

Letter

Acetaminophen enhances pruritus in a mouse model of contact dermatitis induced by suboptimal concentration of hapten

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ABSTRACT — Acetaminophen (APAP) is one of the most commonly used drugs worldwide to reduce fever, particularly in children. It is generally considered to be a safe drug. However, a number of studies have shown that regular use of APAP increases the risk of developing allergic diseases. Nonetheless, no animal models have been used to investigate these findings. Therefore, we aimed to create an animal model of APAP-induced pruritus in mice. APAP (0.25% and 0.5%) was administered via drinking water daily from infancy, and a suboptimal concentration of 2,4,6-trinitrochlorobenzene (TNCB) was applied repeatedly to each ear three times a week for 7 weeks to evoke chronic allergic contact dermatitis. Neither 0.25% nor 0.5% APAP was overtly hepatotoxic after 73 days of daily administration. Repeated challenge with TNCB evoked increase in the number of scratching bouts compared to day 1. This increase in the number of scratching bouts was significant in 0.25% and 0.5% APAP groups but not in the group treated with TNCB alone. Daily administration of 0.5% APAP significantly increased in the number of scratching bouts compared to TNCB alone on day 29. This animal model will be useful for investigating the mechanism underlying the increased risk of development of eczema caused by regular APAP use and for examining safer and more effective therapy with APAP.

Key words: Acetaminophen, Paracetamol, Infant mice, Pruritus, Allergic contact dermatitis, Animal model

INTRODUCTION

The prevalence of allergic diseases has increased over the last four decades; however the reasons for this increase are poorly understood and have not been investigated experimentally. Acetaminophen (APAP) is one of the most commonly used anti-pyretic agents, particularly in children, and although it is generally considered safe, during the last decade there have been a number of studies indicating that regular use of APAP carries a dose-dependent risk for the development of allergic diseases in general and asthma in particular (Newson *et al.*, 2000; Barr *et al.*, 2004; Davey *et al.*, 2005; Eneli *et al.*, 2005; McKeever *et al.*, 2005; Shaheen *et al.*, 2005; Barragán-Mejueiro *et al.*, 2006; Karimi *et al.*, 2006; Koniman *et al.*, 2007; Wong *et al.*, 2007).

In 2008, an analysis based on data from 205,487 children in 31 countries participating in the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three study identified a strong dose-dependent association between use of APAP and symptoms of asthma, as well as rhinoconjunctivitis and eczema, in children aged 6-7 years, with frequent APAP use (Beasley *et al.*, 2008). Similarly, they identified association between use of APAP in the first year of life and the symptoms of asthma, rhinoconjunctivitis and eczema in childhood. These results suggest that the effect of APAP is not restricted to the airways. Several biologic mechanisms have been suggested as an explanation for the increased morbidity caused by regular APAP use. However, no experimental investigation to confirm these suggestions has been conducted because to date there has been no suitable animal

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model. In the present study, we gave APAP to mice every day from 3 weeks of age to focus on the increased risk of eczema caused by regular use of APAP in childhood. Pruritus can occur in patients suffering from numerous different skin diseases, for example all patients with atopic dermatitis experience pruritus (Metz and Ständer, 2010). Pruritus can worsen the skin condition of the patient considerably and is thought to be an important exacerbating factor for skin disorders. Scratching in response to pruritus exacerbates skin inflammation, and the resulting “itch-scratch cycle” leads to chronic allergic dermatitis (Pfenninger and Zainea, 2001; Mahtani *et al.*, 2005).

Therefore, the aim of the present study was to create an animal model of APAP-induced pruritus in mice with chronic allergic contact dermatitis induced by repeated application of suboptimal concentration of 2,4,6-trinitrochlorobenzene (TNCB), with the expectation that the model would help to elucidate the mechanism of increased risk for the development of eczema caused by regular APAP use and contribute to safer and more effective usage of APAP in childhood.

MATERIALS AND METHODS

Animals

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee. Female BALB/c mice, 3 weeks of age, were obtained from Japan SLC Inc. (Shizuoka, Japan), and housed under controlled light (0700–1900 hr) and temperature (24°C) conditions with food and water available *ad libitum*. The hair on the abdominal area was clipped at least 1 day before the start of the experiment.

Reagents

TNCB was obtained from Tokyo Chemical (Tokyo, Japan), dissolved in acetone/olive oil (3:1) as a 0.1% (w/v) solution, and used for both sensitization to and elicitation of chronic allergic contact dermatitis. APAP was obtained from Sigma Chemical (St. Louis, MO, USA).

Drug treatment

APAP, dissolved in distilled water at 0.25% or 0.5% (w/v), was added to the drinking water from 3 weeks of age for 10 weeks (from day -24 to 49). The volume of drinking water in the bottles was measured to estimate dietary intake of water.

TNCB-induced chronic allergic contact dermatitis

The experimental protocols are illustrated in Fig. 1. We defined the first day of the challenge with TNCB as day 0. The hair on the abdominal region was shaved with a hair clipper 2 days before sensitization with TNCB. On day -7, after 17 days of administration of APAP, mice were sensitized by a single epicutaneous application of 50 μ l TNCB solution to the shaved abdomen, and on day 0 challenged with 10 μ l/ear TNCB solution to both ears. TNCB was then applied repeatedly to each ear three times a week until day 49. As a negative control, acetone/olive oil (3:1) was applied to both ears instead of TNCB (Nil).

Measurement of ear thickness

Right and left ear thickness was measured with a micrometer (Mitsutoyo, Kanagawa, Japan), under light ether anesthesia, 24 hr after TNCB challenge. Ear swelling was calculated by averaging the thickness values for both ears.

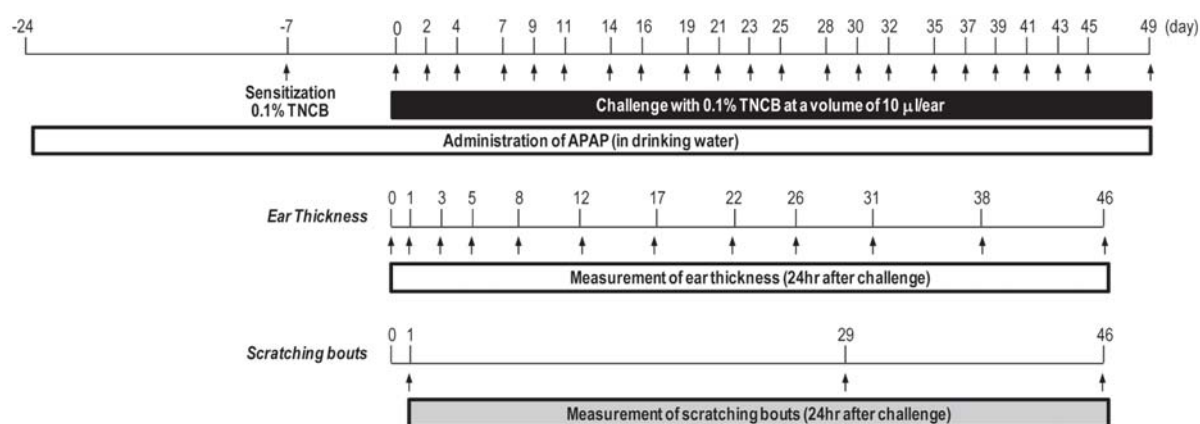


Fig. 1. Schedule for the elicitation of chronic allergic contact dermatitis and application of reagents. APAP, acetaminophen at 0.25% and 0.5%.

Pruritus measurement

The number of bouts of scratching behavior was counted for 2 hr at 24 hr after TNCB challenge on days 0, 28, and 45. Pruritus was evaluated by automatic counting of the scratching bouts using MicroAct (Neuroscience Inc., Tokyo, Japan), as reported previously (Inagaki *et al.*, 2003).

Measurement of serum aminotransferase activities

Blood was collected by retro-orbital bleeding on day 49. Serum was then obtained by centrifugation at $1,000 \times g$ for 20 min at 4°C and stored at -20°C until use. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using Transaminase CII-test Wako (Wako Pure Chemical Institute, Osaka, Japan).

Measurement of serum IgE

Blood was collected by retro-orbital bleeding on day 49. Serum was obtained by centrifugation at $1,000 \times g$ for 20 min at 4°C and stored at -20°C until use. Serum IgE levels were determined using a commercial sandwich ELISA assay (Bethyl Laboratories Inc., Montgomery, TX, USA).

Statistical analysis

All data are presented as mean $\pm$ S.E.M. Statistical significance was analyzed using Dunnett's method or Fisher's least significant difference test for multiple comparisons, and Student's *t* test for two-group comparison. Differences at $p < 0.05$ were considered statistically significant. All statistical analyses were conducted using StatLight software (Yukms Co., Ltd. Tokyo, Japan).

RESULTS

Effects on serum AST and ALT activities

Serum AST and ALT activities were assessed after 73 days of daily administration of APAP (day 49). Neither 0.25% nor 0.5% APAP was overtly hepatotoxic. Mice had normal serum AST and ALT activities, and their body weight was also normal on day 49 (Table 1).

Effects of APAP on scratching behavior

Repeated challenge with a suboptimal concentration of TNCB evoked increase in the number of scratching bouts compared to day 1. This increase in the number of scratching bouts was significant in 0.25% and 0.5% APAP groups but not in the group treated with TNCB alone. Daily administration of 0.5% APAP significantly increased in the number of scratching bouts compared to TNCB alone on day 29 (Fig. 2).

Table 1. Effect of APAP on serum AST and ALT activities.

		Body weight (g)	AST (IU/l)	ALT (IU/l)
Nil		18.3 ± 0.3	28.0 ± 5.1	3.8 ± 0.5
APAP	0%	20.1 ± 0.4	29.3 ± 1.3	4.9 ± 0.8
	0.25%	18.8 ± 0.7	30.1 ± 3.0	6.5 ± 1.2
	0.5%	19.0 ± 0.6	34.2 ± 2.9	4.2 ± 0.8

APAP, dissolved in distilled water at 0.25% and 0.5% (w/v), was administered via the drinking water to mice from 3 weeks of age every day for 10 weeks (from day -24 to 49). Blood was collected 3 hr after the final TNCB challenge on day 49. Nil, non-application of TNCB. Values represent the mean $\pm$ S.E.M. for 4-7 mice.

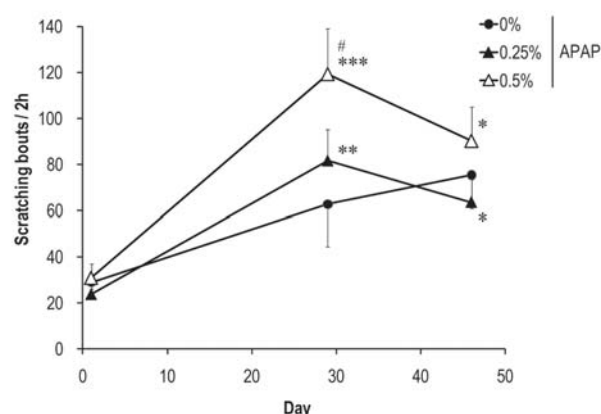


Fig. 2. Effect of APAP on scratching behavior induced by repeated application of TNCB. APAP, dissolved in distilled water at 0.25% and 0.5% (w/v), was administered via the drinking water to mice from 3 weeks of age every day for 10 weeks (from day -24 to 49). The ears of TNCB-sensitized mice were repeatedly challenged with 0.1% TNCB three times a week from day 0 to 49. Twenty-four hr after the TNCB challenge, scratching bouts were counted for 2 hr using MicroAct. Values represent the mean $\pm$ S.E.M. for 7 mice. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs each corresponding value on day 1 (Dunnett's multiple comparisons), # $p < 0.05$ vs 0% APAP (Fisher's least significant difference test).

Effects of APAP on ear thickness

Repeated exposure of mouse ears to a suboptimal concentration of TNCB induced chronic allergic contact dermatitis characterized by skin swelling, which reached a plateau on day 22. Daily administration of 0.25% APAP

significantly inhibited the increase in ear thickness from day 8 to 22. In contrast, 0.5% APAP did not show any obvious inhibitory effect on ear thickness (Fig. 3).

Effects of APAP on total serum IgE concentration

Repeated application of a suboptimal concentration of TNCB caused a significant elevation of the total serum IgE level on day 49. Administration of 0.25% APAP significantly attenuated the increase. In contrast, 0.5% APAP did not show any obvious inhibition (Fig. 4).

DISCUSSION

In a previous study, we showed that repeated application of 1.0-1.1% TNCB induced chronic allergic contact dermatitis characterized by significant skin swelling and scratching behavior throughout the experimental period (Yamaura *et al.*, 2011). In the present study, we used a suboptimal concentration of TNCB (0.1%) as a hapten to induce moderate chronic allergic contact dermatitis in order to examine the exacerbating effect of APAP on the pruritic response in mice. In our preliminary study, repeated challenge with a suboptimal concentration of TNCB (0.1%) evoke about 30-40% of skin swelling compared with that of optimal concentration of TNCB, and

the number of scratching bouts was also lower.

In the present study, the increase in scratching bouts on day 29 was dependent on APAP concentration; this is in agreement with the dose-dependent increased risk of eczema reported by the ISAAC Study Group (Beasley *et al.*, 2008).

Several biological mechanisms have been proposed to explain the association between regular APAP use and asthma and other atopic diseases (Allmers, 2005; Eneli *et al.*, 2005; Beasley *et al.*, 2008). Oxidative stress appears to play an important role in the development and progression of respiratory diseases. Glutathione (GSH), an endogenous antioxidant, is found in the respiratory tract lining fluid (Cantin *et al.*, 1987), and decreased GSH levels are associated with oxidant damage in the lung (Nuttall *et al.*, 2003). Furthermore, high doses of APAP have been shown to reduce levels of GSH in the lung tissue of animals (Micheli *et al.*, 1994). In the present study, we detected GSH in ear skin, but APAP did not decrease GSH levels (data not shown). Therefore, GSH levels do not appear to be associated with the exacerbation of pruritus.

The daily dose of 0.25% and 0.5% APAP, calculated from the estimated intake of drinking water, was 390 and 680 mg/kg, respectively (data not shown). These doses were high enough to cause hepatic injury in mice. Long-term daily administration of APAP via addition to the drinking water for 10 weeks from infancy did not increase serum AST and ALT activity, suggesting that APAP at these concentrations did not cause hepatic injury. In addition, no reduction in body weight or decrease in the levels of GSH in the liver were observed in mice

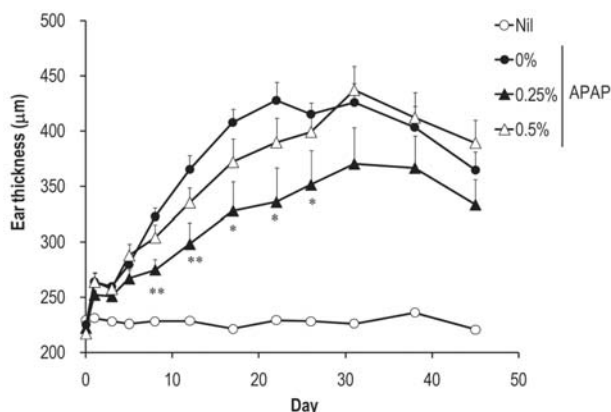


Fig. 3. Effect of APAP on increased ear thickness induced by repeated application of TNCB. APAP, dissolved in distilled water at 0.25% and 0.5% (w/v), was administered via the drinking water to mice from 3 weeks of age every day for 10 weeks (from day -24 to 49). The ears of TNCB-sensitized mice were repeatedly challenged with 0.1% TNCB three times a week from day 0 to 49. Ear thickness was measured 24 hr after each TNCB challenge. Nil, non-application of TNCB. Values represent the mean $\pm$ S.E.M. for 4-7 mice. * p < 0.05 and ** p < 0.01 vs 0% APAP (Fisher's least significant difference test).

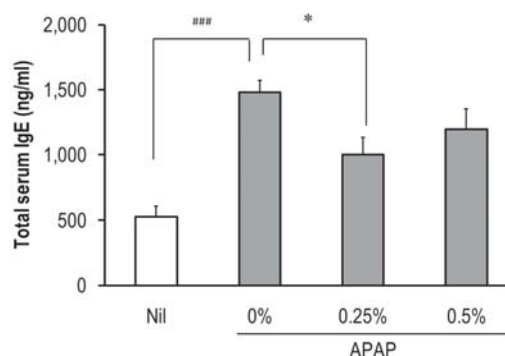


Fig. 4. Effect of APAP on total serum IgE level. Blood was collected on day 49. Nil, non-application of TNCB. Values represent the mean $\pm$ S.E.M. for 4-7 mice. *** p < 0.001 vs Nil (Student's *t* test), * p < 0.05 vs 0% APAP (Fisher's least significant difference test).

that received APAP (data not shown). We estimated the amount of ingested drinking water by measuring the volume remaining in the bottle. We did not take into account spillage, thus the intake volume might have been overestimated and the actual dose might be below hepatotoxic levels. However, it was also reported that repeated APAP exposure provides a protective effect on APAP-induced hepatic injury in mice (Shayiq *et al.*, 1999). This protective effect might, at least in part, explain our results of serum AST and ALT activities in the experimental APAP-treated animals. In addition, young children have been known to be less susceptible to APAP toxicity than adults because of lesser production of toxic metabolites and greater degree of conjugation with sulfate (Wilkes *et al.*, 2005). Oxidation of APAP by fetal liver was approximately ten times slower than by adult liver (Rollins *et al.*, 1979). Therefore, lesser susceptibility to APAP toxicity in childhood might be seen in our study.

We found that regular administration of 0.5% APAP did not increase ear swelling. Furthermore, 0.25% APAP significantly inhibited ear swelling during the early experimental period, and the increase in immunoglobulin E (IgE) levels caused by repeated application of TNCB were also decreased. Previously, we reported the inhibitory effect of APAP on antibody production in mice (Yamaura *et al.*, 2002). Therefore, decreased levels of IgE detected in animals that received 0.25% APAP might be attributed to the inhibitory effect of APAP on antibody production. It was reported that the high correlation between the serum IgE level and skin inflammation (Matsuda *et al.*, 1997; Nagai *et al.*, 1997). IgE-mediated mast cell activation leads to release of various kinds of chemical mediators, which results in infiltration of inflammatory cells into the skin lesion. The inhibitory effect of 0.25% APAP on ear thickness might be associated with the inhibition of serum levels of IgE antibodies. On the other hand, high doses of APAP may exacerbate pruritus and dermatitis via an unknown mechanism. It was reported that that prostaglandin (PG) D₂, which is produced primarily by activated skin mast cells functions as a natural antipruritic agent by suppressing histamine release from mast cells (Hashimoto *et al.*, 2005). We hypothesized that high dose of APAP might suppress the production of PGD₂ due to the inhibition of cyclooxygenase-1 and -2 (Swierkosz *et al.*, 2002). This lead to enhance the pruritus in mice and exacerbated the skin inflammation via "itch-scratch cycle" (Pfenninger and Zainea, 2001).

Therefore, no inhibitory effect on ear swelling might be seen in the presence of 0.5% APAP. However further study is needed to investigate this possibility.

In summary, we showed that long-term daily admin-

istration of APAP exacerbates pruritus in mice repeatedly treated with suboptimal concentration (1/10 of optimal concentration) of TNCB which in the absence of APAP does not evoke a significant increase in the number of scratching bouts. This study will be helpful for further research on the mechanism of increased risk for development of eczema caused by regular APAP use, and for examining safer and more effective APAP treatment regimens.

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REFERENCES

- Allmers, H. (2005): Frequent acetaminophen use and allergic diseases: is the association clear? *J. Allergy Clin. Immunol.*, **116**, 859-862.
- Beasley, R., Clayton, T., Crane, J., von Mutius, E., Lai, C.K., Montefort, S., Stewart, A. and ISAAC Phase Three Study Group (2008): Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6-7 years: analysis from Phase Three of the ISAAC programme. *Lancet*, **372**, 1039-1048.
- Barr, R.G., Wentowski, C.C., Curhan, G.C., Somers, S.C., Stampfer, M.J., Schwartz, J., Speizer, F.E. and Camargo, C.A.Jr. (2004): Prospective study of acetaminophen use and newly diagnosed asthma among women. *Am. J. Respir. Crit. Care Med.*, **169**, 836-841.
- Barragán-Mejíueiro, M.M., Morfin-Maciél, B. and Nava-Ocampo, A.A. (2006): A Mexican population-based study on exposure to paracetamol and the risk of wheezing, rhinitis, and eczema in childhood. *J. Investig. Allergol. Clin. Immunol.*, **16**, 247-252.
- Cantin, A.M., North, S.L., Hubbard, R.C. and Crystal, R.G. (1987): Normal alveolar epithelial lining fluid contains high levels of glutathione. *J. Appl. Physiol.*, **63**, 152-157.
- Davey, G., Berhane, Y., Duncan, P., Aref-Adib, G., Britton, J. and Venn, A. (2005): Use of acetaminophen and the risk of self-reported allergic symptoms and skin sensitization in Butajira, Ethiopia. *J. Allergy Clin. Immunol.*, **116**, 863-868.
- Eneli, I., Sadri, K., Camargo, C.Jr. and Barr, R.G. (2005): Acetaminophen and the risk of asthma: the epidemiologic and pathophysiologic evidence. *Chest*, **127**, 604-612.
- Hashimoto, Y., Arai, I., Tanaka, M. and Nakaike, S. (2005): Prostaglandin D₂ inhibits IgE-mediated scratching by suppressing histamine release from mast cells. *J. Pharmacol. Sci.*, **98**, 90-93.
- Inagaki, N., Igeta, K., Shiraishi, N., Kim, J.F., Nagao, M., Nakamura, N. and Nagai, H. (2003): Evaluation and characterization of mouse scratching behavior by a new apparatus, Micro-Act. *Skin. Pharmacol. Appl. Skin. Physiol.*, **16**, 165-175.
- Karimi, M., Mirzaei, M. and Ahmadi, M.H. (2006): Acetaminophen use and the symptoms of asthma, allergic rhinitis and eczema in children. *Iran J. Allergy Asthma Immunol.*, **5**, 63-67.
- Koniman, R., Chan, Y.H., Tan, T.N. and Van Bever, H.P. (2007): A matched patient-sibling study on the usage of paracetamol and

- the subsequent development of allergy and asthma. *Pediatr. Allergy Immunol.*, **18**, 128-134.
- Mahtani, R., Parekh, N., Mangat, I. and Bhalerao, S. (2005): Alleviating the itch-scratch cycle in atopic dermatitis. *Psychosomatics*, **46**, 373-374.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W. and Ra, C. (1997): Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int. Immunol.*, **9**, 461-466.
- McKeever, T.M., Lewis, S.A., Smit, H.A., Burney, P., Britton, J.R. and Cassano, P.A. (2005): The association of acetaminophen, aspirin, and ibuprofen with respiratory disease and lung function. *Am. J. Respir. Crit. Care Med.*, **171**, 966-971.
- Metz, M. and Ständer, S. (2010): Chronic pruritus--pathogenesis, clinical aspects and treatment. *J. Eur. Acad. Dermatol. Venereol.*, **24**, 1249-1260.
- Micheli, L., Cerretani, D., Fiaschi, A.I., Giorgi, G., Romeo, M.R. and Runci, F.M. (1994): Effect of acetaminophen on glutathione levels in rat testis and lung. *Environ. Health Perspect.*, **102**, Suppl. 9, 63-64.
- Nagai, H., Matsuo, A., Hiyama, H., Inagaki, N. and Kawada, K. (1997): Immunoglobulin E production in mice by means of contact sensitization with a simple chemical, hapten. *J. Allergy Clin. Immunol.*, **100**, S39-S44.
- Newson, R.B., Shaheen, S.O., Chinn, S. and Burney, P.G. (2000): Paracetamol sales and atopic disease in children and adults: an ecological analysis. *Eur. Respir. J.*, **16**, 817-823.
- Nuttall, S.L., Williams, J. and Kendall, M.J. (2003): Does paracetamol cause asthma? *J. Clin. Pharm. Ther.*, **28**, 251-257.
- Pfenninger, J.L. and Zainea, G.G. (2001): Common anorectal conditions: Part I. Symptoms and complaints. *Am. Fam. Physician*, **63**, 2391-2398.
- Rollins, D.E., von Bahr, C., Glaumann, H., Moldéus, P. and Rane, A. (1979): Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science*, **205**, 1414-1416.
- Shaheen, S.O., Newson, R.B., Henderson, A.J., Headley, J.E., Stratton, F.D., Jones, R.W., Strachan, D.P. and ALSPAC Study Team (2005): Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood. *Clin. Exp. Allergy*, **35**, 18-25.
- Shayiq, R.M., Roberts, D.W., Rothstein, K., Snawder, J.E., Benson, W., Ma, X. and Black, M. (1999): Repeat exposure to incremental doses of acetaminophen provides protection against acetaminophen-induced lethality in mice: an explanation for high acetaminophen dosage in humans without hepatic injury. *Hepatology*, **29**, 451-463.
- Swierkosz, T.A., Jordan, L., McBride, M., McGough, K., Devlin, J. and Botting, R.M. (2002): Actions of paracetamol on cyclooxygenases in tissue and cell homogenates of mouse and rabbit. *Med. Sci. Monit.*, **8**, 496-503.
- Yamaura, K., Ogawa, K., Yonekawa, T., Nakamura, T., Yano, S., and Ueno, K. (2002): Inhibition of the antibody production by acetaminophen independent of liver injury in mice. *Biol. Pharm. Bull.*, **25**, 201-205.
- Yamaura, K., Doi, R., Suwa, E. and Ueno, K. (2011): A novel animal model of pruritus induced by successive application of glucocorticoid to mouse skin. *J. Toxicol. Sci.*, **36**, 395-401.
- Yamaura, K., Maki, S. and Ueno, K. (2011): Anthocyanins from bilberry (*Vaccinium myrtillus* L.) alleviate pruritus in a mouse model of chronic allergic contact dermatitis. *Pharmacognosy Res.*, **3**, 173-177.
- Wilkes, J.M., Clark, L.E. and Herrera, J.L. (2005): Acetaminophen overdose in pregnancy. *South. Med. J.*, **98**, 1118-1122.
- Wong, G.W., Leung, T.F., Ma, Y., Liu, E.K., Yung, E. and Lai, C.K. (2007): Symptoms of asthma and atopic disorders in preschool children: prevalence and risk factors. *Clin. Exp. Allergy*, **37**, 174-179.