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Modulatory Effects of Guava Extract on Adriamycin (Doxorubicin) Induced Toxicity in Wistar Rats

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Abstract: Adriamycin, though, a drug of choice in cancer therapy, its use is associated with acute and chronic complications, which are capable of exacerbating the conditions of the patients. Thus, efforts are being directed at evaluating several compounds, antioxidants and natural products that are capable of ameliorating these complications whilst still retaining the potency and efficacy of Adriamycin as an anti cancer agent. The modulatory and ameliorative effects of methanol guava leaf extract (*Psidium guajava* Linn) against Adriamycin/Doxorubicin toxicity was evaluated in Wistar rats. Adriamycin (ADR) injection resulted in considerable liver and kidney damage as seen in the form of elevated liver enzymes, total protein as well as urea and creatinine levels in the blood. It also resulted in increases in the total triglyceride and cholesterol levels. The use of the extract alone on the other hand showed hepatoprotective and nephroprotective properties of guava leaf extract in that the above parameters were significantly lower than those of the untreated control. The protective effect of the extract against ADR toxicity was however limited to the liver. Effects which, may be due to the antioxidant and free radical scavenging properties of some of the components of the extract.

Key words: Psidium guajava · Adriamycin · Hepatotoxicity · Nephrotoxicity

INTRODUCTION

Cancer chemotherapy is known to be associated with many complications. This is not unconnected with the toxic nature of most of the therapeutic drugs used in cancer management or treatment. Chemotherapeutic drugs employed in cancer treatment belong to different classes, they include: alkylating agents such as Cyclophosphamide and Ifosfamide; Anthracycline antibiotics which affect nucleic acids (such as Adriamycin/Doxorubicin, Danuomycin etc.), platinum compounds especially Cisplatin; mitosis inhibitors such as Vincristine; and antimetabolites e.g. 5-Fluorouracil. Others include, Camptothecin derivatives such as Topotecan, hormonal agents such as Tamoxifen, as well as interferons. Of all these anticancer agents, the most potent groups – the alkylating agents, anthracycline antibiotics and the platinum compounds, also appear to be the most toxic, [1].

Many of these compounds have been observed to act via free radicals and reactive oxygen species (ROS) generation in the cell. For example, Adriamycin/ Doxorubicin which is the object of the present study, has been reported to act as an anti-cancer drug by inhibiting DNA synthesis via DNA intercalation or inhibition of polymerase activities as it facilitates growth arrest and disruption of p53 function [2], which is responsible for Adriamycin induced apoptosis [3]. Adriamycin can also trigger apoptosis by producing ceramide (which prompts apoptosis by activating p53 or other downstream pathways such as JNK), the degradation of Akt by serine threonine proteases, the mitochondrial release of Cytochrome C increased FasL (death receptor Fas/CD95 ligand) mRNA production [4]. Other authors have also suggested induction of DNA adduct and DNA cross-linking.

The most widely accepted mechanism of action of Adriamycin, which incidentally is also responsible for its cardiotoxicity action, is the generation of free radicals and reactive oxygen species [5]. These ROS are therefore responsible for direct damage to DNA of cancer cells as well as lipid peroxidation. Adriamycin generates ROS by

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the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the Adriamycin to the corresponding Adriamycin semiquinone. Adriamycin free radicals come from a non-enzymatic mechanism that involves reactions with iron. For example, Fe^{3+} reacts with Adriamycin in a redox reaction after which the iron atom accepts an electron and a Fe^{2+} doxorubicin free radical complex is produced. The iron-Adriamycin complex so formed then reduces oxygen to hydrogen peroxide and other active oxygen species. Adriamycin also localizes or accumulates in the mitochondria and is highly susceptible to enzymatic reduction to generate superoxide radicals and reactive oxygen species [6]. Thus, the cardiotoxicity associated with Adriamycin treatment might be associated with the ROS generated and the low endogenous antioxidant content of the cardiomyocytes. This is widely believed to be the brain behind acute and chronic cardiotoxicity effects of Adriamycin, for example, intravenous infusion of Adriamycin causes acute nausea, vomiting, myelosuppression and arrhythmia. While chronic effects which may be a result cumulative usage include dose dependent cardiomyopathy such as marked hypotension, cardiac dilatation, ventricular failure leading to congestive heart failure. Other side effects include persistent loss of cognitive function [7]. It then follows therefore, that efforts of clinician to reduce or limit the toxic side effects of ADR must be geared at sourcing for potent antioxidants that can counteract the ROS and free radicals generated by ADR treatment on the heart and other organs and also potentiate its actions on tumour cell. This therefore has necessitated the search for antioxidant combination with ADR that will both potentiate its anti tumour activities and reduce the cardiotoxic effects.

Several antioxidants have been evaluated with variable reports; among the candidates that have been evaluated are the metal ion chelators like transferrins, metallothionein, desferrioxamine or proteins that oxidize ferrous ions, such as ceruloplasmin. Others are low molecular-mass agents that scavenge reactive oxygen species and that are synthesized *in vivo* as bilirubin, sex hormones, melatonin, uric acid, or lipoic acid. While several antioxidants such as vitamin E, vitamin C, vitamin A, coenzyme Q, flavonoids, antioxidant components of virgin olive oil and selenium have also been considered by researchers to reduce ADR toxicity and potentiate its anti cancer actions.

As the use of plant extracts and alternative medicine continue to gain ground, in chemotherapy, the use of some of these compounds with antioxidative potentials are also being considered and evaluated in the management of ADR toxicity in cancer therapy. For example, Mukhaerjee *et al*. [8] reported that the use of garlic homogenate prevents acute ADR-induced cardio toxicity and decreases myocardial TNF-α (a principal inflammatory cytokine and mediator in oxidative stress) expression in the heart.

Guava *(Psidium guajava)* is a small tree usually 10 meters high with thin, smooth, patchy and peeling bark. It is believed to be native to Mexico but it is found all over South America, Europe, Africa and Asia. It has opposing, short, petiolate and oval leaves with prominent pinnate veins. Its fruits are fleshy, green to yellow globose or ovoid berry with an edible pink mesocarp and numerous small, hard, white seeds [9]. Guava extract is so called because of its antioxidant potential, due to the presence of several carotenoids such as phytofluene, β Carotene, β crytoxanthin, ã carotene, lycopene, rubixanthin, cryptoflavin, lutein, etc. [10]. It has also been reported to contain phenolic compounds that prevent lipid peroxidation in addition to flavonoids – quercetin, avicularin and guaijaverin. Other components of guava (*P. guajava L)* extract that may have contributed to its antioxidant activities in free radical scavenging assays (DPPH assay) include triterpene, tannins and essential oil. The fruit is also known to contain a high level of ascorbic acid (= 300mg/100g of fresh fruit), which is three to six times higher than that of oranges.

In view of the significance of adverse effects induced by doxorubicin, this work was planned to evaluate the effects of acute ADR/Doxorubicin injection on the liver enzymes, Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP); triglyceride and total cholesterol as well as creatinine and urea as indicators of kidney damage. The modulating and ameliorative effects of various dosages of guava (*P. gujava L.)* leaf extract were also examined in Wistar rats.

MATERIAL AND METHODS

Chemicals: ADR was purchased from Danax Pharmaceuticals, Mokola, Ibadan, Nigeria. All other reagents and chemicals were of analytical grade.

Plant Material Preparation: Guava leaves were harvested from within University of Ibadan, Ibadan, Nigeria and its surrounding suburbs. The leaves were identified and authenticated in the Department of Botany, University of Ibadan. The extraction was carried out as described by

Njar *et al*., [11]. The leaves were air-dried and pulverized with an electric blender. Methanol extract of guava leaf was prepared by soaking the pulverized leaves in methanol for 72 hours, filtered and concentrated in a rotary evaporator under pressure at 40°C. The yield of the extraction process was 9.2%. The resulting extract was harvested and kept at 4°C for use.

Phytochemical Screening: Guava leaf extract was subjected to phytochemical analysis using Sofowora, Evans and Harbourne methods for detection of Alkaloids, Saponins, Tannins, Anthraquinones, Flavonoids and Cardenolides

Experimental Animals and Management: Thirty healthy adult male Wistar strain albino rats aged 16 weeks, with an average weight between 225 and 228g were used for the study. They were obtained and housed in cages at the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan and provided with standard laboratory animal feed (obtained from Ladokun Feeds Limited, Ibadan, Nigeria) and water *ad libitum*. After 14 days of acclimatization, the rats were randomly divided into six groups of five rats each. This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan.

Experimental Procedure: The animals were divided into six groups A – F consisting of five rats per group. Group A, which served as the control was not treated with Adriamycin (ADR) while group B received a single acute dose of ADR, group C received 500mg/kg body weight of guava extract alone. Groups D – F received ADR in combination with 125mg/kg, 250mg/kg and 500mg/kg body weight of Guava leaf extracts, respectively. The treatment lasted for seven days after which blood samples were collected from all the animals into plain bottles.

Biochemical Analysis: The blood was allowed to clot and serum was obtained from each sample by centrifugation at 3000g for 10 min. Commercially available kits were used according to the respective manufacturer's procedure for the measurement of serum liver enzyme activity. From the serum samples, Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total protein (TP), Albumin, (while the globulin was calculated by deduction of the Albumin values from the Total protein) Creatinine and Urea were determined using RANDOX® laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. Total triglyceride (TG) and Total cholesterol (Chol) were analyzed by Ecoline 25 GPO-PAP and Ecoline CHOD-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany) respectively.

Statistical Analysis: Values expressed as mean ± SD were compared between groups using One Way Anova with Prism Graphpad Statistical Software Version 5.01 for Window. $P<0.05$ was considered statistically significant.

RESULTS

The results of the phytochemical screening of the leaf extract of *Psidium guajava* is presented in Table 1. The phytochemical investigation revealed the presence of Flavonoids, Saponins, Phenols, Terpenes, Sesquiterpenes, Tannins and absence of Alkaloids. As shown in Table 2 below, the liver enzyme, Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) values were considerably brought down in those rats given both ADR and 125, 250 and 500mg/kg guava leaf extract ($P>0.05$) when compared to the untreated control (Group A) and even those treated with ADR only (Group B) and 500mg/kg guava leaf extracts (Group C) respectively. However, Aspartate transaminase (AST) did not show any considerable difference across the groups treated with Adriamycin (ADR) and guava leaf extract when compared with the untreated control and those rats that were given ADR or guava leaf extract only.

Total triglyceride (Trigl) and total cholesterol (Chol) on the other hand were only reduced significantly in the rats given the guava leaf extract only (Group C) (Table 3). These values were lower than those treated with ADR+125 and ADR+500mg/kg guava leaves extract at $P>0.05$ and $P>0.01$, respectively but non significantly lower than the others including the untreated control.

Table 1: Phytochemical screening of *P. guajava* leaf extract

S/No	Constituents	Observation
1	Alkaloids	-ve
2	Flavonoids	++ve
3	Saponins	+ve
4	Phenols	++ve
5	Terpenes	++ve
6	Sesquiterpenes	+ve
7	Tannins	+ve

(+ve) = present; (++ve)= abundant; (-ve) = absent

Table 2: Ameliorative effects of guava (*psidium guajava* linn) leaf extract on liver enzymes, cholesterol and triglyceride levels in adr toxicity in wistar rats

Parameters	Grp A (Control)	Grp B (ADR only)	Grp C (500mg/kg Guava leaf extract only	Grp D (ADR +125mg/kg Guava leaf extract	Grp E (ADR + 250mg/kg Guava leaf extract	Grp F (ADR + 500mg/kg Guava leaf extract
AST	115.40± 9.64	116.40± 6.16	110.60± 2.25	132.00± 13.23	119.60± 2.38	107.20± 4.16
ALP	180.40± 12.70^{abc*}	133.80± 4.22	179.40± 14.23^{def*}	88.20± 6.08ad*	96.40± 12.49^{be*}	102.80± 11.39^{ef*}
ALT	71.40± 6.41^{ab}	58.20± 6.66	55.60± 2.02	50.80± 4.60a*	54.60± 3.92	44.00± 2.43^{b**}
TRIGL	58.60± 8.04	60.60± 1.69	44.20± 3.26^{ab}	65.6± 3.42^{a*}	57.80± 3.18	70.00± 2.59^{b**}
CHOL	55.40± 8.27	68.80± 3.32^{a*}	50.40± 2.66^{ab}	67.20± 2.38	68.40± 2.69	68.80± 1.83^{b*}

Values with the same superscript alphabets are significantly different.

* = P <0.05; ** = P <0.01; *** = P <0.001

Values are expressed as means ± SD

Table 3: Ameliorative effects of Guava (*Psidium guajava* Linn) leaf extract on total serum protein, creatinine and urea levels in ADR toxicity in Wistar rats

Parameters	Grp A (Control)	Grp B (ADR only)	Grp C (500mg/kg Guava leaf extract only	Grp D (ADR + 125mg/kg Guava leaf extract	Grp E (ADR + 250mg/kg Guava leaf extract	Grp F (ADR + 500mg/kg Guava leaf extract
TP	5.57± 0.27^{abcd}	6.42 ± 0.05^{a*}	5.24±0.12^{abcd}	6.50±0.08^{b**}	6.04± 0.10^{e***}	6.44 ± 0.02^{d***}
ALB	3.54 ± 0.13	3.50 ± 0.03	3.68 ± 0.10	3.42± 0.04	3.38 ± 0.05	3.56 ± 0.02
GLOB	2.04 ± 0.38	2.92 ± 0.08^{a*}	1.56 ± 0.18^{ab}	2.48± 1.34	2.66 ± 0.33	2.88 ± 0.09^{b*}
CREAT	0.30±0.00^{abcde}	0.58 ± 0.04^{a***}	0.28 ± 0.02^{abcde***}	0.60±0.00^{b***}	0.54± 0.02^{e***}	0.54± 0.02^{d****}
UREA	41.60 ± 4.86	37.60 ± 1.28	33.20 ± 4.07	43.20 ± 2.84	35.80 ± 1.72	43. 00 ± 0.02

Values with the same superscript alphabets are significantly different.

* = P <0.05; ** = P <0.01; *** = P <0.001

Values are expressed as means ± SD

The total protein value was also significantly lower in Group C than were those treated with ADR and 125mg/kg (P>0.01), 250mg/kg and 500mg/kg guava leaf extract (P>0.001), respectively. It was also lower than those of the untreated control and those treated with ADR only (P>0.05). In like manner, the globulin value was significantly lower in Group C than those treated with ADR only (Group B) and ADR+500mg/kg guava leaf extract (Group F).

In a manner similar to that observed in the total protein (TP), the creatinine level was significantly lower (P>0.001) in Group C (extract only) than in all the other groups. Urea level also showed a similar trend, although, non-significantly (Table 3).

DISCUSSION

Treatment with Adriamycin (Doxorubicin) as demonstrated in the present study has an acute devastating effect on the liver and kidney, as exemplified by the increases in the liver enzymes, creatinine, urea and the total protein released into the blood stream as a result of damages on the hepatocytes and the kidney cells by the presence of Doxorubicin. This obviously exacerbates the grave situation being experienced by cancer patients that are being treated with the drug. This is not far fetched, as ADR and other anthracyclines, despite being the drugs of choice in cancer therapy because of their efficacy and efficiency [3] produce considerable and debilitating acute and chronic side effects, some of which are irreversible especially in the heart in the form of cardiomyopathy such as hypotension, cardiac dilatation, tachycardia and congestive heart failure. There could also be a complementary loss of cognitive function, with or without the cardiomyopathy [7].

It is pertinent therefore to prevent as much as possible the side effect of ADR therapy in cancer patients, thus the use of dietary antioxidants and natural products. As observed in the present study therefore, there was reduction of the liver damage by ADR in the presence of the methanol guava leaf extract as shown by the reduced levels of ALT and ALP in the rats that were treated with ADR and the extract. This might not be unconnected with the presence of flavonoids, carotenoids and phenolic compounds as well as the free radical scavengers including triterpene, tannins and essential oil [10]. Pretreatment and concurrent combination of antioxidants with ADR chemotherapy in cancer treatment with considerable success in the reduction of Doxorubicin complications in cancer treatment has been widely reported [5]. The use of Vitamin E (α tocopherol), Vitamin C, Vitamin A and other carotenoids have been used with

considerable success in the amelioration of ADR complications both in experimental animals and in clinical practices [2, 12].

The mechanism involved in the chemotherapeutic action of ADR against cancer has been well elucidated by previous studies. It is believed that ADR destroys cancer cells by inhibiting DNA synthesis via the blockage of Topoisomerase II (TOP2), the enzyme responsible for modification of DNA topology without altering the structure and sequence of deoxynucleotides [3]. ADR also facilitates apoptosis of cancer cells via activation of p53. The most important mechanism of ADR employed in cancer therapy and conversely in the associated side effects is the generation of free radicals and other reactive oxygen species [13].

As enumerated by Granados-Principal *et al*. [3], DOX is transformed into a semiquinone free radical through electron reduction by various NAD(P)H-dependent reductases in the complex I of the electron transport chain (cytochrome P-450 reductase). This semiquinone reacts with molecular oxygen to produce the superoxide radical (O_2^-) and it converts DOX into quinone. This quinone–semiquinone cycle generates large amounts of O_2^-, which subsequently give rise to ROS and RNS species such as hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-) or peroxynitrite [4].

ADR semiquinone, O_2^- and H_2O_2, can also further promote the release of iron from ferritin and cytoplasmic aconitase, thus altering iron metabolism. Subsequently, iron can react with DOX and subsequently produce hydroxyl radicals. ADR activates NAD(P)H oxidases (NOXs) which give rise to free radicals that participate in activating the apoptotic pathway [14] and generate peroxynitrite through the mitochondrial production of ROS.

Furthermore, side chain carbonyl group of the carbon 13 in DOX is converted into a hydroxyl group by aldoketo reductases, giving rise to a secondary alcohol (doxorubicinol), which can release iron from cytoplasmic aconitase, disturbing the iron metabolism and, therefore, causing oxidative stress [7].

Finally, ADR also has the ability to modify the chemical composition, structure and function of biological membranes, mainly at the mitochondrial level, fundamentally due to the peroxidation of membrane lipids, leading to release of protein and cholesterol from the cytosol into the blood stream [15]. This might be responsible for the observed increases in levels of triglyceride and total cholesterol in the rats treated with ADR alone in the present study. Unfortunately however, the use of methanol guava leaf extract in this study did not ameliorate the triglyceride and total cholesterol content even in rats treated with the extract and ADR despite the facts that the extract was capable of lowering the cholesterol and triglyceride levels when used alone in the absence of ADR. In a like manner, the extract could not ameliorate the kidney damage associated with ADR therapy as there was no improvement in the values of creatinine and urea in those rats that were treated ADR and the extract, despite the fact that animals treated with 500mg/kg of the extract alone had values of creatinine and urea that were lower that the untreated control.

We can deduce from this study that methanol guava leaf extract has hepatoprotective, nephroprotective activities. It also demonstrated the capacity to lower blood triglyceride and cholesterol levels, although it could only protect the liver against ADR associated hepatic damage.

The hepatoprotective activity of guava leaf extract has been previously reported at 500mg/kg body weight against CCl_4, Paracetamol and Thioacetamide induced liver damage in the rats as used in our current study. The hepato protective activity was attributed to the presence of antioxidants in the guava leaf extract [16]. In another study by Roy and Das [17] evaluating the hepatoprotective effects of different guava leaf extracts (petroleum ether, chloroform, ethyl acetate, methanol and aqueous) against CCl_4 and Paracetamol induced hepatotoxicity in rats, hepatoprotective activity of the various guava leaf extract was also reported, with methanol extract showing better protection against liver damage than the other forms of extract.

This activity of guava leaf extract is considered to be a function of the antioxidant and free radical scavenging ability of the extract [18, 7]. Present in the leaves are several phenolic compounds such as protocatechuic acid, ferulic acid, quercetin and guavin B [10], quercetin, ascorbic acid, gallic acid and caffeic acid with excellent antioxidant properties. Others include Terpenoids and flavonoids such as Oleanolic acid Nerolidiol, β-sitosterol, ursolic, crategolic and guayavolic acids as well as essential oil including α-pinene, β pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, cineol, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene [9].

Apart from antioxidant activities, anti bacterial, anti diarrheal, anti viral, antitussive and anti-inflammatory as well as anti-diabetic activities of *Psidium guajava* has been reported.

In conclusion, the present study demonstrated the hepatoprotective and nephroprotective properties of methanol guava leaf extract (*Psidium guajava* Linn) as well as its ability to lower blood cholesterol and triglycerides when used alone. However, its use in the amelioration of Adriamycin toxicity was limited to the protection of the liver in this study. Further study on its protective effects against ADR induced cardiomyopathies are also been conducted in our laboratory.

REFERENCES

1. Kastan, M.B., O. Onyekwere, D. Sidransky, B. Vogelstein and R.W. Craig, 1991. Participation of pp: 53 in the cellular response to DNA damage. Cancer Res., 51: 6304-6311.
2. Lamson, D.W. and M.S. Brignall, 1999. Antioxidants in Cancer Therapy; Their Actions and Interactions with Oncologic Therapies. Altern Med Rev., 4(5): 304-329.
3. Granados-Principal, S., J.S. Quiles, C.L. Ramirez-Tortosa, P. Sanchez-Rovira and M.C. Ramirez-Tortosa, 2010. New advances in molecular mechanisms and the prevention of Adriamycin toxicity by antioxidant nutrients. Food and Chemical Toxicology, 48: 1425-1438.
4. Quiles, J.L., J.J. Ochoa, J.R. Huertas, M. López-Frías and J. Mataix, 2006. Olive oil and mitochondrial oxidative stress: studies on adriamycin toxicity, physical exercise and ageing. In: J.L. Quiles, M.C. Ramirez-Tortosa and P. Yaqoob, (Eds.), Olive Oil and Health. CABI Publishing, Oxford, pp: 119-151.
5. Quiles, J.L., J.R. Huertas, M. Battino, J. Mataix and M.C. Ramírez-Tortosa, 2002. Antioxidant nutrients and Adriamycin toxicity. Toxicology, 180: 79-95.
6. Doroshow, J.H., 1983. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. Cancer Res., 43: 460–472.
7. Chen, Y., P. Jungsuwadee, M. Vore, D.A. Butterfield and D.K. St Clair, 2007. Collateral damage in cancer chemotherapy: oxidative stress in non-targeted tissues. Mol. Interv., 7: 147-156.
8. Mukherjee, S., S.K. Banerjee, M. Maulik, A.K. Dinda, K.K. Talwar and S.K. Maulik, 2003. Protection against acute adriamycin-induced cardiotoxicity by garlic: Role of endogenous antioxidants and inhibition of TNF-α expression. BMC Pharmacology, 3(16): 1-9.
9. Gutiérrez, R.M.P., S. Mitchell and R.V. Solis, 2008. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology Journal of Ethnopharmacology, 117(1): 1-27.
10. Thaipong, K., U. Boonprakob, L. Cisneros-Zevallos and D.H. Byrne, 2005. Hydrophilic and lipophilic antioxidant activities of guava fruits. Southeast Asian Journal of Tropical Medicine Public Health, 36: 254-257.
11. Njar, V.C.O., T.O. Alao, J.I. Okogun and H.L. Holl, 1993. 2 methoxycathin-6-one: a new alkaloid from the stem wood of Quassiaamara. Plant Med, 59: 259-61.
12. Wahab, M.H.A., E.E.M.S. Akoul and A.H. Abdelaziz, 2000. Modulatory effects of melatonin and vitamin-E on doxorubicin-induced cardiotoxicity in Ehrlich ascites carcinoma bearing mice. Tumor, 86: 157-162.
13. Takemura, G. and H. Fujiwara, 2007. Doxorubicin-Induced Cardiomyopathy from the Cardiotoxic Mechanisms to Management Progress in Cardiovascular Diseases, 49(5): 330-352.
14. Gilleron, M., X. Marechal, D. Montaigne, J. Franczak, R. Neviere and S. Lancel, 2009. NADPH oxidases participate to doxorubicin-induced cardiac myocyte apoptosis. Biochem. Biophys. Res. Commun., 388: 727-731.
15. Huertas, J.R., M. Battino, V. Barzanti, M. Maranesi, G. Parenti-Castelli, G.P. Littarru, E. Turchetto, F.J. Mataix and G. Lenaz, 1992. Mitochondrial and microsomal cholesterol mobilization after oxidative stress induced by Adriamycin in rats fed with dietary olive and corn oil. Life Sci., 50: 2111-2118.
16. Roy, C.K., J.V. Kamath and M. Asad, 2006. Hepatoprotective activity of *Psidium guajava* Linn. Indian Journal of Experimental Biology, 44: 305-311.
17. Roy, C.K. and A.K. Das, 2010. Comparative evaluation of different extracts of leaves of *Psidium guajava* Linn for hepatoprotective activity. Pakistan Journal of Pharmaceutical Sciences, 23(1): 15-20.
18. Qian, H. and V. Nihorimbere, 2004. Antioxidant power of phytochemicals from *Psidium guajava* leaf. Journal of Zhejiang University Science, 5(6): 676-683.