Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

AMELIORATION OF PARACETAMOL INDUCED NEPHROTOXICITY BY *MAYTENUS EMARGINATA* IN MALE WISTAR RATS

M. M. PATHAN*, M. A. KHAN[1], S. D. MOREGAONKAR[2], A. P. SOMKUWAR[3] AND N. Z. GAIKWAD[4]

*[1]Department of Pathology, [3]Department of Pharmacology and Toxicology, College of Veterinary & Animal Sciences, (MAFSU, Nagpur) Udgir-413517 (M.S.), [2]Department of Pathology, Bombay Veterinary College, Parel, Mumbai, [4]Department of Biochemistry, College of Veterinary & Animal Sciences, Udgir, India. Email: muqtark@gmail.com

Received: 28 July 2013, Revised and Accepted: 23 Aug 2013

ABSTRACT

Objective: To investigate the nephroprotective activity of *Maytenus emarginata* ethanolic extract.

Methods: The study was performed against Paracetamol induced nephrotoxicity in male Wistar rats. The rats were divided in five groups. Rats of Group C1 served healthy control received no treatment, Group C2 served vehicle control received 1ml of Propylene glycol orally. C3 group rats acted as nephrotoxicity control and received Paracetamol orally @ 500 mg/kg B.W. of rats. Group T1 and T2 received *Maytenus emarginata* extract @ 100 mg/kg B.W. and *Maytenus emarginata* extract @ 100 mg/kg B.W. of rats respectively along with Paracetamol @ 500 mg/kg B.W. of rats orally for 28 days. On 28th day blood was collected and hemato-biochemical parameters were estimated. Rats were sacrificed and kidneys were examined histopathologicaly.

Results: Histopathological examination and elevation of biochemical marker enzymes serum creatinine and BUN confirmed the kidney injury produced by Paracetamol. Treatment of rats with *Maytenus emarginata* extract showed significant reduction in levels of serum creatinine near to normal in a dose dependant manner. Dose of 150 mg/kg B.W. of *Maytenus emarginata* extract showed significant effects comparable to the healthy control group rats.

Conclusion: *Maytenus emarginata* extract possesses potent nephroprotective effects against the Paracetamol induced nephrotoxicity.

Keywords: Nephroprotective, Histopathology, Acetaminophen, *M. emarginata*.

INTRODUCTION

Paracetamol (acetaminophen) is an analgesic drug and metabolized by cytochrome-P450 system, which leads to the formation of N-acetyl P-Benzoquinoneimine (NAPQI). Paracetamol is a powerful inducer of cytochrome-P450. The action of P450 system on Paracetamol produces a highly reactive quinoneimine that combines to the sulfhydryl group of proteins. The toxicity occurs because of its reactive metabolite NAPQI. NAPQI exerts its toxicity primarily via its oxidative effect on cellular proteins. The inactivation of proteins leads to death of liver cells. Paracetamol toxicity in animals and man produces hepatic necrosis and a depletion of both mitochondrial and cytosolic pools of reduced glutathione [1-3]. Kidney is the second target organ of Paracetamol toxicity although nephrotoxicity may exists in the absence of hepatotoxicity following Paracetamol overdose [4], kidney has been badly ignored in studies aimed at treatment of Paracetamol toxicity with herbal preparations [5].

In different countries many herbs are used in folk medicine to treat drug or toxin induced renal damage [6] but lack scientific support. *Maytenus emarginata* also named as *Gymnosporia montana* (Roth.) Bemth, *Gymnosporia emarginata,* locally called as Yekkadi (Marathi), Baikal (Hindi) and Vikankata in Ayurveda. *M. emarginata* is a shrub having thorns and 2-6 meter in height; produce creamy-white flowers, ovoid reddish-purple fruits and ovoid chestnut-brown coloured seeds [7]. *Maytenus emarginata* belongs to the family Celastracea, is an evergreen shrub that tolerates various types of stresses of desert. It is found all over in India, (Maharashtra, Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat, Delhi, Bihar and Tamilnadu). The crude plant extract of the Celastracea was used in traditional medicine and agriculture is astonishing and includes stimulant, restoration, anti-tumour anti-leukemic, anti-bacterial, insecticidal and insect repellent activities. *Maytenus emarginata* have significant in-vitro antioxidant activity and act as good source of natural antioxidant [8].

Maytenus emarginata leaves and root extract have been reported to possess antimicrobial property [9]. Presence of high amounts of phenol in the *Gymnosporia Montana* has been reported [10]. De *et al.,* (1991) [11] found the presence of fructose, glucose and seven amino acids on chemical analysis of *Gymnosporia Montana*. Triterpene, quinone, methides, luperone, β-amirine, dulcital and sitosterol have been isolated from timber, root and leaf extract of *Gymnosporia Montana* [12].

Protective effect of *Maytenus emarginata* against hepatotoxicity was observed earlier [13]. However, the protective effect of *Maytenus emarginata* in nephrotoxicity has not yet been studied yet. Thus the present study was designed to investigate the protective effects of *Maytenus emarginata* against Paracetamol induced toxicity.

MATERIAL AND MEHODS

Chemicals

Paracetamol was obtained from SNP College, Pusad. The kits for biochemical estimation were purchased from Merk Ltd, Kalyan-Badlapur Road, Ambernath (INDIA). The solvents and other chemicals were obtained from local dealers.

Animals

The experiment was carried out on male Wistar rats weighing 150 ± 20 g. Rats were maintained in standard laboratory conditions of temperature (27 ± 2°C) and 60% humidity. The rats were fed on standard feed pellets with clean drinking water *ad lib*. The experiment was conducted after getting approval from Institutional Animal Ethics Committee, COVAS, Udgir, Maharashtra, INDIA.

All the rats were divided into five groups. Group C1, C2 and C3 represented healthy control, vehicle control and toxic control respectively. Group T1and T2 acted as treatment groups receiving *M. emarginata* extract @ 100 mg/kg body weight and 150 mg/kg body weight of rats respectively.

Collection of Plant Material

The leaves of *Maytenus emarginata* were collected from nearby areas of Udgir city (M.S.) in the month of October-November and authenticated by Dr. Allapure, HOD, Dept. of Botany, Maharashtra Udaygiri College, Udgir, Maharashtra, INDIA.

Preparation of Extract

Maytenus emarginata leaves were shade dried at room temperature and were powdered by using mechanical grinder to a fine powder. The alcoholic extract of *M. emarginata* was obtained with 95% alcohol using Soxhalet apparatus by extracting at 65 °C till the discoloration of alcohol in the extraction chamber. The extract was kept in air tight vials after complete evaporation and was stored in refrigerator till use.

Induction of Hepatotoxicity

Rats were fasted over night and hepatotoxicity was induced by administration of Paracetamol in pure form (API) in distilled water @ dose rate of 500 mg/kg body weight by gastric gavage to the rats of groups C3, T1 and T2 once daily for the entire duration of study.

Extract treatment

The rats in groups T1 and T2 were given ethanol extract *M. emarginata* @ 100 mg/kg body weight of rats and *M. emarginata* extract @150 mg/kg body weight of rats orally in propylene glycol using oral gavage, respectively daily for twenty eight days period.

Haemato-biochemical Studies

Blood samples were collected from orbital plexus of rats into sterilized vials for collecting serum for biochemical estimations and into heparin coated vials for hematological estimations at the end of experiment. The serum samples were used to estimate serum creatinine levels. After collecting blood the rats were sacrificed, kidneys were removed immediately examined and fixed in 10% formal saline for histopathological studies.

Histopathological Observations

Kidney pieces were cut into suitable size and were collected in 10% formal saline for fixation. After proper fixation these tissue samples were embedded in paraffin and processed as per standard procedures. The sections were sectioned at 3 – 5 µ thickness and were stained with Mayer's haematoxylin and eosin for microscopic examination [14].

Statistical Analysis

Numerical data was expressed as mean value ± standard error and was analyzed statistically by CRD and analysis of variance by using Graph Pad Prism Software. A probability of less than 5% ($p<0.05$) was considered significant.

RESULTS AND DISCUSSION

Present study was aimed to investigate the protective effects of *Maytenus emarginata* extract against the Paracetamol induced nephrotoxicity in Wistar rats. Nephrotoxicity induced by various drugs was assessed by investigating the serum creatinine and urea levels [15]. Table 1 depicts the serum levels of creatinine and Blood Urea Nitrogen (BUN) in the experimental rats of various groups. Rats of C1 and C2 group showed normal levels of serum creatinine and BUN.

Table 1: Effect of *Maytenus emarginata* extract against Paracetamol toxicity on Biochemical parameters

Parameters	Normal control	Vehicle control	Toxic control	MEE 100 mg/kg	MEE 100 mg/kg
Creatinine (mg /dl)	0.86^{bc} ±0.05	0.77^{c} ±0.06	2.04^{a} ±0.10	1.04^{b} ±0.09	0.79^{c} ±0.05
BUN (mg /dl)	18.00^{c} ±0.73	18.33^{c} ±0.88	40.33^{a} ±1.99	21.83^{b} ±0.74	18.83^{c} ±0.54

Table 2: Effect of *Maytenus emarginata* extract against Paracetamol toxicity on Haematological parameters

Parameters	Normal control	Vehicle control	Toxic control	MEE 100 mg/kg	MEE 100 mg/kg
Haemoglobin g%	12.30^{a}±0.22	11.90^{a}±0.22	8.43^{c}±0.20	11.87^{ab}±0.17	12.00^{a}±0.12
Haematocrit (%)	38.17^{a}±0.70	38.67^{a}±0.66	23.00^{c}±0.73	34.83^{b}±1.22	38.17^{a}±0.90
TEC (10^6/µl)	6.85^{ab}±0.21	6.65^{ab} ±0.39	5.71^{c}±0.19	6.41^{bc}±0.08	7.13^{a}±0.23
TLC (10^3/µl)	8.32±0.17	9.25±0.54	8.35±0.50	8.92±0.43	8.55 ±0.45

*Values are expressed as mean ± SEM (n=6) using one way ANOVA followed by CRD. Means bearing different superscripts within rows differ significantly from each other. MEE = *Maytenus emarginata* extract.

Toxic control (C3) group rats showed marked increase in serum creatinine and BUN values as compared to the values of serum creatinine and BUN in rats of healthy control (C1) group. Increased levels of serum creatinine and urea have been considered as index of assessing nephrotoxicity [16-17]. The elevated values in experimental rats indicate the severity of kidney damage by the Paracetamol. Necrosis of liver and kidney cells observed in the present study might be responsible for elevation of these biomarker enzymes. Values of serum creatinine and BUN in T1 and T2 group rats treated with *Maytenus emarginata* extract showed significant ($P<0.05$) improvement and serum creatinine values of T2 group rats were statistically comparable with the values of serum creatinine and BUN in C1 (healthy control) group rats. Previous studies reported the significant decrease in serum creatinine level on treatment with various herbal preparations and extracts which was increased in Paracetamol induced nephrotoxic rats which support the findings of present study [4][18].

Haematological findings of the experiment have been depicted in the table 2. Rats of the group C1 and C2 showed haematological values within the normal range. Rats of C3 group (toxic control) group showed significant decrease in mean values of haemoglobin, Haematocrit and Total Erythrocyte Count while compared with rats of C1 group. Blood perform vital functions in the body including transport of substances making it susceptible to the intoxication by xenobiotics, making hematopoietic system an important target system [19] which might be the reason of effect seen on haematological values thereof. Values of Hb, Hct and TEC in rats of T1 and T3 groups treated with *Maytenus emarginata* were significantly improved than the values of Hb, Hct and TEC in rats of C3 group and were at par with values of Hb in healthy control group rats, indicates that the treatment with extract of *Maytenus emarginata* significantly ($P<0.05$) improved the reduced Hb, Hct and TEC levels in Paracetamol induced nephrotoxicity. Previous studies on haematological changes in Paracetamol toxicity reported significant decrease in Hb, Hct and TEC of Paracetamol treated rats and increase of these parameters near to normal by various plant extract treatment which are accordance with the findings of present study [1][20-21].

Histopathological examination of sections of kidney tissue of C1 (fig 1a) and C2 (fig 1b) groups did not reveal any appreciable histopathological alterations. Kidney sections of rats of C3 Group (fig 1c) treated with Paracetamol showed loss of renal tubular architecture with rearrangement of renal tubules and glomerulus. Kidney sections showed diffuse degenerative changes in the sections. The renal tubules showed cellular swelling invariably with narrowing of lumen to great extent. Sections revealed inter

tubular congestion with T2 group was not evident. Kidney sections reveal normal tubules with no protein cast in their mononuclear inflammatory cell infiltration. There were focal areas of necrosis were present in the renal sections with accumulation of protein casts in the tubular lumen. The Binding of NAPBQ, an oxidative product of Paracetamol, to sulphydryl groups of protein resulting in cell necrosis [22]. Rats treated with *Maytenus emarginata* extract of the group T1 (fig 1d) and T2 (fig 1e) showed maintained renal tubular architecture near to normal. Inter tubular congestion in the sections of T1 and lumen. Sections of kidney revealed some degree of cellular swelling. The protective effects by the extract could have been due to flavonoids and alkaloids [23-24]. These findings are in accordance with earlier studies [4][18]. The biochemical estimations are supported by histopathological findings and coincide with other investigators [25-28]. These findings suggest the nephroprotective effects of *Maytenus emarginata* extract treatment with maximum effects @ 150 mg/kg B.W. of rats.

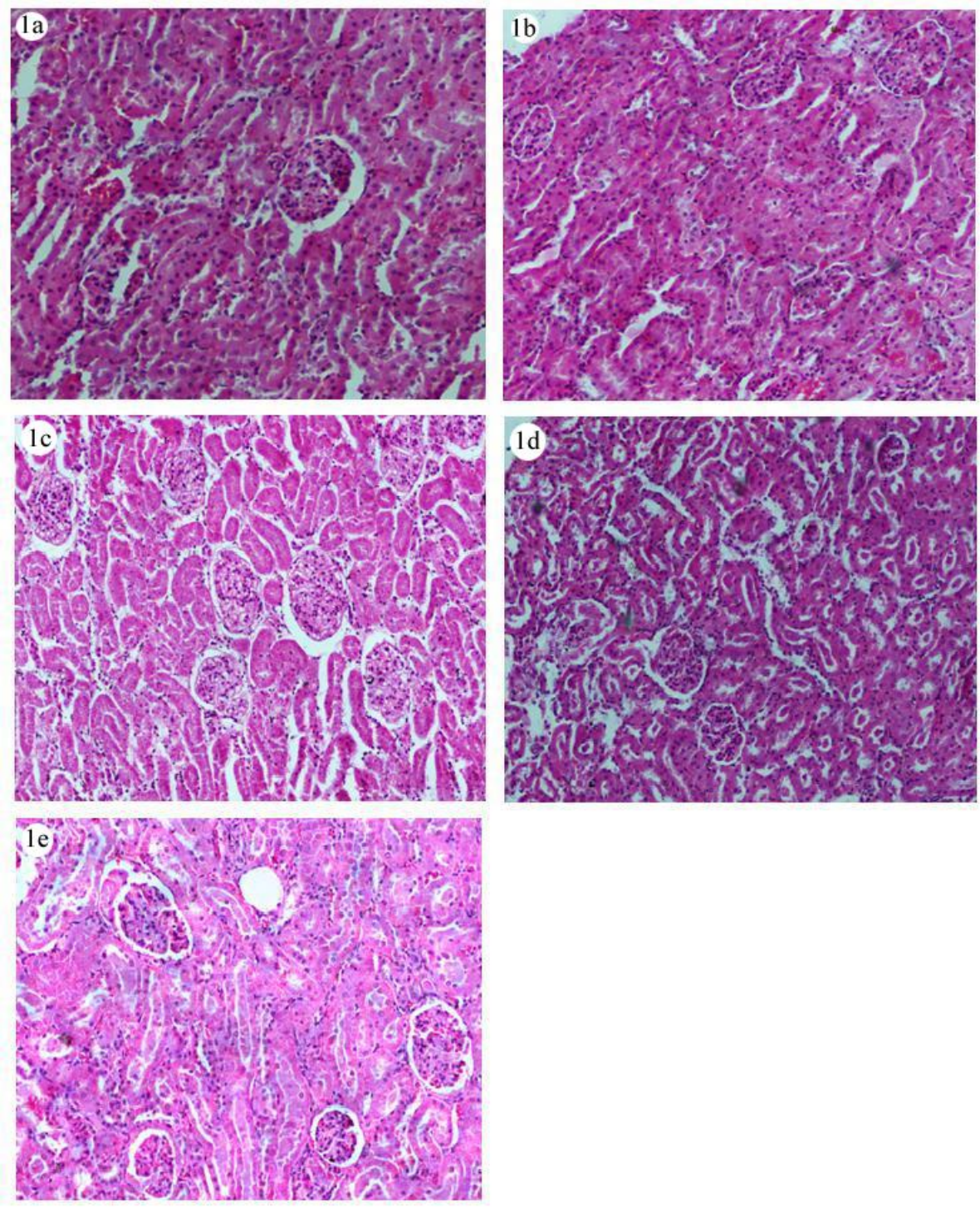

Fig. 1: Nephroprotective effect of *Maytenus emarginata* extract a) Healthy Control b) Vehicle Control c) Toxic Control- Section Showing narrowing of tubular lumen, necrosis and infiltration of inflammatory cells d) MEE (100 mg/kg) treatment- Section showing near to normal histoarchitecture with mild infiltration of inflammatory cells e) MEE (150 mg/kg) Treatment- Section showing maintained histoarchitecture with no evidence of necrosis (H&E X200).

CONCLUSION

In the present investigation it was found that *Maytenus emarginata* extract bring all the parameters affected by Paracetamol near to normal. Thus the ethanol extract of *Maytenus emarginata* has protective effect which minimizes the nephrototoxicity induced by Paracetamol, thereby suggesting its use as a potent nephroprotective agent.

ACKNOWLEDGEMENT

The authors are thankful to Associate Dean, College of Veterinary & Animal Sciences, Udgir (M.S.) for providing the facilities required.

REFERENCES

1. Elhabib, E. M., Homeida M. M. A. and Adam S. E. I. Effect of combined Paracetamol and *Cuminum cyminum* and *Nigella sativa*use in Wistar rats. J. Pharmacol. Toxicol. 2007;2 (7): 653-659.
2. Dasgupta B., Kalita J. C., Chowdhury A. and Kotoky J. Hepatoprotective activity of *Nelsonia canescens* (Lam.) Spreng on acute hepatotoxicity induced by Paracetamol. Int. J. Pharmacy. Pharm. Sci. 2012; 4 (1): 107-112.
3. Sandhu N. S., Chopra D. and Kaur S. Amelioration of Paracetamol-induced hepatotoxicity by a protein isolated from the leaves of the herbs *Cajanus acutifolus* Linn. Int. J. Pharmacy. Pharm. Sci. 2010; 2 (3): 75-80.
4. Gulnaz H., Tahir M., Muni B. and Sami W. Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male Wistar rats. Biomedica. 2010;26: 9-15.
5. Sharma A., Makwana M. and Rathore H. S. Will herbal-Paracetamol combination drug prevent both liver and kidney disease? – Results and Possibilities. Ethnobotanical Leaflets. 2008; 12: 286-298.
6. Palani S., Raja S., Kumar R. P., Jayakumar S. and Kumar B. S. Therapeutic efficacy of *Pimpinella tirupatiensis* (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. Int. J. PharmTech. Res. 2009;1 (3): 925-932.
7. Anjaria J., Minoo P. and Dwivedi S. Ethnovet Heritage Indian Ethno-Veterinary Medicine an overview, Pathik enterprise, Ahmedabad, 2002. p. 242.
8. Sagwan, S., Rao D. V. and Sharma R. A. In-vitro antioxidant activity of *Maytenus emarginata* (Willd.) using DPPH assay. J. Chem. Res. 2011;4 (7): 2276-2278.
9. Tambhekar, D. H. and Khante B. S. Evaluation of antibacterial properties of ethnomedicinal herbs used by korkus in Melghatof India against enteric pathogens. Int. J. Pharma. Bio. Sci. 2010;1 (1).
10. Bhavita, D., Zaveri M. and Lakshmi B. Pharmacognostical and phytochemical study of leaf of *Gymnosporia Montana* (vikalo). J. Global Pharm. Tech. 2011;3 (1): 23-27.
11. De, S., Dave K. K. and Bhavsagar J. C. Priminary phytochemical investigations of the leaves of vikartaka (*Gymnosporia montana*) and prosarani (*Paederia foetida*). BMEBR. 1991;12 (3): 145-149.
12. Winjerantne, D. B. T., Kumar V., Uvais M., Sultanbawa S. and Balsubramaniam S. Triterpenes from *Gymnosporia emarginata.* Phytochemistry. 1982;21 (9): 2422-2423.
13. Patel, P. B., Patel T. K., Shah P., Baxi S. N., Sharma H.O. and Tripathi C. B. Protective effect of ethanol extract of *Gymnosporia Montana* (Roth.) Bemth. in Paracetamol induced hepatotoxicity in rats. Indian J. Pharm. Sci. 2010;72 (3): 392-396.
14. Culling C.F.A. A Hand Book of Histopathological Techniques, 3rd edn., Butter Worth and Co., Ltd. 1974. p. 29-221.
15. Bennit W. M., Parker R. A., Elliot W. C., Gilbert D. and Houghton D. Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity. J. Infec. Diseases. 1982; 145: 370-374.
16. Anwar S., Khan N. A., Amin K. M. Y. and Ahmad G. Effects of Banadiq-al Buzoor in some renal disorders. Hamdard Medicus, Vol. XLII. Hamdard Foundation, Karachi, Pakistan 1999. 4: 31-36.
17. Ali, B., Ben H., Ismail T. H. and Basheer, A. A. Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity: influence of gonadectomy and hormonal replacement therapy. Ind. J. Pharmacol. 2001; 33: 369-373.
18. Palani, S., Raja S., Kumar R. P., Parameswaran P. and Kumar B. S. Therapeutic efficacy of Acorus calamus on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. Acta Pharmaceutica Sciencia. 2010; 52: 89-100.
19. Adeneye, A. A., Olagunju J. A., Elias S. O., Olantunbosun D. O., Mustafa A. O., Adeshile O. I., *et al.* International J. of Applied Res. in Natural Products. 2008;1 (3): 29-42.
20. Nwodo, O. F. C., Joshua P. E., Ozoemena N. F. and Onwuzu N. J. Haematological Evaluation of the Effect of Coconut (Cocosnucifera) Water on Paracetamol- Intoxication in Laboratory Rats. J. of Pharm. Res. 2010;3 (8): 1831-1834.
21. Dougnon, T. J., Kpodekon T. M., Laleye A., Ahissou H. and Loko F. Effects of pineapple (*Ananas comosus*) on haematological and biochemical parameters in albino Wistar rats intoxicated with Doliparen. Afr. J. Biotechno. 2011;10 (28): 5418-5422.
22. Kanchana N. and Sadiq A. M. Hepatoprotective effect of *Plumbago zenlanica* on Paracetamol induced liver toxicity in rats. Int. J. Pharmacy. Pharm. Sci. 2011; 3 (1): 152-154.
23. Donsky, H. M. D. and Clarke D. M. D. Reliévaa *Mahonia aquifolium* extract for the treatment of adult patients with atopic dermatitis. Am J Ther. 2007; 14: 442-46.
24. Lucia R., Marek O., Daniela K., Viktor K. and Lydia B. Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. J Inflamm. 2007;32 (4):15.
25. Corcoran G., Rac W., Smith G. and Mitchell J. Effect of N-acetyl-cysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther. 1985; 232, 864–72.
26. Gardner C. R., Laskin J. D., Dambach D. M., Sacco M. and Durham S. K. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: potential role of tumor necrosis factor-alpha and interleukin-10. Toxicol Appl Pharmacol. 2002; 184, 27–36.
27. Newton J. F., Bailie M. B. and Hook J. B. Acetaminophen nephrotoxicity in the rat. Renal metabolic activation in vitro. Toxico1 Appl Phannaco. 1983; l70: 433-44.
28. Trumper L., Monasterolo L. A. and Elias M. M. Probenecid protects against in vitro acetaminophen induced nephrotoxicity in male Wistar rats. J Pharmacol Exp Ther. 1998; 283: 606-10.