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Research Article

Stressful Effect of Repeated Subcutaneous Injection of Tulathromycin on Social and Grooming Behaviors of Rats

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Abstract

Stressful effect of repeated subcutaneous injection of tulathromycin on serum cortisol level and behavior of rats was investigated. Twenty seven apparently healthy adult male rats were equally divided into three groups. Group 1 was subcutaneously injected with tulathromycin in a dose of (2.5 mg kg^{-1} b.wt.) twice with 15 days interval, group 2 was injected for three successive injections with one week interval and control group rats were subcutaneously injected with 0.1 mL normal saline once weekly for three weeks. Rat's behavior was videotaped. Thereafter, frequency and duration/min of rats behaviors (Self-grooming, feeding, comfort and locomotion) were calculated and their aggression bouts were scored. At the end of the experiment, blood samples were collected and serum was obtained for measuring liver and kidney enzymes and serum cortisol level. Cortisol level was increased to 1.4 and 1.5 times that of control in group 1 and group 2, respectively, while the enzymes activities were not significantly altered after the end of the experiment. At the second week, group 2 rats performed self-grooming for longer time than control rats. However, at the third week of injection, self-grooming duration of rats in both groups was shorter than those in control. Furthermore, group1 rats showed marked social behavior disturbance expressed in prominent aggression in between. Data suggested stressful effect of repeated subcutaneous injection of tulathromycin to rats. Thus, repeated subcutaneous injection of tulathromycin is not recommended for animals.

Key words: Behavior, stress, rat, tulathromycin, cortisol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics are widely used drugs for treatment of infectious diseases in human and animal. Macrloids are antibacterial agents used as veterinary drugs for treatment or prevention of diseases (Er and Yazar, 2010). They are active against Gram-positive bacteria, target the bacterial ribosome and inhibit bacterial protein biosynthesis (Leal *et al*., 2001). Furthermore, macrloids are commonly prescribed to human for lower and upper respiratory tract and soft-tissue infections (McArdell *et al*., 2003). Tulathromycin (TLM) is a new member of the triamilide subclass of macrolide antibiotics, commonly used for the treatment of respiratory infectionsin different animal species such as cattle and swine (Evans, 2005) and rabbits (Abo-El-Sooud *et al*., 2012). It inhibits bacterial growth by preventing essential protein biosynthesis through selective binding to bacterial ribosomes and stimulate dissociation of peptidyl-tRNA from the ribosome during the translocation process (Good *et al*., 2012).

Macrolids were reported to induce biochemical alterations and cardiac toxicty (Er *et al*., 2011) in the treated animals. In addition, injection of macroloids caused severe pain and irritation (Giguere, 2013). Therefore, repeated injection of macroloids including TLM may induce stressful effect on the treated individuals.

Serum cortisol level is a common biomarker to stress (Retana-Marquez *et al*., 2003). In addition, behavioral alterations of individual are usually reflection to his physiological condition, biochemical changes and provide rapid assessment of stress (Lester *et al*., 1996). Therefore, correlation of physiological, biochemical and behavioral changes to drugs is the best approach to assess their impacts on the treated animals.

Recently, there is more focusing on self grooming and social behavior especially aggression alteration as a behavioral copying strategy in response to stress (Babb *et al*., 2014).

To the best of our knowledge the available pharmacodynamic data about tulathromycin is not sufficient and side effects resulted from its injection is not reported. Thus, behavioral, biochemical changes Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and lactate dehydrogenase (LDH) and cortisol level of albino rats (*Rattus norvigious* albinus) in response to repeated subcutaneous injections of TLM were investigated for assessment of its stressful effect on treated animals.

MATERIALS AND METHODS

Drug and chemicals: Tulathromycin (TLM) (100 mg mL^{-1}) was supplied as an injectable solution (Draxxin®) by animal health division Pfizer company, Cairo, Egypt. The LDH kits (Biosystems S.A. Costa Barva 30, Barcelona, Spain), AST and ALT kits (Spinreact, S.A./S.A.U. Ctra Santa Coloma, Spain), creatinine kits (Diamond) and cortisol kits (the ADVIA Centaur and ADVIA Centaur Xp Assay Manual).

Animals and housing: Twenty seven apparently healthy adult albino male rats (Average weight 160-200 g) were obtained from breeding unit of Helwan farm of laboratory animals (Helwan, Egypt). Animals were group housed (3/cage) in (47×25×21) cm dimension. Rats were kept for 2 week prior to the onset of the study in department of Pharmacology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt. Animals within each social group (i.e., homecage) received the same experimental treatment throughout the experiment. Three replicates were used for each group.

Rats were maintained at 21±2°C temperature, 45±5% humidity, with a 12:12 h light: dark cycle (07.00-19.00 h), fed a commercially prepared pellet diet and watered *ad libitum* throughout the study. Bedding was changed twice per week. Cages, feeders and water bottles were washed twice weekly.

Experimental design: After two weeks of acclimatization, rats were equally divided into three groups (9 rats, each 3/cage). Rats in the control group were subcutaneously (S/C) injected with 0.1 mL normal saline once weekly for three weeks, while those in the second group (G1) were S/C injected with TLM in a dose of (2.5 mg kg^{-1} b.wt.) (Er and Yazar, 2010) twice with 15 day interval and the third group (G2) were S/C injected with TLM (2.5 mg kg^{-1} b.wt.) for three successive doses (once weekly).

The study was conducted in accordance with the principles and guidelines of the Canadian Council on Animal Care and approved by department of Animal care and Welfare, Faculty of Veterinary Medicine, Beni-Suef University.

Marking of experimental animals: Rats were marked using three different stains whereas, each rat had a fixed color during the experiment to facilitate tracking of its behavior throughout the experiment. The non toxic stains were smeared on various parts of the animal body. Re-marking of rats was necessary at interval according to disappearance of the stain.

Behavioral data collection and ethogram: Behavior of each three rats (in same cage) was videotaped for 30 min session three days weekly, three times per day (once in the morning 8:30", anther at noon the 12:00" and the last at afternoon time 3:30") using digital video camera (Sony, Japan). Three videos for each group were collected for behavior analysis.

Thereafter, rat's behavior was analyzed using focal observation (Lehner, 1996). In all groups, rats behaviors including, self-grooming (licking and nibbling the fur, washing face with forelimbs, licking ears, head in front of the snout with forelimbs), feeding, comfort (Rest and sleep) and locomotor (walking) behaviors were analyzed. Frequency and duration/min of each behavior pattern were calculated.

Moreover, aggressive behavior of the rats was analyzed using zero-one score method (Lehner, 1996). Rats aggression was scored as 0 when aggressive bouts duration was less than 30 sec (Miczek and De Bore, 2004) while, that was prolonged for more than 30 min was scored as 1.

Blood sampling: After the end of injection period, blood was collected from the retro-orbital venous plexus (Poole, 1987), using clean microcapillary tubes. Collected blood was kept in a clean screw-capped bottle and incubated at 37°C until clotting, centrifuged at 3000 rpm for 15 min to obtain serum which stored at -20°C till use.

Cortisol and biochemical assay: Serum cortisol was assayed using direct chemiluminescent technology (Chodosh and Daniels, 1993). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured colourimetrically, serum creatinine activity was measured by thecolourimetric kinetic method and lactate dehydrogenase (LDH) was measured spectrophotometerically according to the method described by Yang (2001). Serum cortisol level was measured according to the ADVIA Centaur Xp Assay Manual.

Statistical analysis: All statistical analyses were performed using Advanced Models SPSS version 20.0. Data were analyzed by One-Way ANOVA. *Post hoc* analysis was carried out using Dunnett's multiple comparison test. The p-values less than 0.05 were considered significant.

RESULTS

Statistical analysis of serum cortisol level and biochemical data showed significant difference between injected rats and control.

Table 1 illustrates significant decrease ($p = 0.035$ and $p = 0.028$) in serum cortisol level of rats treated with two and three repeated doses of TLM (2.5 mg kg^{-1} b.wt.), respectively, in relation to control group. Serum cortisol level was increased to 1.4 and 1.5 times that of control in rats in group 1 and 2, respectively. Moreover, liver and kidney functions in rats treated with two and three repeated doses of TLM (2.5 mg kg^{-1} b.wt.) showed no significant alterations in ALT, AST, LDH and creatinine levels in serum compared to control group.

Rats behavior alterations in response to repeated S/C injection of TLM in a dose of (2.5 mg kg^{-1} b.wt.) are demonstrated in Fig. 1. Self grooming behavior of rats in group 1 (injected with two repeated doses) was performed for longer ($p = 0.042$) time than control rats. However, rats in both groups (injected with two and three doses) showed shorter ($p = 0.04$ in G1 and $p = 0.02$ in G2) (Fig. 1a) duration of self grooming performance than those of control (Fig. 1b) at the third week of TLM injection.

Feeding of rats in treated groups was not significantly different than those in control (Fig. 1c and d). Similarly, comfort (Fig. 1e and f) and locomotor behaviors (Fig. 1g and h) of rats repeatedly injected with TLM did not differ from control.

The aggression bouts observed per social group (i.e., each cage of 3) are illustrated in Table 2. Aggression bouts were scored as 1 in rats injected for two times while, those injected with three doses of TLM and in control group were scored as 0.

Table 1: Effect of repeated subcutaneous administration of tulathromycin; TLM (2.5 mg kg^{-1} b.wt.) on serum cortisol level, ALT, AST, LDH and creatinine levels of rats

Groups	Serum cortisol level (μg dL^{-1})	ALT (U L^{-1})	AST (U L^{-1})	LDH (U L^{-1})	Creatinine (mg dL^{-1})
Control	0.76±0.11	32.00±3.21	127.00±4.93	65.5±16.45	0.670±0.025
Group 1	1.15±0.11*	35.66±8.76	110.25±4.73	74.3±12.30	0.725±0.047
Group 2	1.16±0.10*	29.66±2.66	139.00±11.69	67.0±13.80	0.700±0.006

Control: Injected with 0.1 mL saline, Group 1: Injected with 2 doses, 2.5 mg kg^{-1} b.wt., of TLM with one week interval and Group 2: Injected with 3 doses, 2.5 mg kg^{-1} b.wt., of TLM with one week interval (Mean±SD), n = 5. *Significance at $p<0.05$ according to *post hoc* analysis, Dunnett's multiple comparison test, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and LDH: Lactate dehydrogenase

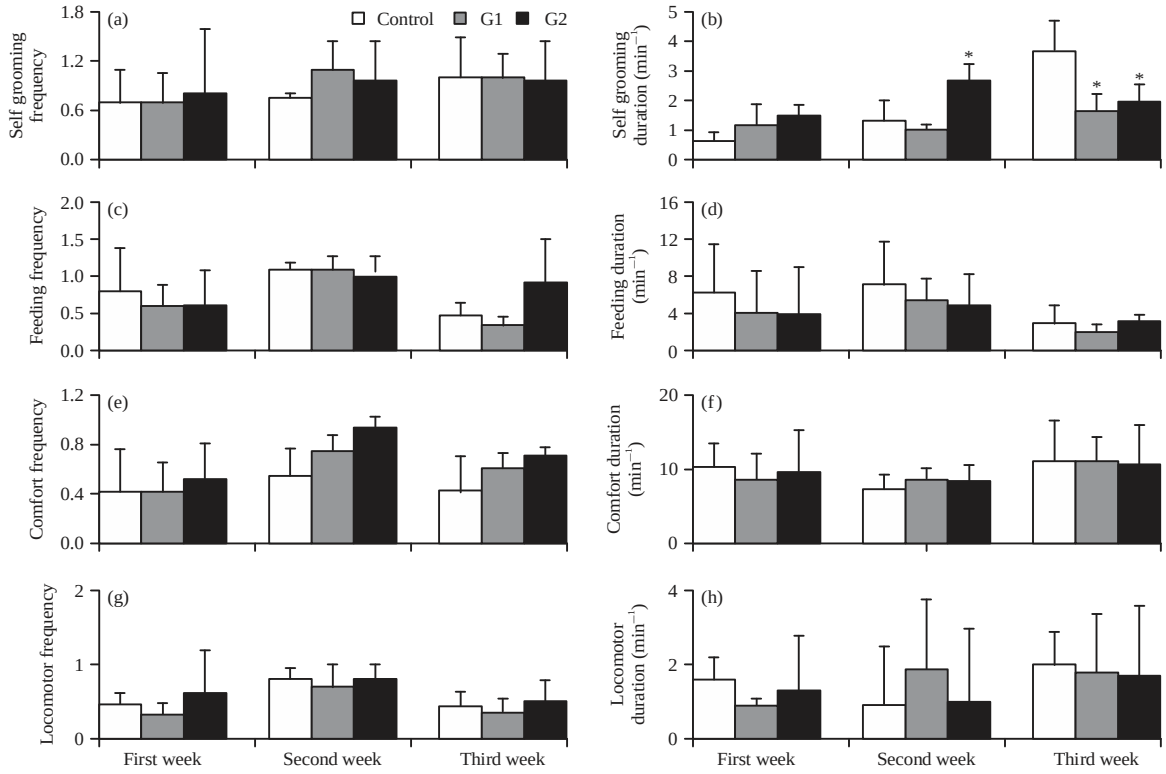

Fig. 1(a-h): Effect of tulathromycin (TLM) on rats (a) Self grooming frequency, (b) Self grooming duration/min, (c) Feeding frequency, (d) Feeding duration/min, (e) Comfort frequency, (f) Comfort duration/min of rats, (g) Locomotor frequency and (h) Locomotor duration/min in control rats (injected with 0.1 mL saline) group 1 (injected with 2 doses, 2.5 mg kg^{-1} b.wt., of TLM with (15 days interval) and group 2 (injected with 3 doses; 2.5 mg kg^{-1} b.wt., of TLM with one week interval) at first, second and third weeks of TLM injection. All values are the Mean±SD (n = 9). *Significant difference between the exposure group and corresponding control (white bars) according to *post hoc* analysis, Dunnett's multiple comparison test at $p<0.05$

Table 2: Effect of tulathromycin on aggressive behavior of rats

Group	First week	Second week	Third week
Control	0	0	0
Group 1	1	1	1
Group 2	0	0	0

Control: injected with 0.1 mL saline, group 1: Injected with 2 doses, 2.5 mg kg^{-1} b.wt., of TLM with one week interval and Group 2: Injected with 3 doses, 2.5 mg kg^{-1} b.wt., of TLM with one week interval, Rats aggression was scored as 0 when aggressive bouts duration was less than 30 sec while, that was prolonged for more than 30 min was scored as 1, (n = 3 social groups; each 3 rats), TLM: Tulathromycin

DISCUSSION

Overall, this study revealed the stressful effect of TLM on rat (*Rattus norvigious* albinus) expressed by marked increase in serum cortisol level and behavioral alterations. The increased cortisol level in serum of TLM (2.5 mg kg^{-1} b.wt.) injected (S/C) rats suggested stressful effect of repeated (two and three) injection of the drug. Similar results were recorded in individuals exposed to stress (Retana-Marquez *et al*., 2003). On the other hand, macrloids caused no significant alterations in serum cortisol level in the treated patients (Kanoh and Rubin, 2010). Therefore, the reported increase in serum cortisol might be as a result of severe pain and irritation of TLM injection (Giguere, 2013).

These findings showed that both two and three S/C repeated injections of TLM (2.5 mg kg^{-1} b.wt.) caused no significant changes in liver and kidney functions. Serum ALT, AST, LDH and creatinine activities were within normal limits. These results were agreeable with Yazar *et al*. (2004) reported no alterations in serum creatinine level after treatment with macrolid antibiotics. On the other hand, CVMP (2002) found that TLM caused slight increase in liver enzymes (ALT and AST) in rats and dogs. In additions, Er *et al*. (2011) recorded an increase in serum creatinine of rabbits treated with TLM. The different data in kidney and liver enzymes in response to macrolids treatment may be due to different doses and treated animals species. Thus, the used TLM dose in this study was safe and bearable for liver and kidney of rats.

The observed data indicated that duration of self grooming behavior showed opposite responses to repeated TLM (2.5 mg kg^{-1} b.wt.) S/C injection. Grooming duration was increased after two successive injections one week in between. However, rats groomed during short duration after the third injection one week later. Similar result was observed in rats injected two doses 15 days in between. In early reports, both results were observed. Smolinsky *et al.* (2009) reported that stress increase grooming behavior of rat. Meanwhile, Kalueff and Tuohimaa (2004) recorded less grooming activity in mice exposed to strong stress. These alterations in grooming performance of rats may be attributed to the increased level of cortisol (Zwiers *et al.*, 1981). Thus, grooming behavior may be useful tool for assessing stressful impacts of repeated subcutaneous TLM injection.

Grooming is critical behavior for health and survival of rats (Borchelt, 1980). Hence, repeated injection of TLM drugs may disturb this behavior and consequently threaten animals health.

This study indicated that feeding, comfort and locomotor behaviors of rats subcutenously injected with TLM (2.5 mg kg^{-1} b.wt.) were not significantly altered. This is in agreement with Silveira *et al.* (2005) who observed no effect of stress on feeding of rats. On the contrary, it was reported that stress may decrease feeding (Haque *et al.*, 2013). Moreover, macrolids (roxithromycin) decreased locomotor behavior and prolonged sleeping period (Deshmukh and Tamboli, 2013). Hence, the used dose did not affect feeding, locomotor and feeding behavior of rat.

The increased aggression bouts of rats that were S/C injected with TLM (2.5 mg kg^{-1} b.wt.) for two times may be argued to increased level of serum cortisol (Guimont, 2009). Similarly, aggressive behavior was increased in response to stress (Sgoifo *et al.*, 1996). Moreover, aggression of the rats three times injected was not marked despite of high serum cortisol level. This data is in agreement with Wood *et al.* (2003) who reported that acute stress did not alter aggressive behavior of rats. These findings support the concept of wide inter-individual aggression differentiation in coping style (Sgoifo *et al.*, 1996) during response to stress. Thus, repeated injection of TLM may result in social disruption due to increase of aggression.

It is worth noting that, the group of rats performed long time grooming showed normal aggression bouts duration and vice versa. It is hypothesize that rats display one pattern only of motor activities (i.e., grooming or aggression) as an active coping strategy in attempting to deal with or escape from pain (Sgoifo *et al.*, 1996).

Further studies are needed to investigate the irreversibility of these effects of the tested drug on the experimental animals.

CONCLUSION

The response of rats to repeated subcutaneous injection of TLM in a dose of (2.5 mg kg^{-1} b.wt.) expressed in high serum cortisol level and increase behaviors of anxiety (self grooming and aggression) suggested its stressful effect on animals. Moreover, prolonged suffering and consequent biochemical and behavioral disruption might threaten animal health. Therefore, repeated subcutaneous injection of TLM is not recommended.

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REFERENCES

Abo-El-Sooud, K., N.A. Afifi and A.M.A. El-Aty, 2012. Pharmacokinetics and bioavailability of tulathromycin following intravenous, intramuscular and subcutaneous administrations in healthy rabbits. Vet. World, 5: 424-428.

Babb, J.A., L.M. Carini, S.L. Spears and B.C. Nephew, 2014. Transgenerational effects of social stress on social behavior, corticosterone, oxytocin and prolactin in rats. Hormones Behav., 65: 386-393.

Borchelt, P.L., 1980. Care of the Body Surface (COBS). In: Comparative Psychology: An Evolutionary Analysis of Animal Behavior, Ray, D.M. (Ed.). Wiley, New York, ISBN: 9780471709305, pp: 362-384.

CVMP., 2002. Tluthromycin, summary report. Committee for veterinary Medicinal Products (CVMP), European Medicines Agency, Veterinary Medicines and Inspections, 842/02-10.

Chodosh, L.A. and G.H. Daniels, 1993. Addison's disease. Endocrinology, 3: 166-181.

Deshmukh, J.B. and S.B. Tamboli, 2013. Study of CNS effects of macrolide antibiotics: An experimental study. J. Evolution Med. Dental Sci., 2: 653-656.

Er, A. and E. Yazar, 2010. Effects of macrolide antibiotics on blood inflammatory mediators and organ damage markers in lipopolysaccharide-induced pulmonary damage rats. Eurasian J. Vet. Sci., 26: 7-13.

Er, A., F. Altan, G. Cetin, B. Dik, M. Elmas and E. Yazar, 2011. Assessment of the cardiotoxicity of tulathromycin in rabbits. Acta Vet. Hungarica, 59: 327-335.

Evans, N.A., 2005. Tulathromycin: An overview of a new triamilide antibiotic for livestock respiratory disease. Vet. Ther., 6: 83-95.

Giguere, S., 2013. Macrolides, Azalides and Ketolides. In: Antimicrobial Therapy in Veterinary Medicine, Giguere, S., J.F. Prescott and P.M. Dowling (Eds.). 5th Edn., Wiley Blackwell, Ames, IA., USA., ISBN: 9781118675076, pp: 211-232.

Good, J.T.Jr., D.R. Rollins and R.J. Martin, 2012. Macrolides in the treatment of asthma. Curr. Opin. Pulmonary Med., 18: 76-84.

Guimont, F., 2009. Stress responses in an outbred, wild-derived hamster, *Phodopus campbelli*: Individuality in the cortisol response to acute restraint stress and the absence of strong association with behavioural responses to acute restraint. Ph.D. Thesis, Queen's University, Ontario.

Haque, Z., N. Akbar, F. Yasmin, M.A. Haleem and D.J. Haleem, 2013. Inhibition of immobilization stress-induced anorexia, behavioral deficits and plasma corticosterone secretion by injected leptin in rats. Stress: Int. J. Biol. Stress, 16: 353-362.

Kalueff, A.V. and P. Tuohimaa, 2004. Contrasting grooming phenotypes in C57Bl/6 and 129S1/SvImJ mice. Brain Res., 1028: 75-82.

Kanoh, S. and B.K. Rubin, 2010. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin. Microbiol. Rev., 23: 590-615.

Leal, C., R. Codony, R. Compano, M. Granados and M.D. Prat, 2001. Determination of macrolide antibiotics by liquid chromatography. J. Chromatogr. A, 910: 285-290.

Lehner, P., 1996. Handbook of Ethological Methods. 2nd Edn., Cambridge University Press, UK., ISBN: 978-0521637503, Pages: 672.

Lester, N., J.C. Lefebvre and F.J. Keefe, 1996. Pain in young adults-III: Relationships of three pain-coping measures to pain and activity interference. Clin. J. Pain, 12: 291-300.

McArdell, C.S., E. Molnar, M.J.F. Suter and W. Giger, 2003. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. Environ. Sci. Technol., 37: 5479-5486.

Miczek, K.A. and S.F. de Bore, 2004. Aggressive, Defensive and Submissive Behavior. In: The Behavior of the Laboratory Rat: A Handbook with Tests: A Handbook with Tests, Whishaw, I.Q. and B. Kolb (Eds.). Oxford University Press, Oxford, ISBN: 9780198036845, pp: 344-348.

Poole, B.T., 1987. The UFA Hand Book on the Care and Management of the Laboratory Animals. Longman Scientific and Technical, USA., Pages: 309.

Retana-Marquez, S., H. Bonilla-Jaime, G. Vazquez-Palacios, R. Martinez-Garcia and J. Velazquez-Moctezuma, 2003. Changes in masculine sexual behavior, corticosterone and testosterone in response to acute and chronic stress in male rats. Hormones Behav., 44: 327-337.

Sgoifo, A., S.F. De Boer, J. Haller and J.M. Koolhaas, 1996. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: Relationship with aggression. Physiol. Behav., 60: 1403-1407.

Silveira, P.P., A.K. Portella, Z. Clemente, G.D. Gamaro and C. Dalmaz, 2005. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. Int. J. Dev. Neurosci., 23: 93-99.

Smolinsky, A.N., C.L. Bergner, J.L. La Porte and A.V. Kalueff, 2009. Analysis of Grooming Behavior and its Utility in Studying Animal Stress, Anxiety and Depression. In: Mood and Anxiety Related Phenotypes in Mice, Gould, T.D. (Ed.). Hum Press, USA., pp: 21-36.

Wood, G.E., L.T. Young, L.P. Reagan and B.S. McEwen, 2003. Acute and chronic restraint stress alter the incidence of social conflict in male rats. Hormones Behav., 43: 205-213.

Yang, D.S., 2001. Effects of Drugs on Clinical Laboratory Tests. 4th Edn., AACC Press, Washington, DC.

Yazar, E., E. Oztekin, A. Sivrikaya, R. Col, M. Elmas and A.L. Bas, 2004. Effects of different doses of tilmicosin on malondialdehyde and glutathione concentrations in mice. Acta Veterinaria Brno, 73: 69-72.

Zwiers, H., V.J. Aloyo and W.H. Gispen, 1981. Behavioral and neurochemical effects of the new opioid peptide dynorphin-(1-13): Comparison with other neuropeptides. Life Sci., 28: 2545-2551.