FOLIA VETERINARIA

The scientific journal of the
UNIVERSITY OF VETERINARY MEDICINE AND
PHARMACY IN KOŠICE — The Slovak Republic

ISSN 0015-5748

3

LIX • 2015

EDITORIAL BOARD

FOLIA VETERINARIA is issued by the *University of Veterinary Medicine and Pharmacy in Košice (UVMP);* address: Komenského 73, 041 81 Košice, The Slovak Republic (tel.: +421 915 984 669, fax: +421 55 632 52 93, E-mail: Milada.Vargova@uvlf.sk).

IČO: 397 474

The journal is published quarterly in English (numbers 1—4) and distributed worldwide.

Subscription rate for 1 year is 120 €. Orders are accepted by *The Department of The Scientific Information — The Library of The University of Veterinary Medicine and Pharmacy in Košice* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; the subscription is accepted by the State treasure.

Bank contact: State treasure, Radlinského 32, Bratislava 15, The Slovak Republic; **account number:** 7000072225/8180.

FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva a farmácie v Košiciach (UVLF),* Komenského 73, 041 81 Košice, Slovenská republika (tel.: 0915 984 669, fax: 055/632 52 93, E-mail: Milada.Vargova@uvlf.sk).

IČO: 397 474

Časopis vychádza kvartálne (č. 1–4) a je distribuovaný celosvetove.

Ročné predplatné 120 €. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva a farmácie v Košiciach* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; predplatné Štátna pokladnica (na nižšie uvedené číslo účtu).

Bankové spojenie: Štátna pokladnica, Radlinského 32, Bratislava 15; **číslo účtu:** 7000072225/8180.

Dátum vydania: 25.3.2014

Tlač: **Univerzita veterinárskeho lekárstva a farmácie** Komenského 73, 041 81 Košice

Sadzba: **Sapfo publishers**, Szakkayho 1, 040 01 Košice

EV 3485/09

For basic information about the journal see Internet home pages: www.uvm.sk; www.uvlf.sk

Indexed and abstracted in AGRIS, CAB, EBSCO

FOLIA VETERINARIA, 59, 3, 2015

CONTENTS

FOLIA VETERINARIA, 59, 3: 153—158, 2015

AMELIORATING EFFECTS OF GUAVA (*PSIDIUM GUAJAVA*) EXTRACT ON ADRIAMYCIN INDUCED REPRODUCTIVE TOXICITIES

Ola-Davies, O. E.[1], Oloye, A. A.[2], Oyeyemi, M. O.[3]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology,
[2]Department of Veterinary Public Health and Reproduction College of Veterinary Medicine
Federal University of Agriculture Abeokuta
Ogun State
[3]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine
University of Ibadan
Oyo State

ooladavies@yahoo.com

ABSTRACT

This study was undertaken to investigate the protective effects of guava extract on Adriamycin induced reproductive toxicities. For this purpose, thirty adult male Wistar rats were randomly divided into 6 treatment groups. Group 1, the control, was administered distilled water while group 2 was treated with Adriamycin (ADR) (30 mg.m^{-2}) alone. Groups 3, 4 and 5 were administered combinations of 30 mg.m^{-2} (ADR) and graded doses (125 mg.kg^{-1}; 250 mg.kg^{-1}; 500 mg.kg^{-1}, respectively) of guava extract. Group 6 was treated with the extract (500 mg.kg^{-1}) alone. The treatments were done for seven days with water and feed provided ad libitum. The effects of these treatments on the reproductive characteristics of the male Wistar rats were thereafter investigated. The results showed that the control group (1) had a significantly higher sperm count (96.25 ± 3.84 × 10^6 cells.ml^{-1}) and motility (80.00 ± 4.08 %) compared to the other treatment groups (P < 0.05). Group 3 had a significantly lower sperm count (40.00 ± 0.00 × 10^6 cells.ml^{-1}) compared with the group 4 (67.33 ± 4.81 × 10^6 cells.ml^{-1}) and 6 (62.60 ± 3.09 × 10^6 cells.ml^{-1}). Group 5 had the lowest percentage livability (50 %), which was significant when compared with the control group, but not significant compared with the other treatment groups (P < 0.05). Groups 3, 5 and 6 had over 20 % sperm cell abnormalities. Most prominent of the abnormalities in groups 3 and 5 were curved tails and curved mid pieces in group 6. This work showed that guava extract at 250 mg.kg^{-1} is safe and gave protective cover to ADR induced reproductive toxicities.

Key words: adriamycin; *Psidium guajava*; reproductive; toxicity

INTRODUCTION

Of all the various chemotherapeutic agents, antracyclines, which are tetracyclic chromophore antibiotics, play

a vital role [3]. ADR is one of such antracyclines antibiotics used as an anti-cancer cytotoxic chemotherapeutic drug. ADR has been used effectively in treating; malignant lymphomas, solid tumours, and acute leukemias [15, 34]. However, cancer chemotherapy is often associated with adverse effects [11]. ADR has severe side effects such as; bone marrow suppression, gastrointestinal toxicity, stomatitis, alopecia, cardiomyopathy, and gonadal injury [9, 26]. Gonadal injury by antineoplastic drugs, though commonly observed, has been less investigated than other adverse effects [36]. These side effects have led to research on the development of specific agents to alleviate ADR toxicity. Hence, the quest for safe and effective agents to minimize ADR toxicity is still an active area of research.

Psidium guajava Linn. (Guava), belonging to the family of Myrtaceae, is a native of tropical America and has long been naturalized in Southeast Asia. It is used as foods and also as folk medicine in the subtropical areas of the globe. The positive effects of guava extracts on human ailments have been described [12, 17, 18, 24, 35, 38]. *Psidium guajava* leaf is a phytotherapeutic used in folk medicine to treat gastrointestinal and respiratory conditions and is also used as an anti-inflammatory medicine [2, 13]. The pharmacological and medicinal uses of the aqueous leaf extract include various disturbances such as; diarrhoea, vomiting, gastric pain, and dysentery [17]. Many other effects already reported include; CNS depressor (33), antimutagenic [5, 7], antiproliferative [14] antibiotic [1], anticough [2], immunomodulatory [16], hypotensive [23], and hypoglycemic [19, 20, 23]. Aqueous extracts from *P. guajava* have antioxidant or radical-scavenging activity. Most of the activity is associated with the polyphenols constituents; however, the guava extracts also contain antioxidants, such as ascorbic acid and carotenoids [2, 37]. The main objective of this study was to investigate the hypothesis that guava extract has an ameliorative effect against the development of ADR reproductive injury.

MATERIALS AND METHODS

Animals

Thirty adult male Wistar rats (3—4 months of age, body weight 240—280 g) bred and maintained in a controlled environment at the Experimental Animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan were used in this study. The temperature was kept at 25.0 ± 2.0 °C [25] and experiments were conducted in accordance with the rules and ethics of the Institutional Committee of Animal Care. Water and feed were provided ad libitum. The rats were randomly divided into 6 groups. Group 1 served as a control and given distilled water, while group 2 was administered Adriamycin (ADR) alone at a dosage rate of 30 $mg.m^{-2}$. Treatment groups 3, 4 and 5 were administered a single dose of ADR (30 $mg.m^{-2}$) together with graded dosages of guava extract (12 $mg.kg^{-1}$, 250 $mg.kg^{-1}$ and 500 $mg.kg^{-1}$). The 6th group was given 500 $mg.kg^{-1}$ guava extract alone. The animals were dosed orally once daily for 7 days using a rat cannula.

Preparation of guava extract

The guava extracts were prepared with leaves of *Psidium guajava* L. [8] obtained from the Campus of the University of Ibadan, Ibadan, Nigeria and collected between June and July of 2012 (plants were free of toxic compounds) and authenticated at the Department of Botany at the same institution. Twenty grams of dry leaves were ground in 200 ml of 0.9 % NaCl (100 °C, 5 min). The crude extract was filtered, centrifuged (1500 RPM, 5 min) to obtain the final extract. The supernatant was considered as the concentration at 100 $mg.ml^{-1}$ of guava leaves.

Sample collection

Rats were anaesthetized using diethyl ether and afterward sacrificed by cervical dislocation. Using the open castration method, a midline incision was made and the testicles were milked out of the incision site with gloved hands. Incising the tunica vaginalis exposed the testicles. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter collected from the cauda epididymis. These methods of collection were similar to that described by Oyeyemi and Ubiogoro [27]. The samples were analysed immediately after collection.

Sperm volume, motility and sperm count

The volume was determined by reading out the volume in a calibrated measuring cylinder. The sperm motility was assessed by the method described by Saba et al. [31]. The spermatozoa were counted by a haemocytometer using the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by Pant and Srivastava [28].

Morphological abnormalities and live/dead ratio

These factors were determined from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains.

The live/dead ratio was determined using 1 % Eosin and 5 % Nigrosin in 3 % sodium citrate dehydrate solution according to the method described by Wells and Awa [31].

Statistical analysis

The data were analysed into descriptive statistics using Graphpad Prism 5. The means were computed together with the Standard Error of the Mean (SEM). Means were compared using the Analysis of Variance. A value of $P < 0.05$ was considered significant.

RESULTS

The secondary metabolites identified in *P. guajava* extracts were; flavonoids, saponins, phenols, terpenes, sesquiterpenes, and tannins, no alkaloid was present (Table1). The control group (1) had a significantly higher sperm count ($96.25 \pm 3.84 \times 10^6$ cells.ml^{-1}) and motility (80.00 ± 4.08 %) compared to the other treatment groups ($P < 0.05$) (Table 2). Group 3 had a significantly lower sperm count (40.00 ± 0.00 cells.ml^{-1}) compared with group 4 (67.33 ± 4.81cells. ml^{-1}) and 6 (62.60 ± 3.09 cells.ml^{-1}) (Table 2). Group 5 had the lowest percentage livability (50 %) that was significant when compared with the control group, but not significant when compared with the other treatment groups ($P < 0.05$). Groups 3, 5 and 6 had over 20 % sperm cell abnormalities (Table 3). Most prominent of the abnormalities in groups 3 and 5 were curved tails and curved mid pieces in group 6 (Table 3). The mean percentage of curved tails and bent mid pieces was higher significantly ($P < 0.05$) in group 2 compared with group 3. The mean percentage of curved tails in group 5, when compared with that of groups 4, 2 and 1, was significantly higher ($P < 0.05$). The mean percentage of curved mid pieces in group 6 was significantly higher when compared with groups 2 and 1 ($P < 0.05$).

DISCUSSION

Table 1. Results of 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. Spermatozoa characteristics of the treatment groups

Group	Treatment	Motility [%]	Livability [%]	Volume [cm^3]	Count [10^6.ml^{-1}]
1	CONTROL	80.00 ± 4.08^{abcde}	96.50 ± 0.87^{a}	5.15 ± 0.03	96.25 ± 3.84^{abcde}
2	ADR alone	20.00 ± 8.17^{a}	60.00 ± 7.07	$5.1\ 3 \pm 0.03$	57.00 ± 8.70^{c}
3	ADR + 125 mg.kg^{-1} extract	20.00 ± 0.00^{d}	60.00 ± 0.00	5.10 ± 0.00	40.00 ± 0.00^{a}
4	ADR+ 250 mg.kg^{-1} extract	23.33 ± 14.53^{c}	61.67 ± 13.02	5.10 ± 0.00	67.33 ± 4.81^{ab}
5	ADR + 500 mg.kg^{-1} extract	20.00 ± 11.55^{b}	50.00 ± 25.17^{a}	3.4 ± 1.70	$52.\ 00 \pm 4.36^{d}$
6	Extract alone	34.00 ± 6.00^{e}	77.00 ± 3.00	5.10 ± 0.00	62.60 ± 3.09^{ae}

N = 5; mean values with same superscripts in the same column differ significantly at $P < 0.05$

Table 3. Morphological abnormalities in the treatment groups

Group	Treatment	Tailless head	Headless tail	Rudimentary tail	Bent tail	Curved tail	Bent mid piece	Curved mid piece	Total abnormal	Total normal	Total cell count
1	Control	15 (1.38 %)	16 (1.48 %)	7 (0.64 %)	31 (2.84 %)	29 (2.66 %)d	30 (2.75 %)	28 (2.57 %)^{b}	156 (14.31 %)	934 (85.69 %)	1090 (100 %)
2	ADR alone	17 (1.46 %)	21 (1.80 %)	5 (0.43 %)	33 (2.83 %)	28 (2.40 %)^{ab}	29 (2.49 %)^{a}	30 (2.58 %)^{a}	163 (13.99 %)	1002 (86.01 %)	1165 (100 %)
3	ADR + 125 mg.kg^{-1} extract	5 (2.33 %)	6 (2.79 %)	1 (0.47 %)	7 (3.26 %)	9 (4.19 %)^{b}	9 (4.19 %)^{a}	7 (3.26 %)	44 (20.47 %)	171 (79.53 %)	215 (100 %)
4	ADR+ 250 mg.kg^{-1} extract	14 (1.68 %)	11 (1.32 %)	6 (0.71 %)	25 (2.99 %)	27 (3.23 %)^{c}	24 (2.87 %)	26 (3.11 %)	133 (15.93 %)	702 (84.07 %)	835 (100 %)
5	ADR + 500 mg.kg^{-1} extract	10 (1.97 %)	8 (1.57 %)	5 (0.98 %)	18 (3.54 %)	22 (4.33 %)^{acd}	19 (3.74 %)	21 (4.13 %)	103 (20.28 %)	405 (79.72 %)	508 (100 %)
6	Extract alone	22 (1.94 %)	23 (2.03 %)	6 (0.53 %)	46 (4.06 %)	41 (3.62 %)	46 (4.06 %)	48 (4.24 %)^{ab}	232 (20.48 %)	901 (79.52 %)	1133 (100 %)

N = 5; mean values with same superscript in the same column differ significantly at $P < 0.05$

Antineoplastic drugs, pesticides and heavy metals are known to affect the structure, functions and biochemical composition of reproductive organs [32]. Many chemotherapeutic drugs are therefore limited in their effectiveness due to their toxic side effects [29]. The antioxidant activity of some compounds could be used to prevent various chronic diseases such as; heart disease, diabetes, cancer, arterial thrombosis, cataracts and may provide health-promoting effects [30]. The chemical analysis of guava extracts, revealed the presence of; flavonoids, saponins, phenols, terpenes, sesquiterpenes, and tannins. This corroborates previous reports of Cuellar et al. [10]; Arima and Danno [4] ; Begum et al. [6] that detected the presence of essential oils, tannins, saponins, carotenoids, flavonoids and triterpenes.

The deleterious effects of the treatment of male Wistar rats with Adriamycin was obvious in this study. Significantly lower motility in all the Adriamycin groups compared to the controls and over 20 % sperm cell abnormalities [21] in groups 3 and 5 indicate a possible testicular degeneration. Noakes et al. [22] had opined that the initial changes in semen quality during testicular degeneration are a decrease in motility and an increase in the percentage of abnormal sperm. Most prominent of the abnormalities in groups 3 and 5 were curved tails and this could be indicative of testicular degeneration [22]. In this work however, it appeared that guava extract did give a semblance of protective covering to Adriamycin-exposed sperm cells at 250 mg.kg^{-1}. At this dosage it had significant percentage livability compared to the ADR + 500 mg.kg^{-1} group, appreciable high sperm counts and acceptable levels of sperm cell abnormalities. The motility was however low, which could be due to the initial reaction to ADR. At this dosage therefore, the effect of ADR against sperm cells seemed to be ameliorated. Below (125 mg.kg^{-1}) and beyond that (500 mg.kg^{-1}) this protective covering seemed to be nonexistent. At 500 mg.kg^{-1} guava extract alone, motility was significantly low ($P < 0.05$) compared with the controls, but the sperm count was appreciable. It therefore appeared that at 500 mg.kg^{-1} guava extract might not be overtly a fertility-inducing agent.

The data presented in this study revealed that guava extract at 250 mg.kg^{-1} is a potent inhibitor of ADR toxicities. Experiments performed to examine the mechanism by which guava extract was exerting its protective effect on ADR toxicities revealed that Guava has an antioxidant property attributed to the polyphenols found in the leaves which

may constitute an important part of its therapeutic effects. This provides evidence that guava extract at 250 mg.kg^{-1} directly protects ADR induced reproductive toxicity.

CONCLUSIONS

In conclusion, the present findings demonstrate that guava extract has multiple therapeutic activities that are beneficial and thus guava extract is a promising agent to ameliorate ADR induced reproductive toxicities. However, additional studies are needed on the chemical characterization of the active principle in the leaf extract of *P. guajava*, that is responsible for the repair of the ADR induced reproductive injury.

REFERENCES

1. Abdelrahim, S. I., Almagboul, A. Z., Omer, M. E., Elegami, A., 2002: Antimicrobial activity of *Psidium guajava* L. Fitoterapia., 73, 713—715.

2. Anil Kumar, 2012: Importance for life – *Psidium guajava*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3, 137.

3. Anthracycline Chemistry and Biology., 2008: Biological occurrence and biosynthesis, synthesis and chemistry: No. 1. **Krohn, Karsten** (Ed.)**:** *Topics in Current Chemistry*, Springer, 321 pp.

4. Arima, H., Danno, G., 2002: Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. *Biosci. Biotechnol. Biochem.*, 66, 1727—1730.

5. Baby, J., Mini Priya, R., Mary Helen, P. A., Sujatha, S., 2010: Bioactive compounds in essential oil and its effects of antimicrobial, cytotoxic activity from the *Psidium guajava* L. leaf. *Journal of Advanced Biotechnology*, 9, 107—114.

6. Begum, S., Hassan, S. I., Siddiqui, B. S., Shaheen, F., Ghayur, M. N., Gilani, A. H., 2002: Triterpenoids from the leaves of *Psidium guajava*. *Phytochemistry*, 61, 399—403.

7. Chen, K. C., Peng, C. C., Chiu, W. T., Cheng, Y. T., Huang, G. T., Hsieh, C. L., 2010: Action mechanism and signal pathways of *Psidium guajava* L. aqueous extract in killing prostate cancer LNCaP cells. *Nutr. Cancer*, 62, 260—270.

8. Conde Garcia, E. A., Nascimento, V. T., Santiago Santos, A. B, 2003: Inotropic effects of extracts of *Psidium guajava* L. (guava) leaves on the guinea pig atrium. *Brazillian Journal of Medical and Biological Research*, 36, 661—668.

9. Cortes-Funes, H., Coronado, C., 2007: Role of anthracyclines in the era of targeted therapy. *Cardiovasc. Toxicol.*, 7, 56—60.

10. Cuellar, A. C., Lara, R. A., Zayas, J. P., 1984: *Psidium guajava* L. tamizaje fitoquímico y estudio del aceite esencial. *Revista Cubana de Farmacia*, 18, 92—99.

11. Dourald, K., Rasul, S., Rasul, K., 2009: Chemotherapy induced cardiomyopathy: pathogenesis, monitoring and management. *J. Clin. Med. Res.*, 1, 8—12.

12. Jeong Yong Moon, Mosaddik, A., Kim, H. et al., 2011: The chloroform fraction of guava (Psidium Cattleianum Sabine) leaf extract inhibits human gastric cancer cell proliferation *via* induction of apoptosis. *Food Chemistry*, 125, 369—375.

13. Jimenez, E. A., Rincon, M., Pulido, R., Saura-Calixto, F., 2001: Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fibre. *J. Agric. Food Chem.*, 49, 5489—5493.

14. Kaileh, M., Berghe, W. V., Boone, E., Essawi, T., Haegeman, G., 2007: Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. *J. Ethnopharmacol.*, 113, 510—516.

15. Kaiserová, H., Šimůnek, T., van der Vijgh, W. J. F., Bast, A., Kvasničková, E., 2007: Flavonoids as protectors against doxorubicin cardiotoxicity: role of iron chelation, antioxidant activity and inhibition of carbonyl reductase. *BBA – Molecular Basis of Disease*, 17772, 1065—1074.

16. Kawakami, Y., Nakamura, T., Hosokawa, T., Suzuki-Yamamoto, T., Yamashita, H., Kimoto, M., 2009: Antiproliferative activity of guava leaf extract via inhibition of prostaglandin endoperoxide H synthase isoforms. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 80, 239—245.

17. Lozoya, X., Reyes-Morales, H., Chavez-Soto, M., Martinez-Garcia Mdel, C., Soto-Gonzalez, Y., Doubova, S. V., 2002: Intestinal anti-spasmodic effect of a phytodrug of *P. guajava* folia in the treatment of acute diarrheic disease. *J. Ethnopharmacol.*, 83, 19—24.

18. Manosroi, J., Dhumtanom, P., Manosroi, A., 2006: Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett.*, 235, 114—120.

19. Mukhtar, H. M., Ansari, S. H., Ali, M., Naved, T., Bhat, Z. A., 2004: Effect of water extract of *Psidium guajava* leaves on alloxan-induced diabetic rats. *Pharmazie*, 59, 734—735.

20. Mukhtar, H. M., Ansari, S. H., Bhat, Z. A., Naved, T., Singh, P., 2006: Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae). *Pharmazie*, 61, 725—727.

21. Noakes, D. E., Parkinson, T. J., England, G. C. W., 2001a: Fertility and Infertility in Male Animals. *Arthurs Veterinary Re-*

production and Obstetrics. Harcourt (Indian) private, limited 8th edn., 741.

22. Noakes, D. E., Parkinson, T. J., England, G. C. W., 2001b: Fertility and Infertility in Male Animals. *Arthurs Veterinary Reproduction and Obstetrics*. Harcourt (Indian) private, limited 8th edn., 747.

23. Ojewole, J. A., 2005: Hypoglycemic and hypotensive effects of *Psidium guajava* L, (Myrtaceae) leaf aqueous extracts Methods Find. *Exp. Clin. Pharmacol.*, 27, 689—695.

24. Ojewole, J. A. Awe, E. O., Chiwororo, W. D. H., 2008: Antidiarrhoeal activity of *Psidium Guajava* Linn. (Myrtaceae) leaf aqueous extract in rodents. *Journal of Smooth Muscle Research*, 44, 195—207.

25. Ola-Davies, O. E., Ajani, O. S., Oyeyemi, M. O., Ekundayo, S., 2014: Testicular and epididymal parameters of *Spondias mombin* L. (Anacardiaceae) protected male Wistar rats exposed to sodium arsenite. Global Veterinaria, 12, 232—238.

26. Outomuro, D., Grana, D. R., Azzato, F., Milei, J., 2007: Adriamycin induced myocardial toxicity: new solutions for an old problem? *Int. J. Cardiol.*, 117, 6—15. [PubMed].

27. Oyeyemi, M. O., Ubiogoro, O., 2005: Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of Large White Boar in Nigeria. *Int. J. Morphol.*, 23, 235—239.

28. Pant, N., Srivastava, S. P., 2003: Testicular and spermatotoxic effect of quinaphos in rats. *Journal of Applied Toxicology*, 23, 271—274.

29. Prasaja, Y., Noorwati S., Retnosari, A., 2015: Incidence of cisplatin-induced nephrotoxicity and associated factors among cancer patients in Indonesia. *Asian Pac. J. Cancer Prev.*, 16, 1117—1122.

30. Qian, H., Nihorimbere, V., 2004: Antioxidant power of phytochemicals from *Psidium guajava* leaf. *Journal of Zhejiang University Science*, 5, 676—683.

31. Saba, A. B., Oridupa, O. A., Oyeyemi, M. O., Osanyigbe, O. D., 2009: Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of Lagenaria Breviflora Roberts. *African Journal of Biotechnology*, 8, 1170—1175.

32. Sehgal, R., Pandey, A. H., 1984: Effect of cadmium chloride on testicular activities in guppy *Lebistes reticulatus*. *Comp. Physiol. Ecol.*, 9, 225—230.

33. Shaheen, H. M, Ali, B. H, Alqarawi, A. A., Bashir, A. K., 2000: Effect of *Psidium guajava* leaves on some aspects of the central nervous system in mice. *Phytother. Res.*, 14, 107—111.

34. Shinde, N., Jagtap, A., Undale, V., Kakade, S., Kotwal, S., Patil, R., 2010: Protective effect of Lepidium sativum against doxorubicin-induced nephrotoxicity in rats. *Res. J. Pharm.. Biol. Chem. Sci.*, 1, 42—48.

35. Wang, B. S., Jiao, H. Liu, 2007: PubMed.gov: Study on antioxidative activities of *Psidium Guajava* Linn leaves extracts. *Wei* Sheng Yan Jiu, 36, 298—300.

36. Ward, J. A., Bardin, C. W., Knight, M., Robinson, J., Gumulus, G., Morris, D. 1998: Delayed effect of Doxorubicin on spermatogenesis and endocrine function in rats. *Reproductive Toxicol.*, 2, 117—126.

37. Yamashiro, S., Noguchi, K., Matsuzaki, T., 2003: Cardioprotective effects of extracts from *Psidium guajava* L and Limonium wrightii, Okinawan medicinal plants, against ischemia-reperfusion injury in perfused rat hearts. *Pharmacology*, 67, 128—135.

38. Yoriko Deguchi, Kouji Miyazaki, 2010: Nutrition & Metabolism: Anti-hyperglycemic and Anti-hyperlipidemic Effects of Guava Leaf Extract. http://www.nutritionandmetabolism.com/content/7/1/9/abstract.

Received June 25, 2015