoshis' treatment to the control animals resulted there was no significant changes in any liver function test (LFT) parameters, such as serum total billirubin, GOT, GPT and ALP when compared with the vehicle control animals. Increase in these LFT parameters would have indicates hepatocyte damage [29]. In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased LFT biosensors level in serum. The normal levels of LFT parameters indicate that the 'Peptoshis' did not interfere with hepatic function and that hepatic integrity was preserved.

CONCLUSION

At the oral doses tested, the formulation was well tolerated and neither produced over signs of clinical toxicity nor any signs of oxidative stress, hepato, pancreato or hematotoxicity. Thus 'Peptoshis' was found to be non-toxic digestive disorders protective formulation when oral acute and sub-acute dose toxicities were performed. The actual mechanism is not clear and further biochemical and pharmacological investigations are needed to isolate and identify the active ingredient(s) within the 'Peptoshis'. Overall, this study provides valuable data on toxicity profile of 'Peptoshis' that should be useful for the planning of future preclinical and clinical studies of the formulation.

Table 2: Determination of median lethal dose (MLD) or LD_{50} of the 'Peptoshis' administered orally

Dose of Peptoshis (mg/kg)	Number of animals used	Number of survival	Number of death	MLD (LD_{50})
25	6	6	0	
50	6	6	0	
100	6	6	0	
200	6	6	0	
400	6	6	0	
800	6	6	0	
1600	6	6	0	
3200	6	6	0	>3200 mg/kg

Table 3: Effect of 'Peptoshis', a polyherbal formulation, on body weight and organo-somatic indices in male albino rat.

Groups	Body weight (gm)		Organs-Somatic Indices (gm %)			
	Initial	Final	Liver	Kidney	Testis	Cerebrum
Vehicle control	212.07±5.21	142.02±4.53	2.93±0.15	0.72±0.07	0.80±0.05	0.62±0.03
Peptoshis treated control	214.57±5.06	144.43±4.76	2.90±0.13	0.73±0.06	0.82±0.06	0.64±0.04

All the values denote Mean ± SEM (n = 6). Statistical analysis followed by student two tail 't' test'. Values in each vertical column did not differ significantly from other, $p>0.05$.

Table 4: Remedial effect of 'Peptoshis' on the activities of hepatic antioxidant enzymes and lipid peroxidation level in male albino rat.

Groups	CAT (mM of H_2O_2 consumption/mg of tissue/min)	Px (Unit/mg of tissue)	SOD (Unit/mg of tissue)	GST (Unit/mg of tissue)	TBARS (nM/mg of tissue)
Vehicle control	4.85±0.74	2.06±0.03	1.25±0.07	2.30±0.07	90.26±4.72
Peptoshis treated control	4.87±0.67	1.72±0.02*	1.26±0.06	2.32±0.08	89.15±5.06

All the values are expressed as Mean ± SEM, n=6. Statistical analysis followed by student two tail 't' test where * indicate $p<0.05$ compare with vehicle control group.

Table 5: Effect of 'Peptoshis' on the activities of pancreatic antioxidant enzymes and lipid peroxidation level in male albino rat.

Groups	CAT (mM of H_2O_2 consumption/mg of tissue/min)	Px (Unit/mg of tissue)	SOD (Unit/mg of tissue)	GST (Unit/mg of tissue)	TBARS (nM/mg of tissue)
Vehicle control	5.02±0.42	2.66±0.06	0.52±0.04	1.54±0.06	69.46±4.91
Peptoshis treated control	4.96±0.39	2.64±0.09	0.46±0.03	1.52±0.04	70.51±4.76

All the values are expressed as Mean ± SEM, n=6. Statistical analysis followed by student two tail 't' test'. Values in each vertical column did not differ significantly from other, $p>0.05$.

Table 6: Remedial role of 'Peptoshis', on liver function test (LFT) biosensors in male albino rat.

Groups	TB (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Vehicle control	1.06±0.04	23.64±3.69	59.21±4.2	118.94±6.46
Peptoshis treated control	1.07±0.03	21.36±3.32	57.69±3.82	110.07±5.95*

All the values are expressed as Mean ± SEM, n=6. Statistical analysis followed by student two tail 't' test where * indicate $p<0.05$ compare with vehicle control group.

Table 7: Remedial effects of 'Peptoshis', on hematological parameters in male albino rat.

Groups	Hb (%)	RBC (10^6/mm^3)	TC (10^3/mm^3)	ESR (mm/1st hr)	PCV (%)
Vehicle control	11.8±0.38	7.22±0.27	5.18±0.06	8.21±0.25	32.88±4. 1
Peptoshis treated control	12.0±0.41	7.25±0.30	5.20±0.08	7.90±0.30*	33.62±3.6

All the values are expressed as Mean ± SEM, n=6. Statistical analysis followed by student two tail 't' test where * indicate $p<0.05$ compare with vehicle control group

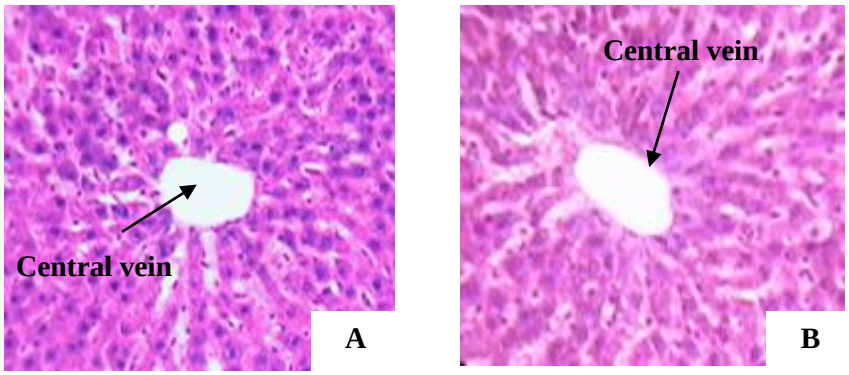

Figure 1: Effect of 'Peptoshis' on histopathological changes of liver that occurred in male albino rats. Plate-A (Vehicle control); Plate-B (Peptoshis treated control). (Hematoxylin- eosin stain, original magnification X 400).

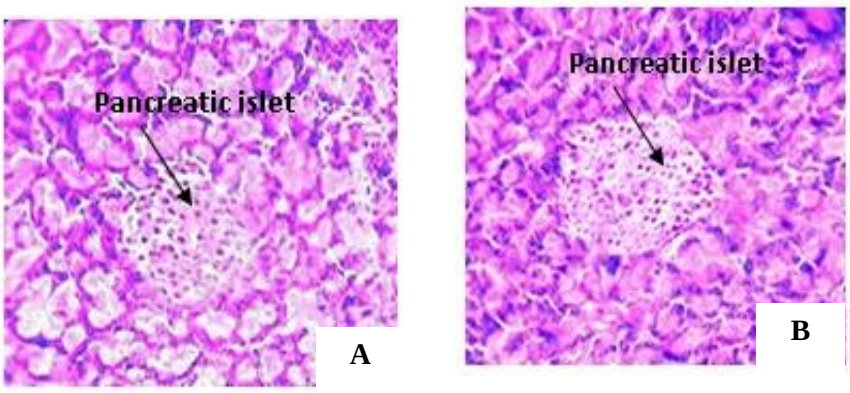

Figure 2: Effect of 'Peptoshis' on histopathological changes of pancreas that occurred in male albino rats. Plate-A (Vehicle control); Plate-B (Peptoshis treated control). (Hematoxylin- eosin stain, original magnification X 400).

REFERENCES

1. O'Mahony R et al. Bactericidal and anti-adhesive properties of culinary and medicinal plants against Helicobacter pylori. World J Gastroenterol 2005; 11:7499-507.
2. Olukoga A, Donaldson D. Liquorice and its health implications. J Res Soc Health 2000; 120: 83-9.
3. Fukai T et al. Anti-Helicobacter pylori flavonoids from licorice extract. Life Sci 2002; 71: 1449-63.
4. Langmead L, Rampton DS. Review article: herbal treatment in gastrointestinal and liver disease-benefits and dangers. Aliment Pharmacol Therap 2001; 15: 1239-52.
5. Richmond BL et al. Compensatory phospholipids digestion is required for cholesterol absorption in pancreatic phospholipase A(2)-deficient mice. Gastroenterol 2001; 120: 1193-1202.
6. Matsuzaka M et al. The decreasing burden of gastric cancer in Japan. Tohoku J Exp Med 2007; 212: 207-19.
7. Bhandary MJ et al. Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol 1995; 47: 149-58.
8. Ray A et al. Antioxidant activity of ethanol extract of rhizome of *Picrorhiza kurra*on indomethacin induced gastric ulcer during healing. Ind J Clin Biochem 2002; 17: 44-51.
9. Kuruvilla A. Herbal formulations as pharmacotherapeutic agents. Ind J Exp Biol 2002; 40: 7-11.
10. Pandey VN et al. An effective ayurvedic hypoglycemic formulation. J Res Ayur Sid 1995; 16:1-14.
11. Mythilpriya R et al. Oral acute and subacute toxicity study with Kalpaamrutha a modified indigenous preparation on rats. J Health Sci 2007; 53: 351-8.
12. Abu TN, et al. Acute and subacute toxicity studies of *Persea americana* Mill (Avocado) seed in rats. Int J Medical Toxicol Legal Med 2008; 11: 10-6.
13. Ghosh NM. Acute toxicity test. Fundamentals of Experimental Pharmacology. Kolkata: Scientific Book Agency, 1984: 178-83.
14. Beers RF, Sizer IW. Spectrophotometric method for measuring the breakdown of hydrogen peroxidase by catalase. J Biol Chem 1952; 195:133-40.
15. Sadasivam S, Manickam A. Peroxidase: In: Methods in Biochemistry. New Delhi: New Age International Pvt. Ltd, 1996: 108-10.
16. Hobig WH et al. Glutathione-S- transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249:7130-9.
17. Marklund S, Marklund G. Involvement of superoxide anione in autooxidation of pyrogallol and a convenient assay of superoxide dismutase. Europian J Biochem 1974; 47: 469-74.
18. Okhawa H et al. Assay for lipid peroxidation in animal tissues thiobarbituric acid reaction. Annal Biochem 1979; 95: 351-8.
19. Doumas BT. Candidate reference method for determination of total billirubin in serum: Development and Validation. Clin Chem 1985; 31: 1779-83.
20. Henry RJ et al. Revised spectrophotometric methods for the determination of glutamate oxaloacetic transaminase, glutamic pyruvate transaminase and lactic acid dehydrogenase. Am J Clin Pathol 1960; 34: 381-98.
21. Horecker BL. Alkaline phosphatase. In: Methods of Enzymology. New York: Academic Press, 1966: 639-42.
22. Godkar PB, Godkar DP. Routine hematological tests. In: Text book of Medical Laboratory Technology. Mumbai: Bhalani Publishing House, 2005: 726-50.
23. Sokal RR, Rohle FJ. Introduction to Analysiy of Variance. In: Methods in Biometry. New York: WH Freeman and Company, 1997: 179-206.
24. Gupta AK, Misra N. Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats. Am J Pharmacol Toxicol 2006; 1: 17-20.
25. Winder CV et al. Comparative bioassay of drugs in adjuvant induced arthritis in rats, flufenamic acid, mefenemic acid and phenyl butazone. Arthr Rheumatol 1969; 12: 472-82.
26. Teo S et al. A 90-days oral gavage toxicity study of D-methyl penidate and DL-methyl penidate in Sprague-dawley rats. Toxicol 2002; 179: 183-96.
27. Bera TK et al. Antidiabetic and antioxidative effects of aqueous extract of seed of *Psoralea corylifolia* (somraji) and seed of *Trigonella foenum-graecum* L. (methi) in separate and composite manner in streptozotocin-induced diabetic male albino rat. Int J Pharm Res Dev 2009; 7: 1-10.
28. Adeneye AA et al. Hematological evaluation of methanol seed extract of citrus. J Ethnopharmacol 2006; 105: 374-9.
29. Aniagu SO et al. Toxicity studies in rats fed nature cure bitters. Afr J Biotechnol 2005; 4: 72-8.