International Journal of Drug Delivery 3 (2011) 340-347

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Acute effect of gibberellic acid on serum enzymes and blood markers in male albino rats

S. Muthu[1], P. Muthuraman[1], V. Muthuviveganandavel[1], K. Srikumar[1]

***Corresponding author:**

K.Srikumar Ph.D., FABMS
1 Dept. of Biochemistry & Molecular Biology,
School of Life Sciences,
Pondicherry University,
Kalapet, Puducherry-605014,
India
Tel: +91-413-2654422
Email: frenzram@gmail.com

Abstract

This study was designed to evaluate the influence of a phytohormone, gibberellic acid (GA_3) on marker enzymes and biomarkers of serum, and blood hemoglobin and its blood cells counts of rat. In order to evaluate the positive/negative effects, the rats were administered 75µg, 150µg and 300µg of GA_3/kg body weight as a single dose. GA3 treatments produced differential effects on the different parameters at dose dependent manner after 4 hours.

The down regulation in specific activities of ALT, ALP, GGT and amylase were noted against the control with significant up regulation of AST activity. GA3 also produced dose dependent effect on biomarkers. There is a substantial reduction in the quantity of glucose, urea, creatinine, calcium, phosphorus, sodium and potassium was recorded against the control. On the other hand, the insignificant increase in content of total protein, albumin and uric acid was observed at all dose of GA3 treatment against the control. GA3 increased the RBC, WBC and neutrophil by decreasing the lymphocyte total numbers. Platelets, monocytes and oesinophils count were not altered by any dose of GA3.

In conclusion, GA3 produced dose dependent effect on different parameters of rat blood serum.

Keywords: phytohormone; gibberellic acid; creatinine; neutrophil

Introduction

Plant growth regulators (PGRs) gave entry into animal cells through diet. Gibberellic acid (GA_3) is an extensively prevalent plant growth regulator due to its use in agriculture. It is a diterpene hormone derived from acetyl Co A through the mevalonic acid metabolic pathway in plants. GA_3 plays a significant role in plant cellular processes such as in cell elongation, breaking dormancy in seeds and buds, in flowering and parthenocarpic fruit development and in the mobilization of food reserves in grass, in seed germination and in juvenility and sex expression (1). GA_3 is known to be ubiquitously present in the plant kingdom.

Recent research has indicated that Plant growth regulators expressed biological potentials in animal cells. It is reported that fecundity, longevity and egg vitality in insects was affected by PGRs treatment (2,3). Ozmen et al (4) observed that abscissic acid and GA_3 affected sexual differentiation and other physiological parameters of mice. PGRs caused increase in the number of splenic plaque forming cells and circulating WBCs, hematocrit value and thymus weight in young deer mice (5). GA_3 induced liver neoplasm in Egyptian toads and the tumours could be diagnosed as hepatocellular carcinomas

(6). Ustan et al (7) have reported that GA_3 induced microabsceses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of mice. PGRs induced oxidative stress, leading to generation of free radicals and caused lipid peroxidation as one of the molecular mechanisms of in PGRs induced toxicity (8). The long term use of high levels of GA_3 showed a significant decrease in testicular weight and sperms count in male albino rats (9). The feeding of GA_3 to Mus musculus doubled the proportion of females producing litters without increase in litter size or number (10). GA_3 had positive influence of on body weight, food conversion rate and fecundity on rats, poultry, pigs and calves (11, 12, 13). GA_3 exhibited estrogenic hormone-like action in mammals (14, 15). In castrated male rats, GA_3 partially restored the weight of prostrate but did not significantly change the weight of epididymis and seminal vesicles (16). Gawienowski had clearly demonstrated that GA_3 not only exihibited synergistic uteropic effect with estradiol in the immature mouse, but also expressedpossessed androgenic properties in male chicks (17). Elkomy et al demonstrated that GA_3 can have testosterone like biological effects on male chicks, was noted for comb and testes weights and secretion of testosterone in male chicks. GA_3 exhibited a direct androgenic-like action on rabbit testis and a positive effect on semen quality and quantity (18, 19).

Therefore, the present study was therefore aimed at investigating the short term effect of GA_3 on male albino rats with regard to the specific activities of marker enzymes (AST, ALT, ALP, GGT and amylase), the content of blood markers (hemoglobin, glucose, cholesterol, triglycerides, total protein, albumin, urea, uric acid and creatinine), serum electrolytes (sodium, potassium, phosphorus and calcium) and blood cell counts (RBC, WBC, platelets, neutrophils, eosinophils, monocytes and lymphocytes). This study highlights the nutritional and biological importance of understanding the influence of environmental (plant) factors on the biology of animal cell functions.

Materials and Methods

Materials

Gibberellic acid (Sigma) was a gift from Dr.Kanabiran, Reader, Pondicherry University, India. All chemicals used for this study were of analytical grade purchased from manufacturers in India. Glass distilled water was used for the preparations of all reagents. Male albino wistar strain rats (160-180g,10-12 weeks old) were obtained from JIPMER, Puducherry, India. The rats were housed at 20±2°C in light/dark cycles. All rats were fed Kamadhenu (India) rat feed pellet (analysed and established composition) and were given water ad libitum. The care of rats followed `guidelines for the care and use of laboratory animals' during experiments.

Methods

The rats were housed in 4 groups of 6 each. A control group was given 50µl of 50% absolute alcohol subcutaneously to serve as control. The experimental group of rats was given 75µg, 150µg and 300µg of GA_3/kg body weight in 50µl of 50% absolute alcohol administered subcutaneously as a single dose. Following this the rats were anaesthetized 4 hours later with anesthetic ether and blood was collected by cardiac puncture. Collected blood was centrifuged for the preparation of serum. Blood collected in EDTA was used for hematological studies. AST and ALT enzyme activity were assayed by the method of Reitman and Frankel (20). ALP was assayed by the method of Bessey et al (21). GGT activity was measured by Orlowski and Meister method (22). Amylase activity was assayed by the method of Bernfeld (23). Blood hemoglobin content was estimated by the method of Sahli's (24). Glucose content was measured by the modified procedure of Asatoor and King method (25). Cholesterol and triglycerides content were measured by using standard kits supplied by Ranbaxy Diagnostic Limited, Mumbai, India. Protein content was measured by the method of Lowry et al (26). The

quantity of serum albumin was measured by the method of Reinhold (27). Urea content was measured by the method of Netelson et al (28) and uric acid by Caraway et al (29). Creatinine content was measured by the method of Owen et al (30). Serum content of calcium, phosphorus, sodium and potassium were determined by the procedure of Halloran et al (31). Total count of RBC and total and differential count of WBC were enumerated by the method of Chesbrough et al (31). Data was expressed as mean ± Standard Error Mean (SEM) for six rats. SPSS 11 program was used for statistical analysis. The ifference between control and treated values was calculated using one way ANOVA and statistical significance was noted ($p<0.05$*) for all the tests.

Results

Treatment of rats with GA_3 caused dose dependent changes in the activities of serum enzymes (Table.1), in the content of glucose, , urea, uricacid, creatinine (Table.2) and in RBC, WBC, platelet count and in the differential counts of WBCs (Table.3). Treatment of rat with different doses of GA_3 altered specific activities of each enzyme studied. The specific activity of AST showed a marginal increase of 14.1%, 16.5% and 2.5% respectively at 75,150 and 300µg use of GA_3 versus the control rats. In contrast, the rat serum ALT, ALP, GGT and amylase specific activities decreased for almost all doses of GA_3 treatment versus the respective controls. The ALT specific activity decreased ($p<0.05$ level) 31% for 75µg, 20% for150µg and 6.2% for 300µg GA_3 used in the study. The activity of GGT decreased at all does of GA_3 used with a maximum decrease (30%) at 300µg GA_3. Amylase specific activity was marginally down regulated at 75µg and 150µg GA_3 doses, while induced considerable decrease (49%) at 300µg of GA_3 dose.

Table 1: Effect of GA_3 on rat serum enzyme specific activities.

		GA_3 treatment (µg/kg body weight)		
Enzymes	**Control**	**75µg**	**150µg**	**300µg**
AST	3.39 ± 0.159	3.78 ± 0.182*	4.49 ± 0.203**	4.95 ± 0.238**
ALT	0.94 ± 0.063	0.65 ± 0.045**	0.75 ± 0.041*	0.88 ± 0.053
ALP	6.48 ± 0.380	4.41 ± 0.205**	5.96 ± 0.399	6.48 ± 0.496
GGT	0.101 ± 0.007	0.072 ± 0.003**	0.084 ± 0.004*	0.098 ± 0.005
Amylase	10.42 ± 0.233	9.32 ± 0.725*	9.22 ± 0.456*	5.31 ± 0.284**

Enzyme specific activities were expressed in IU/gm of wet tissue x 10^{-3}
Each value represents ± SEM of 6 rats.
*$p<0.05$ and **$p<0.001$

Table 2: Effect of GA_3 on rat serum biomarkers and mineral contents

		GA_3 treatment (µg/kg body weight)		
Biomarkers	**Control**	**75µg**	**150µg**	**300µg**
Glucose (mg/dl)	110.0 ± 6.73	82.0 ± 4.61**	76.0 ± 3.83**	72.0 ± 4.47**
Total protein (gm/dl)	6.00 ± 0.44	6.72 ± 0.201*	6.60 ± 0.221	6.12 ± 0.413
Albumin (gm/dl)	3.60 ± 0.21	4.00 ± 0.27*	3.87 ± 0.24	3.87 ± 0.24
Urea (mg/dl)	33.0 ± 1.38	27.1 ± 1.06*	25.1 ± 1.14**	19.3 ± 1.04**
Uric acid (mg/dl)	9.70 ± 0.69	10.9 ± 0.47	12.0 ± 0.55**	13.70 ± 0.6**
Createnine (mg/dl)	1.01 ± 0.06	1.31 ± 0.08**	1.37 ± 0.09**	1.4 ± 0.08**
Calcium (mg/dl)	11.30 ± 0.77	11.8 ± 1.06	10.2 ± 0.88	9.50 ± 0.48*
Phosphorus (mg/dl)	6.12 ± 0.34	5.46 ± 0.354	5.06 ± 0.230*	4.44 ± 0.20**
Sodium (meq/l)	147.0 ± 5.25	144.0 ± 5.59	132.0 ± 5.01*	130.0 ± 3.84*
Potassium (meq/l	4.90 ± 0.45	4.00 ± 0.16*	3.80 ± 0.15*	3.60 ± 0.14**

Each value represents ± SEM of 6 rats. *$p<0.05$ and **$p<0.001$

Similarly, treatment of rat with different doses of GA_3 altered the biomarker content of rat blood in a dose dependent manner (Table 2). There was however, no significant change in hemoglobin content due to any doses of GA_3. Nevertheless, the blood glucose content, significantly decreased at all doses of GA_3 such as, by 26% with 75µg, 31% with 150µg and 49% with 300µg GA_3 compared to corresponding controls. Serum cholesterol was noted to be only marginally increased (25%) by 300µg GA_3, whereas showed 21% for 75µg and 6.2% for 150µg GA_3. Similar to cholesterol, the triglyceride content also marginally decreased at low dose (14%) and increased pattern was observed for all remaining doses of GA_3 treatments. The total serum protein and serum albumin content remained stationary for all doses of GA_3 treatment. Serum urea content decreased 18%, 24% and 42% at 75, 150 and 300µg GA_3 doses respectively. Creatinine, sodium, potassium and phosphorus content also noticeably decreased at all doses of GA_3 treatment in a dose dependent manner. In contrast, the serum uric acid content was however found to be increased significantly ($P<0.05$) at all doses of GA_3. Gibberellic acid produced quantum alterations in the numbers of certain blood cells in a dose dependent manner (Table 3). The RBC number increased 46% at 75 and 105% at 150µg GA_3 dose. The total number of WBC also increased following 150µg dose of GA_3. GA_3 did not produce significant changes in platelet, eosinophil, and monocyte numbers. Surprisingly, a progressive increase followed by decrease in the percentage of neutrophils and lymphocytes respectively was noted for low to high does of GA_3 treatment of the rats.

Discussion

Since GA_3 is a popular plant growth hormone and information on its acute positive or negative in vivo effect on higher animals is almost nil this compound was used for the present study. The data collected was from a single time point by using different doses of GA_3. The results of this study cannot be compared to any other previous study as no previous study on short term effect of GA_3 in rat model exists.

In this study, the acute effect of GA_3 on rat was analyzed by determining changes in selected enzyme activities and blood/serum markers. Measurement of the activities of various enzyme markers in the animal tissues and body fluids had a critical role in disease investigation and diagnosis (32), as also for the identification of drug toxicity including that of natural compounds (33). The present study indicated that GA_3 have differential effects on serum and blood parameters in a dose dependent manner. Each enzyme studied behaved differently towards different doses of the compound administered (Table 1). The marginal increase in serum AST activity was noted 4 hours post GA_3 treatment.

Table 3: Effect of GA_3 on rat serum lipid profile

		GA_3 treatment (µg/kg body weight)		
Lipid fractions	Control	75µg	150µg	300µg
Cholesterol (mg/dl)	78.0 ± 4.44	72 .0 ± 4.67	78.0 ± 4.42	84.0 ± 5.79
Triglycerides (mg/dl)	56.0 ± 3.92	54.0 ± 3.94	58.0 ± 3.69	62.0 ± 3.67
HDL (mg/dl)	17.1 ± 0.69	21.1 ± 1.11**	20.2 ± 1.04*	20.1 ± 1.11
LDL (mg/dl)	39.0 ± 1.12	42.0 ± 2.91	45.0 ± 2.27*	47.0 ± 2.01**
VLDL (mg/dl)	22.1 ± 1.21	10.1 ± 0.62**	12.2 ± 0.61**	17.2 ± 1.04**

Each value represents ± SEM of 6 rats.
*$p<0.05$ and **$p<0.001$.

Table 4: Effect of GA_3 on rat blood cells count.

		GA_3 treatment (µg/kg body weight)		
Blood cells	Control	75µg	150µg	300µg
RBC ($x10^6$/cumm)	5.9 ± 0.24	8.73 ± 0.29**	8.27 ± 0.336**	6.82 ± 0.32*
WBC ($x10^3$/cumm)	6.00 ± 0.24	5.15 ± 0.27*	6.29 ± 0.37	7.7 ± 0.30**
Platelets (x 10^5/cumm)	2.72 ± 0.18	1.62 ± 0.07**	2.01 ± 0.07**	2.85 ± 0.20
Neutrophils(%)	52.0 ± 4.25	58.0 ± 3.77	62.0 ± 2.68*	64.0 ± 3.68*
Eosinophils(%)	2.0 ± 0.13	2.0 ± 0.13	2.0 ± 0.26	2.0 ± 0.12
Monocytes(%)	1.0 ± 0.13	1.0 ± 0.14	1.0 ± 0.16	1.0 ± 0.13
Lymphocytes(%)	46.0 ± 2.28	40.0 ± 3.55*	35.0 ± 1.02*	34.0 ± 1.26*
Hemoglobin (gm%)	13.9 ± 1.04	14.6 ± 0.91	14.0 ± 1.17	13.1 ± 1.11

Each value represents ± SEM of 6 rats.
*p<0.05 and **p<0.001.

The concrete reason for such a change is not clearly understood. The significant increase in serum AST activity denoted increased release of this enzyme from tissues as well as due to the increased transaminase activity. It may also be due to the effect of GA_3 on tissues with the possibility of damage of the cardiac and hepatic tissues of rat, as they remain the prime source for serum AST activity. Serum enzymes are generally derived from tissues through extrusion and damage to cell membranes (34). It may also be possibly mediated by changes in the aspartate amino transferase metabolism in the cardiac and liver tissue caused by GA_3. Reson et al reported that increase of transaminase activities were related to aminoacid imbalance that initiated protein catabolism (34). Therefore, enzymes from diseased tissues and organs may manifest in the serum, resulting in increased serum enzyme activity. Alterations in biochemical as well as haematological parameters may be considered toxicity indices of compounds/drugs as result of their chronic use (35). On the other hand, reduction in the activities of serum ALT, ALP, GGT and amylase was noted in GA3 treated rats, surprisingly below the control values. The specific reason for this observation is not presently known. This decrease in enzyme activity may possibly be due to decreased synthesis and release of these enzymes from their sources and or inhibition or reduction of enzyme activities. Serum content of these enzyme activities reflected the overall status of the animal health when subjected to exogenous modulants, infection or injury. Decreased transaminase levels are commonly observed for substances that induced liver cytochrome P^{450}. Down regulation of ALT synthesis would affect the ability of the liver to metabolize amino acids for energy production in the cell. This would limit the capacity of the hepatocytes to generate appropriate energy levels in this tissue through pyruvate generation. Gamma glutamyl transpeptidase, activity found highest in the kidney, was significantly reduced with medium and higher doses of GA_3. The decrease in activity of this enzyme may probably be due to an imbalance in metabolism of glutathione that is needed for detoxification of foreign compounds. There was also significant reduction in amylase enzyme activity recorded using 300µg of GA_3. GA_3's suppressive effect on amylase is probably through encouraging tissue lipogenesis.

A progressive decrease in the quantity of glucose was noted at all doses of GA_3 treatment. This may be lead to a hypoglycemic effect of GA_3 on rat tissues. Further it suggests increased glucose utilization by cells resulting in greater glucose entry into the cells of each tissue. Utilization of the G-6-P generated, either through the glycolysis or through the HMP pathway remains the

established biochemical measure to counteract enhanced glucose load in tissues. In contrast, the amount of total free cholesterol and triglyceride in the serum of rat was found to be elevated when 300µg of GA_3 was used. The reason for an elevated cholesterol and triglyceride due to GA_3 possibly represents increased mobilization of these lipids from tissues, or due to their decreased transport from blood to tissues. The total protein and albumin content was not significantly altered by any dose of GA_3, indicating that the protein metabolism was not affected by GA_3.

Urea, an end product of protein metabolism, was significantly reduced at all doses of GA_3 treatment, a positive indication of normal kidney function with no adverse effect on protein metabolism. From the above facts it is clearly understood that the GA_3 treatments did not produce significant changes in protein metabolism. The amount of urea excretion depended upon the glomerular filtration rate and when this process failed, the plasma urea level gets raised. Uric acid, the end product of purine metabolism has proved to be a selective antioxidant, capable especially of reacting with hydroxy radicals and hypochlorous acid[46]. Increased level of uric acid in GA_3 treated rats was noted in this study. A reduction in MDA content was also noted in all the tissues studied for the different doses of GA_3 treatment (data not shown). There seems to be correlation between increase in uric acid and the decrease in MDA content noted in this study. The serum creatinine was found to be reduced by GA_3 treatment. Serum creatinine level and BUN are the only parameters used for the determination of nephrotoxicity[47]. BUN is produced endogenously by tissue creatinine breakdown, and a change increase in serum creatinine level depended upon the glomerular filtration rate. When creatinine excretion failed to balance the production, serum creatinine level increased (36). Results of this study led to conclude that GA_3 promoted greater creatinine excretion. GA_3 treatment also were reduced the content of serum minerals. The reason for the reduction in the quantity of sodium, potassium, calcium and phosphorus noted was not clearly understood. It may be due to alterations in the permeability of electrolytes across cell membranes/ or due to abberations in the membrane ATPase function. A decrease in these ions may lead to severe complications in body functions.

The total numbers of RBC, WBC and neutrophil were significantly increased at all doses of GA_3 treatment, possibly due to an action of GA_3 on hemopoiesis. Decrease in platelet counts except at 75 and 300µg GA_3 dose and of lymphocytes at all doses of GA_3, while the eosinophil and monocyte numbers remained stationary. Reason for the differential response of blood cells to exogenous GA_3 administration was not clear. In conclusion, treatment of rat with GA_3 produced dose dependent effect on serum enzymes and blood markers. This study highlights the possible varied effects of GA_3 in the rat and possibly in other mammals as a short term response to its presence in the animal tissue. Further detailed study is required to report on its wider effects.

Acknowledgements

The authors gratefully acknowledge financial support from the Dept. of Science and Technology, New Delhi, India through the grant SR/SO/AS-16/2004 awarded to KS and for the DST FIST support to department. Technical help received from G. Nirmal Kumar and J. Vikramathithan is gratefully acknowledged.

References

1. Salisbury FB, Ross CW. Plant physiology. Belmont, CA: Wadsworth; 1992;p357-407:531-548.
2. Guarra AA. Effect of biological active substances in the diet on development and reproduction of Heliothis spp. J Econ Entomol 1970;63:1518-1521.
3. Visscher NS. Regulation of grasshopper fecundity, longevity and egg lay viability by plant growth hormones. Experimentia 1980;36:130-131.

4. Visscher NS. Special report dietary plant growth hormones affect insect growth and reproduction. Bull Plant Growth Reg Soc Am 1983;11:4-6.
5. De Man W, De Loof A, Briers T, Huybrechts R. Effect of abscisic acid on vitellogenesis in Sareophaga bullata. Entomol Exp Appl 1991;29:259-267.
6. Alanso C. The effects of gibberellic acid upon developmental processes in Drosophila hydlei. Entomol Exp Appl 1971;14:73-82.
7. Ozmen M, Topcuoghu SF, Bozcuk S, Bozcuk NA. Effects of abscisic acid and gibberellic acid on sexual differentiation and some physiological parameters of laboratory mice. Tr J Biol1995;19:357-364.
8. Olson IJ, Hinsdill RD. Influence of feeding chlorocholine chloride and glyposine on selected immune parameters in deer mice Peromiseus moniculatus. Toxicology 1984;30:103-114.
9. El-mofty MM, Sakr SA. Introduction of neoplasms in the Egyptian Toad by gibberellin A_3. Oncology 1988;45:61-64.
10. Ustan H, Tecimer T, Ozmen M, Topcuoghu, Bozcuk NA. Effects of gibberellic acid and benzopurin on mice. Hispathologic Rev Ank Patho Bult 1992;9:36-40.
11. Candeias LP, Folkes LK, Porssa M, Parrick J, Wardman P. Enhancement of lipid peroxidation by indole-3-acetic acid and derivatives:Substituent effects. Free Rad Res 1995;23:403-418.
12. Ravikumar S, Srikumar K. Metabolic dysregulation and inhibition of spermatogenesis by gibberellic acid in rat testicular cells. J Environ Biol 2005;26(3):567-569.
13. Olson P. The stimulating Effect of Phytohormone, Gibberellic acid, on Reproduction of Mus musculatus. Australian Wild life Research 1981;8(2):321-325.
14. Alkhiat AA, Morsy H, Shehata E, Abdellaty A. Veterinary Pharmacology and toxicology. Ministry of High Education and Scientific Research Iraq 1981.
15. Madacsi JP, Parrish FW, Mc Naaughton JL. Anim Feed Science and Technol 1988;20:69.
16. Abdelhamid AM, Dorra TM, Ali MA, Abou-Egla EH. Effect of gibberellic acid on broiler chicken performance and some metabolic parameters. Arch Anim Nutr 1994;46(3):269-276.
17. Gawienowski AM, Stadnicki SS, Stacewicz-sapuntzakir M. Synergistic uterotrophic effect of gibberellic acid and estradiol in the immature mouse. Life Sciences 1977a;20:785-788.
18. Gawienowski AM, Chatterijee D. Effect of prostaglandin inhibitor on the uterotrophic response of estradiol and gibberellic acid. Life Sciences 1980;27:1393-1396.
19. Bouton C, Maillet M, Feintuch MJ. CR Soc Biol 1969;163:1484-1487.
20. Gawienowski AM, Stadnicki, Stacewicz-sapuntzakir M. Androgenic properties of gibberellic acid in the Chicks comb bioassay. Experientia 1977b;33:1544-1545.
21. Elkomy AE, Samar A, Einagar, Azza El-Sebai. Steroidogenic effects of gibberellic acid (GA_3) on chicks. Egypt Poult Sci 2007;27(4):1239-1255.
22. Kamel KI, Elkomy AE, El-Sbeiy. The Androgenic Action of Gibberellic Acid (GA_3) on Reproductive Performance of New Zealand White Rabbit Bucks. World Journal of agricultural Sciences 2009;5(1):40-48.
23. Reitman S, Frankel S. A colorimetric determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminase. Am J Clin Path 1957;28:56-63.
24. Bessey OA, Lowry OH, Brock MJ. One point colorimetric method of determining

ALP in serum or plasma. J Biol Chem 1946;164:321.
25. Orlowski M, Meister M. γ-Glutamyl P-nitroanilide, a new convenient substrate for determination and study of L and D-γ-glutamyl transpeptidase activity. Biochem Biophys Acta 1963;73:679-681.
26. Bernfeld P. Amylase α and β. Methods in enzymology 1955;1:149.
27. Asatoor AM, King EJ. Simplified colorimetric blood sugar method. Practical Clinical Biochemistry, Harold Varley, Arnold-Heineman Publishers;India, 2nd edition 1954:56-58.
28. Zak B, Dickenman RC, White EG, Burenett H, Cherney PJ. Rapid estimation of free and total cholesterol. Am J Clin Patho1954;24:1307-1315.
29. Bucolo G, Dravid H. Quantitative determination of serum triglycerides by use of enzyme. Clin Chem 1973;19:476-482.
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-275.
31. Reinhold JG. Standard methods in clinical chemistry. In:Reiner M, edn. New York;Academic Press, 1953;88.
32. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biological fluids by means of the reaction between diacetyl and urea, Am J Clin Path 1951;21:275-281.
33. Caraway WT. Uricacid. Standard methods in Clinical Chemistry. In:Saligson D, edn. New York;Academic Press, 1963;4:239-247.
34. Owen JA, Iggo B, Scandrett FJ, Stewart CP. The determination of creatinine in plasma or serum and in urine; a critical examination. Biochem J 1954;58:426-437.
35. Halloran O., et al. Determination of trace elements in biological and environmental samples by atomic absorption spectroscopy, Methods in Biotech 2; Bioremediation protocol; edn by Sheehan, New Gersey, 1998;pp.210-211.
36. Chesbrough M and McArthur J. In: A laboratory manual for rural tropical hospitals. The English Language Book Society and Churchil Livingstone, London, 1972;P.145.