Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 10 (1): 68-74, 2016
ISSN 1819-3420 / DOI: 10.3923/rjet.2016.68.74

Detrimental Effects of Chlorpyrifos and Cypermethrin on Reproductive Physiology of Male Albino Rats

E.V. Ikpeme, L.E. Okonko and O.U. Udensi
Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

Corresponding Author: L.E. Okonko, Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

ABSTRACT

This study was designed to evaluate the effects of chlorpyrifos and cypermethrin, singly and in combination on reproductive physiology of male albino rats. Thirty six mature male rats were used for this study. Completely randomized design was used in a 3×3 factorial format. Meaning there were 3 treatments (chlorpyrifos, cypermethrin and chlorpyrifos+cypermethrin) and each treatment had 3 groups (A, B and C) and each group had 4 rats. Group A was the control and received distilled water. Group B received 5 mg kg^{-1} b.wt., of treatment while Group C received 10 mg kg^{-1} b.wt., of treatment. Treatments were administered via oral gavage and lasted for 65 days. The rats were sacrificed; blood and sperm samples were collected and examined. The result revealed that the treatments significantly ($p<0.05$) reduced epididymal sperm motility, viability and count of rats. While sperm head abnormality increased significantly in treated rats. Furthermore, follicle stimulating hormone, luteinizing hormone, testosterone and prolactin levels reduced significantly ($p<0.05$) in treated rats compared to those in the control group. The findings of this study therefore indicate that repeated oral exposure to chlorpyrifos and cypermethrin, both singly and in combination had adverse effects on the reproductive physiology of male albino rats.

Key words: Chlorpyrifos, cypermethrin, pesticide combination, sperm, albino rat

INTRODUCTION

Pesticides are often considered a quick, easy and cheap means of controlling 'Pests' in urban and rural areas. Thus, their use has increased crop yields as well as food quality. A huge quantity of crops is lost yearly due to the effect of pests and diseases. These crop losses could double if pesticide uses were abandoned. However, because the use of pesticides improves crop production, the cost of food is also impacted. Without pesticides, food production would decline, thus leading to shortage in supply and increase in price of food crops. Pesticides allow farmers to produce high quality crops free of insect blemish and contamination.

Conversely, the indiscriminate use of pesticides has resulted in serious problems to man and the environment (Grewal *et al*., 2010; Gold *et al*., 2001). Almost every part of our environment has been contaminated by these chemicals as their residues are found in soil and air, in surface and ground water and on food crops. The application of pesticides is often not precise and as such, unlimited exposures pose high risk to non-target organisms, ranging from soil microorganisms to insects, plants, fish, birds and man. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species (Miller, 2004). Pesticides are one of the causes of water pollution and some pesticides are persistent organic pollutants and contribute to soil

contamination. In addition, the use of pesticides reduces biodiversity and nitrogen fixation contributes to pollinator decline, destroys habitat (especially for birds) and threatens endangered species (Miller, 2004; Palmer *et al*., 2007).

Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems and also causing cancer (EPA., 1999). Strong evidence also exists for other negative outcomes of pesticide exposure which include neurological effect, birth defects, foetal death and neuro-developmental disorder (Sanborn *et al*., 2007). The World Health Organization and the United Nations Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning annually (Jeyaratnam, 1990). A study by the California Department of Public Health found that women in the first eight weeks of pregnancy who live near farm fields sprayed with organochlorine pesticides, dicofol and endosulfan are several times more likely to give birth to children with autism (Roberts *et al*., 2007).

Chlorpyrifos and cypermethrin are pesticides widely used by cocoa farmers in Nigeria. These farmers face the risk of becoming poisoned during mixing and spraying of these pesticides. They are exposed to these chemicals through inhalation or direct contact arising from spills, splashes and leakages.

In view of this situation, this study was carried out to evaluate the detrimental effects of chlorpyrifos and cypermethrin, singly and in combination on reproductive physiology of male albino rats.

MATERIALS AND METHODS

Experimental materials: The experiment was carried out in the Animal house of Genetics and Biotechnology Department, University of Calabar, Nigeria.

Chlorpyrifos and cypermethrin were purchased from Department of Agrochemicals, Ministry of Agriculture, Calabar.

Experimental animals/procedure: Thirty six sexually mature male albino rats of body weight ranging from 170-200 g were purchased from the Department of Physiology, University of Calabar, Nigeria. They were kept in aluminum cages covered with wire mesh, fed with growers mash and allowed unrestricted access to clean water. They were handled in accordance with the guidelines for care and use of laboratory animals as mandated by the Animal Genetics research committee of the Department (GBT/2012/008).

The experiment was laid out in a 3×3 factorial format using Completely Randomized Design (CRD) as shown in Table 1.

The animals were sacrificed under chloroform anesthesia 24 h after the last dose was administered.

Estimation of sperm quality and quantity: Testes and epididymes were surgically removed and weighed using an electronic weighing balance. The epididymes were placed in physiological saline in the ratio of 1:10 (w/v) (Ikpeme *et al*., 2007) and macerated to release the sperm cells. After pipetting, the suspension was filtered using an 80 mm stainless wire mesh and used for estimation of the sperm parameters.

Table 1: Completely Randomized Design (CRD)

Treatment groups	No. of animal	Experimental period
Chlorpyrifos		65 days
A (0 mg kg^{-1})	4	
B (5 mg kg^{-1})	4	
C (10 mg kg^{-1})	4	
Cypermethrin		
A (0 mg kg^{-1})	4	
B (5 mg kg^{-1})	4	
C (10 mg kg^{-1})	4	
Chlorpyrifos+Cypermethrin		
A (0 mg kg^{-1})	4	
B (5 mg kg^{-1})	4	
C (10 mg kg^{-1})	4	

Estimation of sperm motility: Two drops of sperm suspension were put on a sterile slide and cover slip placed on it. Five slides were prepared in quick succession for each sample. The number of motile cells divided by the total number of spermatozoa counted under 40x lens was determined and expressed in percentage (Ekaluo *et al*., 2013).

Determination of sperm viability: This was done using the eosin-nigrosin staining technique (Ikpeme *et al*., 2007). Two drops of sperm suspension was mixed with equal volume of stain and smeared on clean slides. Five air-dried smears were prepared for each sample and then viewed under 40x lens of light microscope. Live sperm cells appeared white while dead ones took up the stain and appeared pink. The number of live cells divided by the total number of cells was expressed in percentage.

Estimation of sperm head abnormality: Two drops of sperm suspension was mixed with an equal volume of 1% eosin Y solution, smeared on glass slides and allowed to air-dry. Five air-dried smears were prepared for each sample and examined with 100x lens in a light microscope. The number of abnormal sperm heads in every 200 spermatozoa was determined and expressed in percentage (Ikpeme *et al*., 2007).

Estimation of sperm count: A cover slip was placed on the improved Neubaeuer ($2.5\times10''^{4}$ mm^3, Hawksley, England) and a fine pore capillary tube was used to charge the haemocytometer with sperm suspension (Ikpeme *et al*., 2007). Sperm cells were counted under 40x lens of the light microscope and expressed in million cells mL^{-1}.

Estimation of reproductive hormones: Blood sample was collected from the rats through cardiac puncture and centrifuged at 2500 rpm to obtain serum. Serum samples were assayed for levels of testosterone, prolactin, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Diagnostic Automation Inc.).

Statistical analysis: All data obtained were subjected to analysis of variance (ANOVA) using PASW (version 18.0). Least Significant Difference (LSD) was used to separate the means of groups that were significant at $p<0.05$.

RESULTS

Weight of testes and epididymes: The result revealed that there were no significant differences ($p>0.05$) between the testes weight of rats in the control and those treated with pesticide. The mean

testes weight of rats in the control group was 1.08 while the least testes weight (0.93) was recorded in rats administered 10 mg kg^{-1} b.wt., of chlorpyrifos+cypermethrin (Table 2). Also, there were no significant differences ($p>0.05$) between the mean epididymal weight of rats in the control and pesticide treated groups. Least epididymal weight (0.35) was recorded in rats administered 10 mg kg^{-1} b.wt., of cypermethrin, whereas the epididymal weight of rats in the control was 0.40 (Table 2).

Sperm quality and quantity: Mean value of sperm motility, viability and count of rats administered pesticide treatment reduced significantly ($p<0.05$) and dose dependently compared with those in the control group. In the control group, mean sperm motility, viability and count were 71.75, 78.50% and 33.75 M mL^{-1}, respectively; however, their least mean values (27.00, 39.25% and 18.00 M mL^{-1}, respectively) were recorded in rats treated with 10 mg kg^{-1} b.wt., of chlorpyrifos+cypermethrin (Table 2). Conversely, mean sperm head abnormality increased significantly ($p<0.05$) in rats treated with pesticides compared to those in the control. Rats in the group administered 10 mg kg^{-1} b.wt., of chlorpyrifos+cypermethrin had the highest mean sperm head abnormality (8.88%) compared with those in the control, which had the least (4.88%).

Reproductive hormones: The result showed that pesticide treatment caused a significant decrease ($p>0.05$) in Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), testosterone and prolactin levels. The mean FSH and LH values of rats in the control group were 6.43 ng mL^{-1} and 9.21 mIU, respectively. The least mean value of FSH and LH (2.73 ng mL^{-1} and 5.13 mIU, respectively) was recorded in rats treated with 10 mg kg^{-1} b.wt., of chlorpyrifos. Furthermore, mean testosterone and prolactin values of rats in the control group were 1.58 and 1.18 ng mL^{-1} respectively, whereas the least testosterone (0.15 ng mL^{-1}) and prolactin (0.93 ng mL^{-1}) values were recorded in rats treated with 10 mg kg^{-1} b.wt., of chlorpyrifos+cypermethrin (Table 3).

Table 2: Epididymal sperm parameters and organ weight of control and pesticide treated rats

	Chlorpyrifos			Cypermethrin			Chlorpyrifos+cypermethrin		
Sperm parameters	A	B	C	A	B	C	A	B	C
Motility (%)	71.75±1.54^{c}	47.25±3.75^{b}	45.50±2.72^{b}	71.75±1.54^{c}	47.50±2.10^{b}	43.00±1.83^{b}	71.75±1.54^{c}	31.50±1.55^{a}	27.00±2.67^{a}
Viability (%)	78.50±1.44^{d}	53.75±1.75^{bc}	47.75±2.29^{b}	78.50±1.44^{d}	49.50±1.85^{b}	46.50±3.42^{ab}	78.50±1.44^{d}	41.50±1.76^{a}	39.25±2.17^{a}
SHAT (%)	4.88±0.43^{a}	6.13±0.75^{a}	6.50±0.35^{a}	4.88±0.43^{a}	7.13±0.85^{ab}	8.50±0.79^{b}	4.88±0.43^{a}	7.13±0.55^{ab}	8.88±0.89^{b}
Count (M mL^{-1})	33.75±1.19^{c}	27.00±1.24^{b}	23.00±0.79^{b}	33.75±1.19^{c}	21.00±0.75^{a}	18.38±1.09^{a}	33.75±1.19^{c}	20.38±1.09^{a}	18.00±0.98^{a}
Testes weight (g)	1.08±0.03^{a}	1.03±0.03^{a}	1.03±0.00^{a}	1.08±0.08^{a}	1.05±0.03^{a}	1.05±0.03^{a}	1.08±0.03^{a}	1.05±0.03^{a}	0.93±0.03^{a}
Epididymes weight (g)	0.40±0.00^{a}	0.38±0.03^{a}	0.40±0.00^{a}	0.40±0.00^{a}	0.38±0.03^{a}	0.35±0.03^{a}	0.40±0.00^{a}	0.40±0.00^{a}	0.40±0.00^{a}

Values are presented as Mean±SEM. Means followed by the same case letter along the horizontal array indicate no significant difference ($p>0.05$). A: Control (0 mg kg^{-1} b.wt.,), B: 5 mg kg^{-1} b.wt., of pesticide, C: 10 mg kg^{-1} b.wt., of pesticide

Table 3: Reproductive hormones of control and pesticide treated rats

	Chlorpyrifos			Cypermethrin			Chlorpyrifos+cypermethrin		
Parameters	A	B	C	A	B	C	A	B	C
FSH (ng mL^{-1})	6.43±0.03^{g}	4.47±0.03^{e}	2.73±0.08^{a}	6.43±0.03^{g}	4.10±0.05^{d}	3.32±0.03^{b}	6.43±0.03^{g}	5.02±0.05^{f}	3.66±0.02^{c}
LH (mIU)	9.21±0.10^{d}	6.21±0.02^{b}	5.13±0.02^{a}	9.21±0.10^{d}	7.30±0.02^{c}	6.01±0.31^{b}	9.21±0.10^{d}	7.36±0.02^{c}	6.42±0.03^{b}
Testosterone (ng mL^{-1})	1.58±0.03^{e}	1.48±0.03^{d}	1.15±0.05^{b}	1.58±0.03^{e}	1.60±0.04^{e}	1.28±0.03^{c}	1.58±0.03^{e}	1.18±0.03^{b}	0.15±0.03^{a}
Prolactin (ng mL^{-1})	1.18±0.03^{c}	1.08±0.03^{ab}	1.03±0.03^{a}	1.18±0.03^{c}	1.08±0.03^{ab}	1.08±0.03^{ab}	1.18±0.03^{c}	0.98±0.02^{a}	0.93±0.04^{a}

Values are presented as Mean±SEM. Means followed by the same case letter along the horizontal array indicate no significant difference ($p>0.05$). A: Control (0 mg kg^{-1} b.wt.,), B: 5 mg kg^{-1} b.wt., of pesticide, C: 10 mg kg^{-1} b.wt., of pesticide, FSH: Follicle stimulating hormone, LH: Leutinization hormone

DISCUSSION

Indiscriminate use of these pesticides has triggered serious health and environmental concerns. This study was therefore designed to evaluate detrimental effects of these chemicals on reproductive physiology of male albino rats.

The result obtained showed that pesticide treatments had no significant reduction ($p>0.05$) on testicular and epididymal weights of rats. The decrease in weight of testes may be due to reduction in tubule size, decrease in number of germ cells or elongated spermatid (Choudhary *et al*., 2008). Decrease in organ weight may also be as a result of decrease in level of serum testosterone, FSH and LH as observed in this study, which is also in agreement with the findings of others (Wang *et al*., 2009).

The result for sperm analysis revealed that pesticide treatments significantly ($p<0.05$) reduced epididymal sperm motility, viability and count, whereas, sperm head abnormalities increased significantly. Reduction in sperm count of treated rats may be due to decrease in serum testosterone level as seen in this study. This is because low testosterone production could result in suppression of spermatogenesis. Inhibition of spermatogenesis may also be as a result of low FSH and LH levels (Sharma *et al*., 2014). The decrease in sperm count is in consonance with the findings of other researchers (Assayed *et al*., 2008; Zidan, 2009; Liu *et al*., 2010; Joshi *et al*., 2011). Furthermore, reduction in sperm motility could be due to decreased mitochondrial activity, altered fructose synthesis or corrosion of microtubule structure of spermatozoan (Okamura *et al*., 2005; Uzunhisarcikli *et al*., 2007). The inhibition of sperm motility in pesticide treated rats could be because of low ATP level (Bai and Shi, 2012; Heikal *et al*., 2014). Interestingly, reduction in sperm viability corroborates the decrease in sperm motility. This reduction could be due to the impact of pesticides on the epididymes of treated rats (Oyeyipo *et al*., 2011). Sperm abnormalities induction indicates point mutations in germ cells (Narayana *et al*., 2002). These abnormalities usually occur during transit, maturation and storage (Tulsiani *et al*., 1998). Reactive oxygen species is induced by these chemicals and is known to adversely affect sperm motility, viability and increase sperm head abnormalities (El-Demerdash *et al*., 2004; Kumar *et al*., 2004; Joshi *et al*., 2011). The decrease in sperm motility, viability and increase in sperm head abnormalities are in conformity with the reports of Zidan (2009), Sai *et al*. (2014) and Sharma *et al*. (2014).

Reductions in FSH and LH on exposure to pesticide have been previously reported (Biswas and Ghosh, 2004; Pareek *et al*., 2007). Decrease in testosterone, prolactin, FSH and LH was also observed in animals treated with chlorpyrifos, cypermethrin and the combination. Concurrent decrease in these hormones reveals extra testicular target by pesticides (Sharma *et al*., 2014). This reduction suggests that pesticides may also affect the hypothalamus pituitary axis (Sharma *et al*., 2014). The LH stimulates leydig cells to produce testosterone and so, decrease in LH may also be a contributing factor to low testosterone production. Moreover, low testosterone, FSH and LH levels inhibit effective spermatogenesis and development of seminiferous tubules, thus causing infertility (Monet-Kuntz *et al*., 1984).

Conclusively, the present study shows that chlorpyrifos and cypermethrin, singly and in combination had adverse effects on the reproductive physiology of male albino rats. However, further investigation should be carried out to assess and confirm the interaction of these pesticides on a broader range of physiological and histological parameters.

REFERENCES

Assayed, M.E., H.A. Salem and A.A. Khalef, 2008. Protective effects of garlic extract and vitamin C against cypermethrin reproductive toxicity in male rats. Res. J. Vet. Sci., 1: 1-15.

Bai, J.P. and Y.L. Shi, 2012. Inhibition of calcium ion channels in mouse spermatogenic cells by male antifertility conpounds. Tripterygium Wilfordii Hook Contraception, 65: 441-445.

Biswas, N.M. and P. Ghosh, 2004. Effect of lead on male gonadal activity in albino rats. Kathmandu Univ. Med. J., 2: 43-46.

Choudhary, N., R. Goyal and S.C. Joshi, 2008. Effect of malathion on reproductive system of male rats. J. Environ. Biol., 29: 259-262.

EPA., 1999. Chlorpyrifos Fate and Environmental Risk Assessment. U.S. Government Printing Office, Washington, DC., USA.

Ekaluo, U.B., Y.B. Ibiang, E.V. Ikpeme, B.E. Ekanem and F.A. Erem, 2013. Effect of deltamethrin and ridomil on sperm parameters and reproductive hormones of male albino rats. J. Toxicol. Environ. Health Sci., 5: 9-14.

El-Demerdash, F.M., M.I. Yousef, F.S. Kedwanyand and H.H. Baghdadi, 2004. Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. J. Environ. Sci. Health B, 93: 443-459.

Gold, L.S., T.H. Slone, B.N. Ames and N.B. Manley, 2001. Pesticide Residues in Food and Cancer Risk: A Critical Analysis. In: Handbook of Pesticide Toxicology, Volume 1: Principles, Krieger, R.I. and W.C. Krieger (Ed.). 2nd Edn., Chapter 38, Academic Press, San Diego, CA., USA., ISBN: 978-0-12-426260-7, pp: 799-843.

Grewal, K.K., G.S. Sandhu, R. Kaur, R.S. Brar and H.S. Sandhu, 2010. Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicol. Int., 17: 94-98.

Heikal, T.M., A.T.H. Mossa, A.W. Ibrahim and H.F. Abdel-Hamid, 2014. Oxidative damage and reproductive toxicity associated with cyromazine and chlorpyrifos in male rats: The protective effects of green tea extract. Res. J. Environ. Toxicol., 8: 53-67.

Ikpeme, E.V., O. Udensi, U.B. Ekaluo, E.A. Uyoh, B.O. Asuquo, F.V. Udoh and P.B. Udoh, 2007. Effect of crude extract of *Carica papaja* seeds on the reproductive efficiency of male albino rats. Global J. Pure Applied Sci., 13: 365-368.

Jeyaratnam, J., 1990. Acute pesticide poisoning: A major global health problem. World Health Stat. Q., 43: 139-144.

Joshi, S.C., B. Bansal and N.D. Jasuja, 2011. Evaluation of reproductive and developmental toxicity of Cypermethrin in male albino rats. Toxicol. Environ. Chem., 93: 593-602.

Kumar, S., A.K. Gautam, K.R. Agarwal, B.A. Shah and H.N. Saiyad, 2004. Demonstration of sperm head shape abnormality and clastogenic potential of cypermethrin. J. Environ. Biol., 25: 187-190.

Liu, L., J.X. Hu, H. Wang, B.J. Chen, Z. He and L.C. Xu, 2010. Effects of beta-cypermethrin on male rat reproductive system. Environ. Toxicol. Pharmacol., 30: 251-256.

Miller, G.T., 2004. Sustaining the Earth. 6th Edn., Thompson Learning Inc., Pacific Grove, CA., USA., pp: 211-216.

Monet-Kuntz, C., M.T. Hochereau-de Reviers and M. Terqui, 1984. Variations in testicular androgen receptors and histology of the lamb testis from birth to puberty. J. Reprod. Fertil., 70: 203-210.

Narayana, K., U.J.A. Souza and K.P.S. Rao, 2002. Ribavirin-induced sperm shape abnormalities in wistar rat. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 513: 193-196.

Okamura, A., M. Kamijima, E. Shibata, K. Ohtani and K. Takagi *et al*., 2005. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicology, 213: 129-137.

Oyeyipo, I.P., Y. Raji, B.O. Emikpe and A.F. Bolarinwa, 2011. Effects of nicotine on sperm characteristics and fertility profile in adult male rats: A possible role of cessation. J. Reprod. Infert., 12: 201-207.

Palmer, W.E., P.T. Bromley and R.L. Brandenburg, 2007. Wildlife and Pesticides-Peanuts. North Carolina Cooperative Extension Service, Raleigh, NC., USA.

Pareek, T.K., A.R. Joshi, A. Sanyal and R.R. Dighe, 2007. Insights into male germ cell apoptosis due to depletion of gonadotropins caused by GnRH antagonists. Apoptosis, 12: 1085-1100.

Roberts, E.M., P.B. English, J.K. Grether, G.C. Windham, L. Somberg and C. Wolff, 2007. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California central valley. Environ. Health Perspect., 115: 1482-1489.

Sai, L., X. Li, Y. Liu, Q. Guo and L. Xie *et al*., 2014. Effects of chlorpyrifos on reproductive toxicology of male rats. Environ. Toxicol., 29: 1083-1088.

Sanborn, M., J.K. Kerr, L.H. Sanin, D.C. Cole, K.L. Bassil and C. Vakil, 2007. Non-cancer health effects of pesticides: Systematic review and implications for family doctors. Family Phys., 53: 1712-1720.

Sharma, P., A.U. Huq and R. Singh, 2014. Cypermethrin-induced reproductive toxicity in the rat is prevented by resveratrol. J. Hum. Reprod. Sci., 7: 99-106.

Tulsiani, D.R.P., A. Abou-Haila, C.R. Loeser and B.M.J. Pereira, 1998. The biological and functional significance of the sperm acrosome and acrosomal enzymes in mammalian fertilization. Exp. Cell Res., 240: 151-164.

Uzunhisarcikli, M., Y. Kalender, K. Dirican, S. Kalender, A. Ogutcu and F. Buyukkomurcu, 2007. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pestic. Biochem. Physiol., 87: 115-122.

Wang, X.Z., S.S. Liu, Y. Sun, J.Y. Wu, Y.L. Zhou and J.H. Zhang, 2009. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, 72: 599-611.

Zidan, N.E.H.A., 2009. Evaluation of the reproductive toxicity of chlorpyrifos methyl, diazinon and profenofos pesticides in male rats. Int. J. Pharmacol., 5: 51-57.