



Research Article

EFFECT OF METHANOLIC ROOT EXTRACTS OF ETHNOMEDICINAL PLANTS ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

Sujani Kamble* and B. Ganga Rao

*Dept. of Pharmacognosy, Government College of Pharmacy, Bangalore -560027

²A. U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam

Abstract

Purpose : To evaluate the hepatoprotective activity of methanolic root extracts of *Memecylon malabaricum* (MM), *Andrographis serpyllifolia* (AS) and *Leucas aspera* (LA) against the paracetamol induced hepatotoxicity.

Approach : The methanolic root extracts of MM (family: Melastomaceae), AS (family: Acanthaceae) and LA (family: Lamiaceae) was studied at different doses and compared with standard Silymarin (25 mg/kg b.w). All the doses were administered orally. Thereafter, paracetamol induced hepatotoxicity in elevation of the serum levels of biochemical parameters like SGOT, SGPT, ALKP, TBL and CHL were studied.

Findings : The rats treated with the methanolic root extracts of MM, AS, LA and silymarin showed a significant ($p < 0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p < 0.05$) in TPTN and ALB levels at dose dependent manner. Pretreatment with Silymarin and methanolic root extracts MM, AS and LA produced significant antihepatotoxic activity.

Conclusion : The reduction of elevated serum levels were explored by evaluated hepatoprotective activity of methanolic root extracts of MM, AS and LA against the paracetamol induced hepatotoxicity.

Key words: *Biochemical parameters, Hepatoprotective, Hepatotoxicity, Medicinal Plants, Methanolic extract.*

Received on : 07-12-2016

Revised on : 19-02-2017

Accepted on : 28-03-2017

INTRODUCTION

Plants are rich sources of medicinal properties. Since time immemorial, plants have been utilized for their medicinal benefits. Despite the availability of advanced technologies and large-scale researches, plants have not been explored largely to study their medicinal properties to bring new drugs into the market. Only 10% of the plant species have been studied for medicinal properties till date.^{1,2} As natural

product research continues to be an important part of the drug discovery, we were keen in taking up the phytochemical investigation of selected plant species to explore hepatoprotective activity. Natural products are associated with extensive molecular diversity and this property is the primary advantage to consider as a source of lead compounds. Recent technological advances have enabled the possibility to evaluate extracts and identify the biological activity at sub milligram scale levels.³ In the present research *Memecylon malabaricum*, *Andrographis serpyllifolia* and *Leucas aspera* roots were selected with the focus of hepatoprotective activity. Earlier research revealed that MM leaf has anti-inflammatory activity⁴, wound healing activity⁵, fruits have significant antioxidant, anti-microbial and

*Corresponding Author :

Sujani Kamble

Dept. of Pharmacognosy,
Government College of Pharmacy,
Bangalore - 560 027
Email: sujik2000@gmail.com

Cytotoxic activities⁶ and are useful in the treatment of toothache, plaque, caries, pyorrhea and aphthae⁷. AS plant traditionally showed antidiabetic⁸, jaundice, digestive problems, snake bites, fever, cancer, inflammation, wound⁹, hypolipidemia¹⁰, malaria¹¹ whereas LA showed antibacterial¹², antioxidant¹³ as well as hepatoprotective activity¹⁴. Based on the traditional applications and as per the recent research review there is a lack of study on these combined plants on hepatoprotective study and hence the present investigation was carried out to identify hepatoprotective activity in the methanolic root extracts of MM, AS and LA plants in paracetamol induced experimental rat models.

MATERIALS AND METHODS

Collection : Roots of *M. malabaricum*, *A. serpyllifolia* and *L. aspera* were collected from Ananthagiri Forest Region, Visakhapatnam and identified by Prof . M. Venkaiah, Department of Botany, Andhra University.

Extraction : The root materials were shade dried and were extracted separately (250 g) in a Soxhlet apparatus with methanol for 6-8 hrs. The solvent was removed by the process of distillation and the crude extract was dried under vacuum and stored in a desiccator prior to further investigation.

Preliminary extract screening

The extract was preliminarily screened for various constituents present as per the method described by Kokate, 1994¹⁵.

Experimental animals :

Wistar albino rats of either sex weighing between 150-200 g were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 + 2°C with an alternating 12h light-dark cycle and relative humidity of 50±15%, one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/A/CPCSEA). Rats were fed with standard diet and water ad libitum during the experiment.

Paracetamol suspension (PCML) : Paracetamol powder was suspended in 1% Sodium CMC and administered at the dose of 3g/kg b.wt. p.o.

Suspensions of test substances : All the selective extracts were suspended in 1% Sodium CMC and administered at the dose levels of 200, 400 and 800 mg/kg. Silymarin being positive control was suspended in 1% Sodium CMC and administered at a dose level of 25 mg/kg.

Paracetamol induced hepatotoxicity : The set of experiment was divided into groups consisting of control, toxicant, standard, and test. The protocol followed for paracetamol induced hepatotoxicity and grouping of animals are given in table-1 and table-2 respectively.

Table 1 : Administration of drugs

Group	Day 1	Day 2	Day 3	Day 4	Day 5
control	Vehicle	Vehicle	Vehicle	Vehicle	With
PCML	Vehicle	Vehicle	Vehicle+ PCML	Vehicle	drawl
Standard	Silymarin	Silymarin	Silymarin+PCML	Vehicle	of
Test	Extract	Extract	Extract +PCML	Vehicle	blood

♦ Vehicle: 1% Sodium CMC, test: Extracts prepared in 1% Sodium CMC.; PCML: Paracetamol.

Table 2 : The Group and the administered dose details

Group and order	Dose given and species of methanolic extract
Group-I	Control
Group-II	Standard
Group-III	Received methanolic extract of <i>M. malabaricum</i> 200 mg/kg in paracetamol induced animal
Group-IV	Received methanolic extract of <i>M. malabaricum</i> 400 mg/kg in paracetamol induced animal
Group-V	Received methanolic extract of <i>M. malabaricum</i> 800 mg/kg in paracetamol induced animal
Group-VI	Received methanolic extract of <i>A. serpyllifolia</i> 200 mg/kg in paracetamol induced animal
Group-VII	Received methanolic extract of <i>A. serpyllifolia</i> 400 mg/kg in paracetamol induced animal
Group-VIII	Received methanolic extract of <i>A. serpyllifolia</i> 800 mg/kg in paracetamol induced animal
Group-IX	Received methanolic extract of <i>L. aspera</i> 200 mg/kg in paracetamol induced animal
Group-X	Received methanolic extract of <i>L. aspera</i> 400 mg/kg in paracetamol induced animal
Group-XI	Received methanolic extract of <i>L. aspera</i> 800 mg/kg in paracetamol induced animal

The rats of control group Received a single daily dose of 1% Sodium CMC (1 ml/kg p.o.) The rats of Paracetamol group received a single daily dose of vehicle for three days and a single dose of Paracetamol (3 g/kg) 30 min after the administration of the vehicle, on the third day of experiment. The animals in silymarin group Received daily dose of silymarin (25 mg/kg p.o.) for three days. Paracetamol (3 g/kg p.o.) was administered 30 min after the third dose of silymarin while test groups were given orally a single daily dose of extracts in sodium CMC for three days and a single dose of paracetamol (3 g/kg p.o.) on the third day 30 min after the administration of respective test suspensions. After 48 h of

paracetamol intoxication blood was collected and serum was analyzed for the biochemical parameters.

Assessment of Biochemical parameters

Blood was collected from each group by puncturing retro orbital plexus and was allowed to clot at room temperature. The serum was then separated by centrifuging at 3000 rpm for 10 minutes which was later used for the estimation of biochemical parameters to determine the liver functions. Serum Glutamic oxaloacetic transaminase (SGOT), Serum Glutamic pyruvic transaminase (SGPT) were estimated using UV – Kinetic method based on the international federation of clinical chemistry method as reference¹⁶. Alkaline phosphatase (ALKP) was estimated by PNPP method,¹⁷ while total bilirubin (TBL) by Jendrassik and Gr of method,¹⁸ total cholesterol (CHL),¹⁹ total protein (TPTN) by color complexation with copper ions in an alkali solution²⁰. Albumin was estimated by bromo cresol green 250 method²¹. All the estimations were carried out using standard kits on auto analyzer of Merck make (300 TX, E.Merck-Micor Labs, Mumbai).

Statistical analysis:

Results for hepatotoxicity was evaluated by mean $\pm$ SEM method, where n=6 animals.

Results and discussion :

Methanolic extract of all the three drugs were carried out and the % yield was calculated as 14.57 %, 13.27 % and 13.48 % for MM plant, AS plant and LA plant respectively.

Phytochemical screening :

Phytochemical screening for the said plants were carried out and revealed the presence of various phytoconstituents that is depicted in Table-3.

Table 3 : Phytochemical screening of various plant root methanol extracts

Constituents	Methanolic root extracts		
	Memecylon malabaricum	Andrographis serpyllifolia	Leucas aspera
Glycosides	+	+	--
Alkaloids	--	+	+
Tannins	+	+	+
Phenols	++	++	++
Flavonoids	+	++	+
Proteins	--	--	--
Carbohydrates	+	--	--
Saponin	+	++	+
Aminoacid	--	--	--
Steroids	+	+	++
Resins	--	--	--

• + = identification of constituents; ++ = prominent identification of constituents.

Paracetamol induced hepatotoxicity:

The experiment was carried out as per the protocol followed for paracetamol induced hepatotoxicity and the results were tabulated in table-4. Table 4 shows Silymarin the standard drug at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL with the values 100.2 ± 0.76 , 100.1 ± 1.2 , 206.4 ± 1.99 , 1.58 ± 0.50 , and 110.9 ± 0.64 , respectively and increased the levels of TPTN and ALB 6.36 ± 0.19 and 3.21 ± 0.22 , respectively. All the extract showed dose dependent activities. Methanolic extract of *M. malabaricum* at 800mg/kg produced 139.1 ± 2.61 , 116.6 ± 1.29 , 234.6 ± 6.01 , 1.92 ± 0.19 , 156.4 ± 2.36 , 4.64 ± 0.46 and 4.65 ± 0.46 respectively. Whereas *A. serpyllifolia* at 800mg/kg dose, showed 149.3 ± 2.16 , 88.04 ± 3.41 , 192.6 ± 2.38 , 2.16 ± 0.05 , 121.4 ± 2.61 , 5.68 ± 0.19 and 3.99 ± 0.10 , respectively. Thereafter *L. aspera* methanolic extract at 800mg/kg produced 123.9 ± 1.42 , 131.1 ± 0.19 , 219.9 ± 2.16 , 1.99 ± 0.08 , 165.6 ± 1.66 , 3.83 ± 0.19 and 3.41 ± 0.19 , respectively.

Table 4 : Effect of methanolic extract of MM, AS and LS on paracetamol induced hepatotoxicity in rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dL)	CHL (mg/dl)	TPTN (g/dL)	ALB (g/dL)
Control	115.6 $\pm$ 1.95	100.4 $\pm$ 0.96	206.6 $\pm$ 1.92	1.01 $\pm$ 0.06	124.6 $\pm$ 2.3	6.50 $\pm$ 1.92	4.92 $\pm$ 0.68
Paracetamol	455.1 $\pm$ 1.95	316.2 $\pm$ 2.00	615.1 $\pm$ 2.61	4.91 $\pm$ 0.44	351.6 $\pm$ 1.91	3.45 $\pm$ 0.31	1.94 $\pm$ 0.45
Silymarin	119.4 $\pm$ 2.19*	105.4 $\pm$ 1.99*	215.4 $\pm$ 2.31*	1.31 $\pm$ 0.10*	129.2 $\pm$ 1.41*	6.95 $\pm$ 1.00**	4.45 $\pm$ 0.92**
MMM 200 mg/kg	376.9 $\pm$ 6.01	266.4 $\pm$ 3.29	540.2 $\pm$ 7.00	3.01 $\pm$ 0.19	274.6 $\pm$ 5.99	2.99 $\pm$ 0.19	2.40 $\pm$ 0.23
MMM 400 mg/kg	150.4 $\pm$ 2.01*	132.1 $\pm$ 3.46*	272.4 $\pm$ 4.23*	1.92 $\pm$ 0.19*	191.1 $\pm$ 8.19*	5.6 $\pm$ 0.19**	3.62 $\pm$ 0.36**
MMM 800 mg/kg	139.1 $\pm$ 2.61*	116.6 $\pm$ 1.29*	234.6 $\pm$ 6.01*	1.92 $\pm$ 0.19*	156.4 $\pm$ 2.36*	4.64 $\pm$ 0.46**	4.65 $\pm$ 0.46**
ASM 200 mg/kg	404.2 $\pm$ 2.91	285.4 $\pm$ 4.19	407.6 $\pm$ 2.14	4.39 $\pm$ 0.09	234.5 $\pm$ 2.64	3.64 $\pm$ 0.09	2.91 $\pm$ 0.14
ASM 400 mg/kg	156.2 $\pm$ 1.62*	93.46 $\pm$ 0.19*	225.4 $\pm$ 0.28*	1.79 $\pm$ 0.21*	114.2 $\pm$ 2.64*	5.10 $\pm$ 0.24**	4.92 $\pm$ 0.19**
ASM 800 mg/kg	149.3 $\pm$ 2.16*	88.04 $\pm$ 3.41*	192.6 $\pm$ 2.38*	2.16 $\pm$ 0.05*	121.4 $\pm$ 2.61*	5.68 $\pm$ 0.19**	3.99 $\pm$ 0.10**
LAM 200 mg/kg	343.8 $\pm$ 1.76	251.6 $\pm$ 2.61	494.4 $\pm$ 4.16	2.61 $\pm$ 0.19	260.4 $\pm$ 2.11	2.70 $\pm$ 0.18	2.42 $\pm$ 0.04
LAM 400 mg/kg	135.6 $\pm$ 1.61*	149.6 $\pm$ 1.37*	278.2 $\pm$ 2.01*	2.09 $\pm$ 0.06*	199.4 $\pm$ 1.91*	3.55 $\pm$ 0.20**	2.99 $\pm$ 0.22**
LAM800 mg/kg	123.9 $\pm$ 1.42*	131.1 $\pm$ 0.19*	219.9 $\pm$ 2.16*	1.99 $\pm$ 0.08*	165.6 $\pm$ 1.66*	3.83 $\pm$ 0.19**	3.41 $\pm$ 0.19**

• Data expressed in mean $\pm$ s.e.m, n=6; Significant reduction compared to hepatotoxic group (p<0.05); Significant increase compared to hepatotoxic group (p<0.05)

• ALKP = Alkaline phosphate; ALB = albumin; ASM = methanolic extract of *A. serpyllifolia* and CHL = cholesterol; MMM = methanolic extract of *M. malabaricum*; SGOT = Serum glutamic oxaloacetic transaminase; SGPT = serum Glutamic pyruvic transaminase; TBL = total bilirubin; TPTN = total protein; LAM = methanolic extract of *L. aspera*

The results clearly depicted that paracetamol intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, whereas

decreased the levels of TPTN, ALB significantly when compared to control indicating acute hepatocellular damage and centrilobular necrosis. The rats treated with the methanolic extracts of *M. malabaricum* and silymarin showed a significant ($P<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The rats treated with the methanolic extracts of *M. malabaricum* and silymarin showed a significant ($p<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The rats treated with the methanolic extracts of *A. serpyllifolia* and silymarin showed a significant ($p<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The rats treated with the methanolic extracts of *L. aspera*, and silymarin showed a significant ($p<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. Effect of Methanolic extracts of *M. malabaricum*, *A. serpyllifolia*, *L. aspera* and silymarin on percentage protection against Paracetamol induced hepatotoxicity in rats is presented in Table 5.

Table 5 : Effect of Methanolic extracts of *M. Malabaricum*, *A. serpyllifolia* and *L. aspera* on Percentage protection against Paracetamol induced hepatotoxicity in rats

Group	SGOT	SGPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin	73.76	66.50	64.98	73.31	63.25	101.4	129.38
MMM 200 mg/kg	17.18	15.74	12.17	38.69	21.89	13.33	23.71
MMM 400 mg/kg	66.95	58.22	55.71	60.89	45.64	62.31	86.59
MMM 800 mg/kg	69.43	63.12	61.85	60.89	55.51	34.49	139.69
ASM200 mg/kg	11.18	9.74	33.73	10.59	33.3	5.50	50
ASM400 mg/kg	65.67	70.44	63.35	63.54	67.51	47.82	153.60
ASM800 mg/kg	67.19	72.15	78.93	56.00	65.47	64.63	105.67
LAM 200 mg/kg	23.57	20.43	17.36	46.84	25.93	21.73	24.74
LAM 400 mg/kg	70.20	52.68	54.77	57.43	43.28	2.89	54.12
LAM 800 mg/kg	72.77	58.50	64.24	59.47	55.46	11.01	75.77

• ALKP = Alkaline phosphate; ALB = albumin; ASM = methanolic extract of *A. serpyllifolia* and CHL = cholesterol; MMM = methanolic extract of *M. malabaricum*; SGOT = Serum glutamic oxaloacetic transaminase; SGPT = serum Glutamic pyruvic transaminase; TBL = total bilirubin; TPTN = total protein; LAM = methanolic extract of *L. aspera*

The methanolic extracts of *M. malabaricum*, *A. serpyllifolia* and *L. aspera* were subjected to hepatoprotective activity in vivo in rats using Paracetamol induced hepatotoxicity. All the extracts were administered at dose levels of 200, 400 and 800 mg/kg body wt. Silymarin being the positive control was administered at dose level of 25 mg/kg b.w.

Paracetamol intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, whereas decreased the levels of TPTN, ALB significantly when compared to control indicating acute hepatocellular damage and biliary obstruction leading to necrosis. The rats treated with the methanolic extracts of *M. malabaricum*, *A. serpyllifolia* *L. aspera* and silymarin showed a significant ($P<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($p<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg.

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through detoxification²². Paracetamol being a drug capable of causing liver disorders in higher dose. The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the of hepatotoxicity have been reported earlier^{23, 24}. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver²⁵. In the present study, hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transaminase, alanine transaminase represents 90% of total enzyme and high level of alanine transaminase in the blood is better index of liver injury, but the elevated levels of enzymes are decreased to near normal levels with the treatment of selected plant extracts which indicates protection by preserving the structural integrity of the hepatocellular membrane against paracetamol. Chronic administration of paracetamol produced a marked elevation of the serum levels of enzymes in treated animals when compared with that of the control group. Treatment with said plant extracts at a dose of 800 mg/kg significantly reduced the elevated levels of those enzymes.

Paracetamol seems to cause impairment in lipoprotein metabolism²⁶ and also alterations in cholesterol metabolism. The levels of cholesterol and triglyceride were significantly increased in paracetamol treated rats, when compared to control, silymarin and extracts treated rats. Elevation of tryglycerides level during paracetamol intoxication could be due to increased availability of free fatty

acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids. Administration of methanolic extracts at higher dose significantly decreased serum lipid profile in paracetamol toxicity induced rats because of its hypolipidemic effects.

CONCLUSION

Pretreatment with Silymarin, ethyl acetate and methanolic extracts of *M. malabaricum*, *A. serpyllifolia* and *L. aspera* produced significant antihepatotoxic activity in all the selected models. The exact bioactive principle responsible for the reduction of elevated serum levels remains to be explored. The active metabolites for producing significant activity must be identified.

REFERENCES

1. Rates SMK. Plants as source of drugs. Toxicon. 2009; 39: 603-613.
2. Mabberley DJ. The plant-book: A Portable Dictionary of the Vascular Plants., 2nd Edition, Cambridge University Press; Cambridge, U.K. 1997.
3. Robert PB. Natural product research: perspectives from a major pharmaceutical company. J Ethnopharmacol. 1996; 51:29-38.
4. Syed IT, Gopalakrishnan S, Hazeena BV, Elango V. Anti-inflammatory activity of *Salacia oblonga* Wall. and *Azima tetraacantha* Lam. J. of Ethnopharmacol. 1997; 56(2): 145-152.
5. Jaswanth A, Begum VH, Akilandeswari S, Begum TN, Manimaran S, Ruckmani K. Effects of *Azima tetraacantha* on dermal wound healing in rats. Hamdard Medicus. 2001; 44: 13-16.
6. Fatimi AM, Martina W, Gudrun S, Ulrike L. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. of Ethnopharmacol. 2007; 111(3): 657-666.
7. Hebbar SS, Harsha VH, Shripathi V, Hedge GR. Ethnomedicine of Dharwad district in Karnataka, India - plants used in oral health care. J of Ethnopharmacol. 2004; 94(2-4): 261-266.
8. Ashok Tiwari K, Madhusudhan Rao J. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Current Sci. 2002; 83(1): 30-38.
9. Sekhar J, Penchala PG, Sudarsanam G, Prasad GP. Ethnic information on treatments for snake bites in Kadapa district of Andhra Pradesh. Life sci leaflets. 2006; 67:25-26.
10. Chelluboina B, Manga K. Hypolipidemic activity of *A. serpyllifolia* Wt. Ic. Pharm Res. 2010; 3(4):769-770.
11. Chithrashree, Murthy NK, Srinivas C. Phytochemical screening and In vitro assessment of antimicrobial and antioxidant potential of *A. serpyllifolia* - An endemic medicinal plant from South India. Inter J of Advanced Res. 2014; 2(2): 917-928.
12. Srinivasan R, Lakshmana G, Rajash Kumar D, Anjaneyulu B, Shaleem Raju K, Anil kumar D. Analgesic, antipyretic, antimicrobial activity of whole *Leucas aspera* ethanolic extract. Pharmanest. 2014; 5(2): 1980-1986.
13. Sandeep B, Balaji B, Premkumar B. Hepatoprotective and antioxidant activity of *Leucas aspera* against d-galactosamine induced liver damage in rats. Pharmaceutical Biology. 2012; 50(12): 1592-1595.
14. Mohd Abdul I, Mohammed Fazil A, Syed Asad B, Mohammed I. Hepatoprotective activity of *Leucas aspera* Spreng against simvastatin induced hepatotoxicity in rats. Annals of Phytomedicine. 2012; 1(2): 88-92.
15. Kokate CK. Practical Pharmacognosy, 4 th edition, Vallabh Prakashan, New Delhi, 1994. pp. 4, 29.
16. Bergmeyer HU, Horder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1). J Clin Chem Clin Biochem. 1986; 24:497-510.
17. Yamini J, Anil Kumar K, Venkataramana V, Divya L, Reddy SN, Rajasigamani K. Estimation of alkaline phosphatase in the gingival crevicular fluid during orthodontic tooth movement in premolar extraction cases to predict therapeutic progression. Journal of Natural Science, Biology and Medicine. 2015; 6(2): 343-346.
18. Garber CC. Jendrassik - Grof analysis for total and direct bilirubin in serum with a centrifugal analyzer. Clin Chem. 1981; 27(8):1410-16.
19. Parekh AC, Jung DH. Cholesterol determination with ferric acetate-uranium acetate and sulfuric acid-ferrous sulfate reagents. Anal Chem 1970; 42: 1423-27.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall. R.J. Protein measurement with Folin's Phenol reagent. J Biol Chem 1951; 193:265-75.
21. Affonso A, Lasky FD. Bromocresol purple dye-binding method for the estimation of serum albumin adapted to the SMA 12/60. Clin Biochem. 1985; 18(5): 285-89.
22. Larrey D. Drug induced liver disease. J Hepatol 2003; 32:77-88.
23. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from *Andro-graphis paniculata* against CCl₄. Ind J Med Res (B). 1990; 92:276-83.
24. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen induced hepatic necrosis: (II) Role of covalent binding in vivo. J Pharmacol Exp Ther. 1973; 187: 195-202.
25. Drotman RB, Lawhorn GT. Serum enzymes are indicators of chemical induced liver damage. Drug Chem Toxicol. 1978; 1:163-71.
26. Kobashigania JA, Kasiska BL. Hyperlipi-demia in solid organ transplantation. Transplantation 1997; 63: 333-38.