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# The effect of Phyllanthus amarus leaf extract on the lipid profile of gentamicin-induced hepatotoxicity in albino rats

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## Abstract

*Phyllanthus amarus* is a medicinal plant, used in traditional practice for treating diseases like hypertension, liver damage and diabetes mellitus. This study aimed to provide a potent therapeutic alternative to conventional drugs in managing liver diseases. Ethanol leaf extract of *Phyllanthus amarus* at 50, 100 and 200 mg/kg body weight was investigated for its acclaimed hepatoprotective activity in gentamicin-induced hepatotoxicity in albino rats. Thirty albino rats weighing 120-150 g were assigned into six groups (I-VI) of five animals each. Rats in group I (control) received distilled water orally. Rats in groups II, III, IV, V and VI were induced with 100 mg/kg of gentamicin for seven days. Groups III-V were treated with 50, 100 and 200 mg/kg body weight of the extract, respectively while group VI was treated with 200 mg/kg bw of silymarin. The lipid profile that relates to hepatotoxicity was assessed using standard methods. The significantly increased serum and liver lipid profile: cholesterol, triglyceride, Low-density lipoprotein and reduced high-density lipoprotein in the untreated animals were attenuated by the extract. Overall, the extract possesses hepatoprotective activity with 200 mg/kg body weight being the most effective dose. The coronary Heart Index (CRI) was not significantly different from the control. Therefore, the ethanol leaf extract of *Phyllanthus amarus* could be explored in controlling some of the metabolic dysfunctions usually associated with hepatotoxicity.

**Keywords:** *Phyllanthus amarus*; Hepatoprotective Activity; Gentamicin; Lipid Profile

## 1. Introduction

Global interest has been shown in the therapeutic potential of medicinal plants to create an alternative, healthy, efficient and accessible complement with little to no side effects that could be in the form of medicinal drugs, food or nutritional supplements, in the treatment and management of different diseases (Mshelia *et al.,* 2017). Long before mankind observed the existence of microbes, the idea that plants contained healing potentials was accepted (Adesokan *et al.,* 2008). Records of early civilization in all parts of the world revealed that many drugs used in modern medicine have been in use since ancient times (Adesokan *et al.,* 2008). Herbal medicine has generated a considerable amount of interest worldwide due to its contribution to overall healthcare delivery. This is predicated on the fact that an estimated 80% of the population in developing countries depend on natural products or herbal medicine (Okigbo *et al.,* 2008).

Despite the improved health system and longevity in the US and Europe, millions of people in these countries are turning back to herbal medicines to prevent or treat many illnesses (Okigbo *et al.,* 2008), and to circumvent the resistance of many human pathogens to conventional drugs, some of which produced side effects like hypersensitivity and immuno-suppression (Adesokan *et al.,* 2008). Medicinal plants contain inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.,* 2008). Their properties could be based on the antioxidant, antimicrobial antipyretic, and enzyme-inhibitory effects of the phytochemicals present in them (Adesokan *et al.,* 2008). It was estimated that 25% of all prescribed medicines today are substances derived from plants (Okigbo *et al.,* 2008). Medicinal plants that show

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improvement in liver function are rich in various bioactive compounds that possess anti-inflammatory, antioxidant, immunomodulatory hepatoprotective and biological response-modifying effects (Foghis. 2023)

The liver regulates most of the chemical levels in the blood and produces bile to carry away waste products. All blood from the stomach and intestines passes through the liver. The liver processes the blood and metabolizes drugs into non-toxic forms or forms that are easier for the body to use. Other functions of the liver include; the conversion of ammonia to urea via the urea cycle. It breaks down insulin and other hormones, breaks down toxic substances (e.g. methylation) and metabolizes drugs, produces and excretes bile, responsible for the mainstay of protein metabolism, synthesis as well as degradation among others (Cotran *et al.*, 2005). Despite the role of the liver, the only treatment for chronic liver disease at present is a liver transplant.

*Phyllanthus amarus* herb has found its traditional usefulness in several health problems such as dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery (Patel, 2011). Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions. Its effect on the excretory system is due to its antiurolithic properties and is used in the treatment of kidney/gallstones, other kidney-related problems, appendix inflammation and prostate problems (Bose Mazumdar Ghosh *et al.,* 2022; Khatoon *et al*., 2008). Because of its efficacy in the field of gastrointestinal disorders, it is used in the treatment of disorders like dyspepsia, colic, diarrhoea, constipation and dysentery. The herb has found use in several female problems such as leucorrhoea, menorrhagia and mammary abscess and can act as a galactagogue.

The choice of this plant was predicated on its use by traditional herbal healers in the Southwestern area of Nigeria in the treatment of liver damage. Thus, the study aims to investigate the acclaimed hepatoprotective effect of *Phyllanthus amarus* on gentamicin-induced hepatotoxicity in albino rats.

## 2. Material and methods

### 2.1. Collection of sample

Plant materials, *Phyllanthus amarus* (PA), leaves were collected from the nearby farm around the hostels at The Federal Polytechnic Ado Ekiti, Nigeria in September 2023, air dried in the laboratory, pulverized and then stored in an airtight container. Sample identification and aunthetication was carried out at the Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Ekiti state Nigeria..

### 2.2. Reagents and Chemicals

All chemicals and all other reagents used were of the analytical grade.

### 2.3. Preparation of Extract

Plant material leaves were washed with sterile water, allowed to drain and air-dried for 28 days, at room temperature. The air-dried samples were ground to a fine powder using a blender. 500 g of sample was soaked in 2000 ml of ethanol for 72 hours. This was filtered and later air-dried to obtain the extract powder. The extract was kept in the freezer at 4 $^0$C for further studies.

### 2.4. Animal protocol

30 Wistar albino rats weighing 120 kg – 150 kg were obtained from the animal house at Ekiti State University, Ado-Ekiti. They were acclimatized in the animal house of the Department of Science Technology, The Federal Polytechnic, Ado Ekiti for 2 weeks, housed in clean wire meshed cages under standard conditions temperature (24 ± 1$^o$C), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed free access to food (commercial pelletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

### 2.5. Experimental Design

30 male Wistar albino rats were randomly divided into six groups (I-VI) of five animals in each group.

### 2.6. Animal treatment

The animal treatment is shown in the table below

**Table 1** Animal treatment

| Groups | Treatment |
|---|---|
| Group 1: Non-Hepatotoxic control (NHC) | Distilled water only for 14 days |
| Group 2: Hepatotoxic Control (HC) | Gentamicin 100 mg/kg alone for 7 days |
| Group3 | Gentamicin 100 mg/kg for 7 days + 50 mg/kg *Phyllanthus amarus* leaf extract for 14 days |
| Group 4 | Gentamicin 100 mg/kg for 7 days + 100 mg/kg *Phyllanthus amarus* leaf extract for 14 days |
| Group 5 | Gentamicin 100 mg/kg for 7 days + 200 mg/kg *Phyllanthus amarus* leaf extract for 14 days |
| Group 6 | Gentamicin 100 mg/kg for 7 days + 200 mg/kg Silymarin for 14 days |

### 2.7. Dissection of animals

The rats were dissected and blood was collected in clean sample bottles and allowed to stand for 1 hour.

### 2.8. Preparation of serum

Serum was prepared by centrifugation at 3000 rpm for 15 min at 25°C. The clear supernatant was collected and used for the estimation of serum biochemical parameters.

### 2.9. Preparation of Homogenates

The livers were excised using scissors and forceps. They were trimmed of fatty tissue, washed in ice-cold 1.15% potassium chloride solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 6000 g at 4°C for 30 mins. The supernatant obtained was collected and stored under $4^0$C and then used for biochemical analyses.

### 2.10. Lipid Profile Analysis

#### *2.10.1. Estimation of Total Cholesterol Level*

Total cholesterol level was determined based on the method of Trinder (1969) using commercially available kits (Randox laboratories, UK).

#### *2.10.2. Evaluation of Concentration of Triglyceride*

Triglyceride level was determined by the method of Tietz (1990) using commercially available kits (Randox laboratories, UK).

#### *2.10.3. High-Density Lipoprotein (HDL-c)-Cholesterol Assay*

The method of Grove (1979) was adopted in the estimation of the concentration of HDL- cholesterol in the serum.

#### *2.10.4. Low-Density Lipoprotein (LDL) - Cholesterol Determination*

The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedwald *et al.,* (1972)

#### *2.10.5. Very Low-Density Lipoprotein (VLDL) - Cholesterol Determination*

The concentration of very low-density lipoprotein in the serum was calculated using the formula of Friedwald *et al.* (1972)

*2.10.6. Coronary Risk Index Estimation*

The coronary risk index was calculated using the formula of Friedwald *et al.* (1972) as given below:

$$CRI = \frac{Cholesterol}{High\ Density\ Lipoprotein}$$

### 2.11. Statistical Analysis

All values are expressed as mean ± SD. Statistical evaluation was done using IBM SPSS 25.0 statistics for Windows (Armonk, 2017). The significance level was set at $p < 0.05$.

## 3. Results

**Table 2** Effect of *Phyllanthus amarus* leaf extract on serum lipid profile in gentamicin-induced hepatotoxicity in albino rats

| Parameters | CHOL(mg/dl) | TRIG(mg/dl) | HDL(mg/dl) | LDL(mg/dl) | VLDL(mg/dl) | CRI |
|---|---|---|---|---|---|---|
| NHC | 66.29±2.06$^{a}$ | 34.33±1.05$^{a}$ | 24.03±0.87$^{e}$ | 35.39±1.04$^{a}$ | 6.87±0.40$^{a}$ | 2.76±0.10$^{a}$ |
| HC (GEN ALONE) | 127.49±3.04$^{f}$ | 67.88±1.15$^{f}$ | 14.77±0.52$^{a}$ | 99.14±3.10$^{f}$ | 13.58±0.36$^{e}$ | 8.63±0.06$^{e}$ |
| GEN+ PA (50mg/kg) | 91.53+1.07$^{e}$ | 50.76±1.00$^{e}$ | 17.23±0.69$^{b}$ | 64.15+1.31$^{e}$ | 10.15+0.73$^{d}$ | 5.31+0.10$^{d}$ |
| GEN+ PA (100mg/kg) | 82.17+1.14$^{d}$ | 45.06±1.23$^{d}$ | 18.84±0.59$^{c}$ | 54.32+1.02$^{d}$ | 9.01+0.69$^{c}$ | 4.36+0.11$^{c}$ |
| GEN+ PA (200mg/kg) | 72.69±2.08$^{b}$ | 37.59±1.20$^{b}$ | 25.48±0.38$^{f}$ | 39.62 ±2.11$^{b}$ | 7.59±0.53$^{b}$ | 2.85±0.09$^{a}$ |
| GEN+ SIL (200mg/kg) | 74.50+1.01$^{c}$ | 39.12±1.02$^{c}$ | 23.01±0.76$^{d}$ | 43.67+1.37$^{c}$ | 7.82+0.45$^{b}$ | 3.24+0.10$^{b}$ |

Values are expressed as mean ± standard deviation (n=5). Values with the different superscript(s) in a column are significantly different (P<0.05).
**Key:** CHOL=Cholesterol,TRIG =Triacylglyceride,HDL =High density lipoprotein,LDL=Low density lipoprotein,VLDL= Very low density lipoprotein, PA= *Phyllanthus amarus*

The results in Table 2 give the effects of *Phyllanthus amarus* leaf extract on serum lipid profile: cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c) and Coronary Risk Index (CRI) in gentamicin-induced hepatotoxicity albino rats. The result showed that there was a statistically significant (P<0.05), increase in serum cholesterol, triglyceride, low-density lipoprotein and very low-density lipoprotein. There was also a decrease in the serum high-density lipoprotein in the experimental animals that received gentamicin compared to the normal control. Extract-dependent decrease in serum concentration of cholesterol, triglycerides, high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c) and Coronary Risk Index (CRI) were observed in animals treated with all dosages (50 mg/kg, 100 mg/kg and 200 mg/kg) of *Phyllanthus amarus* leaf extract when compared with the untreated group. *Phyllanthus amarus* leaf extract (50 mg/kg, 100 mg/kg and 200 mg/kg) significantly (P<0.05) increased the concentration of high-density lipoprotein when compared with the untreated group.

**Table 3** Effect of *Phyllanthus amarus* leaf extract on Liver Lipid Profile in Gentamicin-induced hepatotoxicity in Albino albino Rats

| Parameters | CHOL(mg/dl) | TRIG(mg/dl) | HDL(mg/dl) | LDL(mg/dl) | VLDL(mg/dl) | CRI |
|---|---|---|---|---|---|---|
| NHC | 87.58±2.14$^{b}$ | 53.70±1.22$^{a}$ | 30.33±1.42$^{e}$ | 46.51±1.96$^{b}$ | 10.74±0.70$^{a}$ | 2.89±0.08$^{a}$ |
| HC (GEN ALONE) | 114.63±3.05$^{f}$ | 91.09±2.10$^{f}$ | 19.09±0.83$^{a}$ | 77.32±2.09$^{e}$ | 18.22±0.62$^{c}$ | 6.00±0.10$^{d}$ |
| GEN+ PA (50mg/kg) | 105.80±2.13$^{e}$ | 82.18±2.31$^{e}$ | 23.82±0.64$^{b}$ | 65.54±2.02$^{d}$ | 16.44±0.39$^{d}$ | 4.44±0.02$^{c}$ |
| GEN+ PA (100mg/kg) | 94.28±1.79$^{d}$ | 70.37±1.65$^{d}$ | 26.37±0.56$^{c}$ | 49.05±1.48$^{c}$ | 18.86±0.64$^{c}$ | 3.57±0.03$^{b}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| GEN+ PA(200mg/kg) | 85.54±1.84$^{a}$ | 58.60±1.17$^{c}$ | 32.30±0.90$^{f}$ | 41.52±1.18$^{a}$ | 11.72±0.55$^{b}$ | 2.65±0.07$^{a}$ |
| GEN+ SIL(200mg/kg) | 89.60±1.724$^{c}$ | 55.03±1.68$^{b}$ | 29.41±0.49$^{d}$ | 49.18±1.26$^{c}$ | 11.01±0.49$^{bc}$ | 3.05±0.03$^{b}$ |

Values are expressed as mean ± standard deviation (n=5). Values with the different superscript(s) in a column are significantly different (P<0.05).
**Key:** CHOL=Cholesterol, TRIG=Triacylglyceride, HDL=High density lipoprotein, LDL=Low density lipoprotein, VLDL= Very low density lipoprotein, PA= *Phyllanthus amarus*

The results in **Table 3** give the effects of *Phyllanthus amarus* leaf extract on liver lipid profile: cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c) and Coronary Risk Index (CRI) in gentamicin-induced hepatotoxicity in albino rats.

The result showed that there was a statistically significant (P<0.05), increase in serum cholesterol, triglyceride, low-density lipoprotein and very low-density lipoprotein while there was a decrease in the serum high-density lipoprotein in the experimental animals that received gentamicin compared to the normal control.

Extract-dependent decrease in serum concentration of cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c) and Coronary Risk Index (CRI) were observed in animals treated with all dosages (50 mg/kg, 100 mg/kg and 200 mg/kg) of *Phyllanthus amarus* leaf extract when compared with the untreated group.

*Phyllanthus amarus* leaf extract (50 mg/kg, 100 mg/kg and 200 mg/kg) significantly (P<0.05) increased the concentration of high-density lipoprotein when compared with the untreated group.

## 4. Discussion

Hepatotoxicity is one of the damages caused by free radicals. This study demonstrated the antioxidant potential and hepatoprotective activity of *P. amarus* leaf extract on gentamicin-induced liver injury in rats. The deleterious increase in the level of cholesterol, triglyceride and low-density lipoprotein with a decrease in the high-density lipoprotein in the liver following exposure to gentamicin toxicity (Tables 2 and 3) was an indication of oxidative stress due to the generation of free radicals. It has been found that people with dyslipidemia have an increased chance of liver disease (Kathak, 2022). A high level of cholesterol is a sign of dyslipidemia (Jialal and Singh, 2019).

Drug-induced hepatotoxicity is still a major and significant public health problem for healthcare professionals (physicians and nurses) as well as the healthcare products regulatory bodies, including the WHO and US FDA. A high proportion of usually used medications and even herbal drugs have the potential to cause liver injury (Björnsson and Björnsson, 2022). Many of the drug-induced liver damage do not show symptoms. However, jaundice is commonly seen (Kuna *et al.,* 2018). The challenges posed by this pathologic condition affect mainly the development of new pharmaceutical drugs and the withdrawal of pharmaceutical promising medicines from the pharmaceutical market (Lee, 2003).

The generation of reactive oxygen species (ROS), proinflammatory mediators and direct action on the cellular organelles of hepatocytes have been reported as some of the mechanisms underlying the induction, onset and occurrence of drug-induced hepatotoxicity (Wallace, 2004). The hepatotoxicants that act by the formation of ROS include acetaminophen (Wallace,2004), halothane (Sarich *et al*., 2006), isoniazid (Wallace, 2004; Lee, 2003), allyl alcohol and bromobenzene. They undergo biotransformation to chemically reactive toxic metabolites which can covalently bind to crucial cellular macromolecules thus inactivating, inhibiting, or blocking critical cellular functions.

The present study revealed that *Phyllanthus amarus* extract has a profound effect on the serum and liver lipid profile of rats. The significantly increased serum lipid profile: cholesterol, triglyceride, Low-density lipoprotein and reduced high-density lipoprotein in the untreated animals were attenuated by the extract. The values obtained showed that silymarin and the extracts, at all dosages, caused a significant reduction in TC, TG and LDL-C while there was an increase in the level of HDL when compared to the control group. The hepatoprotective effect of the extract is dose-dependent. The highest dose used for treatment (200 mg/Kg) showed better activity than silymarin, the standard drug. The CRI of the group treated with the highest dose was not significantly different from the control.

## 5. Conclusion

In conclusion, this study has demonstrated that ethanol leaf extract of *P. amarus* modulates the liver and serum lipid profile level of gentamicin-induced hepatotoxicity in rats in a dose-dependent manner. It also exhibits antioxidant properties and could prevent impairment of the liver in the hepatotoxicity state. The highest dose used has a CRI not significantly different from the control.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

Animal use and care committee of the federal Polytechnic Ado Ekiti after critical evaluation and review approved this study (approval number FPA/EC/24/0005) National Institutes of Health's Guidelines for using and caring for laboratory animals and relevant methods were followed to ensure that the animals were not subjected to excessive stress and discomfort during this experiment

## References

[1] Adesokan, A. A., Yakubu, M. T., Owoyele, B. V., Akanji, M. A., Soladoye, A. and Lawal, O. K. (2008): Effect of Administration of Aqueous and Ethanolic Extracts of Enantia chlorantha Stem Bark on Brewer's Yeast-induced Pyresis in Rats. African Journal of Biochemical Research; 2 (7): 165-169.

[2] Armonk, N. Y. (2017): IBM SPSS Statistics for Windows, Version 25.0 IBM Corp.

[3] Björnsson, H., and Björnsson, E. (2022). Drug-induced liver injury: Pathogenesis, epidemiology, clinical features, and practical management. European Journal of Internal Medicine, 97, 26–31.

[4] Bose Mazumdar Ghosh, A., Banerjee, A. and Chattopadhyay, S. An insight into the potent medicinal plant *Phyllanthus amarus* Schum. and Thonn. Nucleus 65, 437–472 (2022). https://doi.org/10.1007/s13237-Jialal and Singh, 2019-00409-z

[5] Cotran, V., Ramzi, S., Kumar, C., Vimay, M., Fausto, F., Nelson, F., Robins, S., Abbas, K., and Abul, K., (2005): Pathologic Basis of Disease (7th ed.) St. Louis, mo; Elsevier Saunders pg 878

[6] Foghis, M., Bungau, S. G., Bungau, A. F., Vesa, C. M., Purza, A. L., Tarce, A. G., Tit, D. M., Pallag, A., Behl, T., Ul Hassan, S. S., and Radu, A. (2023). Plants-based medicine implication in the evolution of chronic liver diseases. Biomedicine and Pharmacotherapy, 158, 114207. https://doi.org/10.1016/j.biopha.2022.114207

[7] Friedwald, W.T., Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in the Plasma, without the use of Preparative Ultracentrifuge. Clinical Chemistry; 18: 499-502.

[8] Grove, T.H. (1979): Effects of Reagent pH on the Determination of High-Density Lipoprotein Cholesterol by Precipitation with Sodium Phosphotungstate-Magnesium. Clinical Chemistry. 25: 560-564.

[9] Jialal, I. and Singh, G. Management of diabetic dyslipidemia: An update. World J Diabetes 10, 280–290 (2019).

[10] Kathak, R.R., Sumon, A.H., Molla, N.H., Hasan, M., Miah, R., Tuba., H. R., Habib, A., and Ali, N. (2022) The association between elevated lipid profile and liver enzymes: a study on Bangladeshi adults. Sci Rep 12, 1711 https://doi.org/10.1038/s41598-022-05766-

[11] Khatoon, S., Rai, V., Singh Rawat, A.K., and Mehrotra, S., (2006): Comparative Pharmacognostic Studies of three Phyllanthus species. Journal of Ethnopharmacology 104, 79–86

[12] Kuna, L., Bozic, I., Kizivat, T., Bojanic, K., Mrso, M., Kralj, E., Smolic, R., Wu, G. Y., Smolic, M. (2018): Models of Drug-Induced Liver Injury (DILI) - Current Issues and Future Perspectives. Curr Drug Metab. 19(10):830-838.

[13] Lee, W. M. (2003): Drug-induced Hepatotoxicity. N. Engl. J. Med. 349, 474–485 • Provides an elaborate meta-analysis review of many studies on this subject matter, it was a repository of reference. Phytother Res.;15:456–8.

[14] Mshelia, H. S., Karumi, Y. and Dibal, N. I. (2017): "Therapeutic Effect of Momordica balsamina Leaf Extract on Ethanol-induced Gastric Ulcer in Wistar Rats" Annals of Research Hospital, Vol. 1, Issue 3, pp. 1-5,

[15] Okigbo, R. N., Eme, U. E. and Ogbogu, S. (2008): Biodiversity and Conservation of Medicinal and Aromatic Plants in Africa. Biotechnology and Molecular Biology Review; 3(6): 127-134.

[16] Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., and Dixit, V. K. (2011). Phyllanthus amarus: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology, 138(2), 286–313.

[17] Sarich, T. C., Youssefi, M., Zhou, T., Adams, S. P., and Wall, R. A. (2006): Role of Hydrazine in the Mechanism of Isoniazid Hepatotoxicity in Rabbits. Arch. Toxicol. 70, 835–840

[18] Tietz, N.W. (1990): Clinical Guide to Laboratory Tests. Second Edition W.B. Saunders Company, Philadelphia, USA 554–556.

[19] Trinder, P. (1969): Determination of Glucose in Blood using Glucose Oxidase with an Alternative Oxygen Acceptor. Annals of Clinical Biochemistry; 6: 24-27.

[20] Wallace, J.L., (2004): Acetaminophen Hepatotoxicity: NO to the Rescue. Br. J. Pharmacol.,143(1): 1-2.