The LEC rat: a useful model for studying liver carcinogenesis related to oxidative stress and inflammation

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Short refereed paper

The LEC rat: a useful model for studying liver carcinogenesis related to oxidative stress and inflammation

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Growing evidence indicates oxidative stress as a mechanism of several diseases including cancer. Oxidative stress can be defined as the imbalance between cellular oxidant species production and antioxidant capability shifted towards the former. Lipid peroxidation is one of the processes that takes place during oxidative stress. Lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), are closely related to carcinogenesis as they are potent mutagens and they have been suggested as modulators of signal pathways related to proliferation and apoptosis, two processes implicated in cancer development. Mechanisms by which oxidative stress leads to tumor formation are still under investigation. The need of suitable *in vivo* models that could reflect that inflammation-related human carcinogenesis is evident. In this regard, the mutant strain Long Evans Cinnamon-like (LEC) rat provides a promising model for investigation of the relationship between hepatitis induced by oxidative stress and hepatocarcinogenesis because it has been demonstrated to develop spontaneous liver tumor formation related to copper accumulation and oxidative stress. In this review, the findings regarding oxidative stress and its relation with liver pathologies in LEC rats are discussed; we focus on the mechanisms proposed for HNE carcinogenesis.

Keywords: LEC rat, oxidative stress, hepatocarcinogenesis, HNE

INTRODUCTION

Oxidative stress can be defined as the imbalance between reactive oxygen species (ROS) production and the cellular antioxidant capacity shifted towards the former. ROS can interact with biomolecules such as DNA, RNA, proteins and lipids, leading to their oxidation and, as a consequence, to cellular damage, genomic instability, apoptosis and cell cycle alterations.[1] There is strong evidence for the involvement of oxidative stress in carcinogenesis. In the case of liver, it is evident that oxidative stress plays a critical role during tumor development related to chronic inflammation mechanisms. Oxidative and nitrative DNA damage are induced in humans and animals under inflammatory conditions. In patients with hepatitis C, 8-nitroguanine has been found in large amounts,[2] and there is a high incidence of 8-hydroxyguanine formation in peripheral leukocytes in human populations highly susceptible to hepatocarcinogenesis suggesting a relation between oxidative stress and hepatocellular carcinoma (HCC).[3]

Carcinogenesis is a multistep process with complex biological mechanisms that are not yet well defined. In humans, the major HCC etiologies involve chronic viral hepatitis, alcohol and metabolic disorders that lead to increased cellular turnover induced by chronic liver injury, regeneration and fibrosis.[4] Hepatic oxidative stress is present throughout these disorders. Chronic hepatitis C leads to mitochondrial dysfunction, ROS production, lipid peroxidation and oxidatively modified proteins; alcohol consumption increases hepatic oxidative stress that is associated with fibrosis and HCC progression.[5] In spite of the strong evidence of oxidative stress in human hepatocarcinogenesis, the mechanism of hepatocyte transformation is not yet fully understood and suitable models for studying the mechanisms of inflammation-

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related hepatocarcinogenesis are necessary. In this regard, the Long Evans Cinnamon-like (LEC) rat model is of interest since it generates spontaneous acute hepatitis, fibrosis and liver tumors as a consequence of an abnormal liver copper (Cu) accumulation and subsequent oxidative stress.[6] This rat strain shows chronic liver damage, hepatocyte death and regeneration, at the promotion stage of carcinogenesis. Such a natural history of HCC development in LEC rats is similar to that of human HCC.

In this review, current knowledge about LEC rat carcinogenesis is discussed, and the use of the LEC rat as a model in chemoprevention is highlighted.

Copper accumulation in LEC rats

The LEC rat is an in-bred strain of a mutant rat that was originally isolated from a closed colony of Long Evans rats. LEC rats are characterized by excessive Cu accumulation in the liver and impaired biliary Cu excretion.[7] Cu accumulation produces great quantities of ROS, mainly the hydroxyl radical (HO•), which is believed to be the origin of the acute hepatitis and the subsequent HCC that is observed in this rat strain.[8]

LEC rats have a deletion in the gene homologous to the Wilson's disease gene, the *ATP7B* gene.[9] The atp7b or Wilson's disease protein is a Cu-transporting P-type ATPase responsible not only for Cu loading into the trans-Golgi network but also for biliary Cu efflux.[10] Wu *et al*.[11] identified the LEC *Atp7b* gene mutation. The disease causing mutation is a deletion of 900 bp in the 3′-terminus which removes the critical ATP binding domain of the wild-type gene leading to a non-functional protein.[11] Introduction of the human *ATP7B* gene into LEC rats restores biliary Cu excretion and prevents hepatic abnormalities, showing that *Atp7b* gene mutation is solely responsible for LEC rat pathologies.[12]

Hepatocyte Cu distribution in LEC rats progresses from being initially well distributed in cytoplasm, bound to metallothionein (Cu-Mt complexes) to an accumulation of the Cu-Mt complexes in the lysosome just before acute hepatitis. The acid conditions in the lysosome result in the degradation of these complexes resulting in the formation of a partially polymerized Cu-Mt containing reactive copper.[13] This Cu initiates lysosomal lipid peroxidation, leading to hepatocyte necrosis and fulminant hepatitis.

Plasma Cu concentration in LEC rats is low because of the lack of holo-ceruloplasmin (hcp) formation in the Golgi apparatus in the liver. However, toward and after the onset of chronic hepatitis, plasma Cu concentrations increase in the form of hcp, while the liver Cu concentration is maintained at a constant level without re-occurrence of fulminant hepatitis. The mechanisms of Cu balance in chronic hepatitis are not clear. Komatsu *et al*.[14] suggested that the increased hcp during acute and chronic hepatitis was explained by the delivery of Cu to ceruloplasmin outside the Golgi apparatus in the liver; however, more investigations are needed.

Clinicopathological characteristics of LEC rats

LEC rats present elevated hepatic Cu levels, reduced biliary Cu excretion, hemolysis, ceruloplasmin deficiency and increased hepatic iron levels. This mutant strain also possesses reduced hepatic selenium that may induce a reduction in the antioxidant capacity against copper-induced free radical damage.[15]

Around 11–16 weeks old, LEC rats suffer from an acute hepatitis period, with symptoms of jaundice. Symptoms include decrease in body weight, yellowish skin on ears, tail and genital region, hematuria, oliguria, subcutaneous hemorrhage and sluggish movement. During this period, activities of serum enzymes, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltranspeptidase (GGT), as well as bilirubin levels, are increased significantly.[7] Kasai *et al*.[16] have described the clinicopathological characteristics of acute hepatitis in the LEC rat. Around 1 week from the first hepatitis signs, LEC rats suffer of a fulminant hepatitis period in which 40–50% of rats die. The remaining animals survive for more than 1 year with chronic hepatitis and develop preneoplastic and neoplastic lesions of the liver such as hepatocellular carcinoma (HCC) together with cholangiofibrosis. Most of the liver cancers are histologically classified as well-differentiated HCC. Sex differences exist in hepatitis index and liver tumor formation. Male LEC rats have higher HCC formation frequency while females develop cholangiocarcinoma more frequently. Moreover, hepatitis in female LEC rats takes place earlier than in males.[16]

The study of liver lesion incidence shows a sequential development of liver foci, nodules and HCC similar to those seen in chemical hepatocarcinogenesis models. Furthermore, the phenotype of preneoplastic and neoplastic lesions in the LEC rat is the same as that in chemical hepatocarcinogenesis – increased levels of the positive tumor markers glutathione-S-transferase type π (GSTP), γ-glutamyltranspeptidase (GGT) and α-fetoprotein (AFP) and decreased levels of the negative markers glucose-6-phosphatase (G6Pase) and adenosine triphosphatase (ATPase). These results indicate that, in both models, spontaneous and chemically-induced cancer may result from similar preneoplastic processes.[17]

Mechanism of hepatocarcinogenesis in LEC rats

Hepatocarcinogenesis in LEC rats is related to the liver Cu and iron accumulation, since in rats fed a low Cu and

iron diet or treated with copper-chelating agents, the preneoplastic lesions almost disappear.[18,19] Copper-associated liver injury is regarded as resulting ultimately from oxidative stress. The majority of the hydroxyl radical (HO•) *in vivo* comes from the metal catalyzed breakdown of hydrogen peroxide (H_2O_2), according to the Fenton reaction:

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + HO^{\bullet} + HO^{-} \qquad \text{Eq. 1}$$

Yamamoto *et al*.[9] have quantified the HO• production in plasma and liver by trapping HO• with salicylic acid. They found an increased HO• production in rats suffering from hepatitis as compared with Wistar rats and LEC rats showing no signs of hepatitis. When they treated the LEC rats with the HO• scavenger D-mannitol, they observed reduced ALT and bilirubin concentrations together with a reduction in mitochondrial lipid peroxidation. That re-inforces the idea that oxidative stress mediated lipid peroxidation is important in the pathogenesis of Cu-induced hepatotoxicity.

Lipid peroxidation refers to oxidative degradation of lipids. It most often affects polyunsaturated fatty acids (PUFAs) because they contain multiple double bonds interrupted by methylene groups that have especially reactive hydrogen atoms.[20] The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE).[21] Both MDA and HNE have the ability to interact with biomolecules such as proteins and nucleic acids. MDA is mutagenic in bacterial and mammalian cells, and it reacts with DNA to form the premutagenic pyrimido[1,2-a]purin-10(3H)-one (M1G) adduct.[22] M1G adduct is mutagenic in *Escherichia coli*, inducing transversions to T and transitions to A.[23] Similarly, HNE is genotoxic for hepatocytes and cerebral endothelial cells. Treatment of hepatocytes with HNE leads to a spectrum of DNA alterations from sister chromatid exchanges to microsomal aberrations.[24] The genotoxicity and mutagenicity of HNE and MDA may be implicated in hepatocarcinogenesis initiation in LEC rats. Increased mutagenic exocyclic DNA adducts were observed in the liver of LEC rats. These adducts come from the addition to DNA of lipid peroxidation products.[25] Levels of etheno-DNA adducts, 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine, increase with age reaching a peak at 8 weeks and 12 weeks in nuclear and mitochondrial DNA, respectively.[26] The mechanisms by which oxidized DNA bases are repaired include the base excision repair (BER) pathway. M1G, etheno-DNA adducts, 8-hydroxyguanine and other oxidized base lesions are removed by this pathway.[1] Acute hepatitis in LEC rats hinders the repair of oxidative DNA-base damage by altering the expression of DNA glycosylases, endonuclease II and 8-oxoguanine DNA-glycosylase which initiate the BER pathway.[27] Interestingly, Feng *et al*.[28] have demonstrated that HNE can inhibit BER of DNA damage induced by benzo[*a*]pyrene diol epoxide as well as damage induced by UV light radiation in human cells. These findings suggest that during LEC rat hepatitis, HNE production may contribute to mutagenesis and carcinogenesis by both direct adduction to DNA and diminishing the DNA repair pathways.

Carcinogenesis is a process in which the balance between apoptosis and cell proliferation is altered. In normal tissues, cell proliferation and apoptosis are strictly regulated by complex mechanisms that include cell-cycle regulation by p53 and cyclins. During hepatitis, p53 expression and hepatocyte apoptosis are higher in LEC rats than in age-matched control rats.[29] Even with the increment of p53 expression, Ba *et al*.,[30] using a yeast-based functional assay, demonstrated the presence of p53 mRNA mutations in LEC rat liver. The authors suggested that during hepatitis the cellular damage degrades transcriptional fidelity and that mutations in p53 may have an effect on p53 function and, hence, cell-cycle control.[30] During chronic hepatitis, there is a continuous cellular turnover. However, the proliferation/apoptotic index ratio indicates an imbalance in favor of cellular proliferation.[29] The analysis of G_1-phase-related cell cycle cyclins suggests that while cyclin D1 may be involved in the regeneration of hepatocytes during chronic hepatitis, cyclin-dependent kinase 4 (Cdk4) may play an important role in the development of HCC since it is significantly increased in HCC compared to precancerous and chronic hepatitis LEC rat livers.[31] Together, these results indicate that during HCC development in LEC rats there is an increase in hepatocyte proliferation rather than a diminution in apoptosis, as is the case in human HCC.

LEC rats as a model in chemoprevention

The LEC rat is accepted as good model for Wilson's disease. Several studies using different compounds have been made in order to test their efficiency in diminishing hepatic failure and HCC development. Cu-chelating agents like D-penicillamine, trientine dihydrochloride and N-benzyl-D-glucamine dithiocarbamate have good results in inhibiting hepatitis and HCC formation by preventing the Cu-dependent ROS production in LEC rats.[18,19,32] D-Penicillamine is nowadays the treatment of choice for Wilson's disease patients. D-Penicillamine not only prevents the development of hepatitis, but it can reverse the hepatitis stage by dissolving the polymerized Cu-Mt complexes and diminishing ROS production.[33] Antioxidant treatment has successfully diminished the incidence of hepatic failure in LEC rats. N-acetylcysteine,[34] proline, ascorbic acid, thioredoxin in combined administration have significantly delayed the appearance of jaundice and rat mortality.[35] Treatment of LEC rats with quercetin or curcumin has not succeeded in inhibiting liver failure; contrarily, these compounds increase liver Cu

accumulation and, hence, rat mortality.[34,36] These findings highlight the deleterious effects of flavonoids in diseases where Cu or iron accumulation is involved, and the importance of testing the different antioxidants in adequate models of inflammation and oxidative stress.

From the fact that oxidative stress in LEC rats affects mitochondrial stability and function, several compounds like L-carnitine and D,L-lipoic acid have been tested.[37,38] L-Carnitine protects mitochondria from ROS attack while D,L-lipoic acid is a good antioxidant and can chelate Cu and iron ions. Interestingly, dietary PUFAs prevent the hepatitis stage in female LEC rats by altering expression of genes involved in fatty acid oxidation, energy metabolism, and metal-related genes like transferrin and ceruloplasmin,[39] indicating a better management of Cu accumulation and lipid oxidation. Furthermore the authors suggest that PUFAs could serve as scavengers rather than proliferators of free radicals produced in the liver of LEC rats.

Conclusions

Several questions remain to be elucidated in hepatocarcinogenesis mechanisms. Suitable models that reflect human carcinogenesis are needed to understand the mechanisms that lead to cell transformation in order to search for new therapies to avoid cancer. The LEC rat is considered to be a good model in this regard, since it mimics liver tumor formation related to oxidative stress and inflammation as seen in human hepatocarcinogenesis. It is clear that lipid peroxidation is involved in hepatocyte initiation in this model, but also it could be associated with cancer promotion by altering protein function related to cell proliferation and apoptosis. LEC rats can be used also for chemoprevention studies. But, essentially, we conclude that LEC rats are an interesting model for testing oxidative-stress cellular damage and growth regulation in a complex process like carcinogenesis. In this regard, we are now evaluating gene expression and metabolic changes in LEC rats at the onset of cancer initiation.

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References

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; **160**: 1–40.
2. Kawanishi S, Hiraku Y. Oxidative and nitrative DNA damage as biomarker for carcinogenesis with special reference to inflammation. *Antioxid Redox Signal* 2006; **8**: 1047–1058.
3. Peng T, Shen HM, Liu ZM *et al*. Oxidative DNA damage in peripheral leukocytes and its association with expression and polymorphism of hOGG1: a study of adolescents in a high risk region for hepatocellular carcinoma in China. *World J Gastroenterol* 2003; **9**: 2186–2193.
4. Moradpour D, Blum HE. Pathogenesis of hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2005; **17**: 477–483.
5. Wang T, Weinman SA. Causes and consequences of mitochondrial reactive oxygen species generation in hepatitis C. *J Gastroenterol Hepatol* 2006; **21**: S34–S37.
6. Mori M, Hattori A, Sawaki M *et al*. The LEC rat: a model for human hepatitis, liver cancer, and much more. *Am J Pathol* 1994; **144**: 200–204.
7. Li Y, Togashi Y, Satoh S *et al*. Spontaneous hepatic copper accumulation in Long-Evans Cinnamon rats with hereditary hepatitis. A model of Wilson's disease. *J Clin Invest* 1991; **87**: 1858–1861.
8. Yamamoto H, Watanabe T, Mizuno H *et al*. *In vivo* evidence for accelerated generation of hydroxyl radicals in liver of Long-Evans Cinnamon (LEC) rats with acute hepatitis. *Free Radic Biol Med* 2001; **30**: 547–554.
9. Yamaguchi Y, Heiny ME, Shimizu N, Aoki T, Gitlin JD. Expression of the Wilson disease gene is deficient in the Long-Evans Cinnamon rat. *Biochem J* 1994; **301**: 1–4.
10. Takeda K, Eguchi H, Soeda S, Shirahata A, Kawamura M. Fe(II)/Cu(I)-dependent P-type ATPase activity in the liver of Long-Evans Cinnamon rats. *Life Sci* 2005; **76**: 2203–2209.
11. Wu J, Forbes JR, Chen HS, Cox DW. LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. *Nat Genet* 1994; **7**: 541–545.
12. Meng Y, Miyoshi I, Hirabayashi M *et al*. Restoration of copper metabolism and rescue of hepatic abnormalities in LEC rats, an animal model of Wilson disease, by expression of human ATP7B gene. *Biochim Biophys Acta* 2004; **1690**: 208–219.
13. Klein D, Lichtmannegger J, Heinzmann U, Müller-Höcker J, Michaelsen S, Summer KH. Association of copper to metallothionein in hepatic lysosomes of Long-Evans Cinnamon (LEC) rats during the development of hepatitis. *Eur J Clin Invest* 1998; **28**: 302–310.
14. Komatsu Yutaka, Ogra Yasumitsu, Suzuki KT. Copper balance and ceruloplasmin in chronic hepatitis in a Wilson disease animal model, LEC rats. *Arch Toxicol* 2002; **76**: 502–508.
15. Downey SJ, Bingle CD, Cottrell S *et al*. The LEC rat possesses reduced hepatic selenium, contributing to the severity of spontaneous hepatitis and sensitivity to carcinogenesis. *Biochem Biophys Res Commun* 1998; **244**: 463–467.
16. Kasai N, Osanai T, Miyosh I, Kamimura E, Yoshida MC, Dempo K. Clinico-pathological studies of LEC rat with hereditary hepatitis and hepatoma in the acute phase of hepatitis. *Lab Anim Sci* 1990; **40**: 502–505.
17. Sawaki M, Enomoto K, Takahashi H, Nakajima Y, Mori M. Phenotype of preneoplastic and neoplastic lesions during spontaneous liver carcinogenesis of LEC rats. *Carcinogenesis* 1990; **11**: 1857–1861.
18. Hayashi M, Miyane K, Hirooka T *et al*. Inhibitory effects of trientine, a copper-chelating agent, on induction of DNA strand breaks in hepatic cells of Long-Evans Cinnamon rats. *Biochim Biophys Acta* 2004; **1674**: 312–318.
19. Jong-Hon K, Togashi Y, Kasai H, Hosokawa M, Takeichi N. Prevention of spontaneous hepatocellular carcinoma in Long-Evans Cinnamon rats with hereditary hepatitis by the administration of D-penicillamine. *Hepatology* 1993; **18**: 614–620.

20. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002; **30**: 620–650.
21. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; **11**: 81–128.
22. Plastaras JP, Riggins JN, Otteneder M, Marnett LJ. Reactivity and mutagenicity of endogenous DNA oxopropenylating agents: base propenals, malondialdehyde, and N-(epsilon)-oxopropenyllysine. *Chem Res Toxicol* 2000; **13**: 1235–1242.
23. Fink SP, Reddy GR, Marnett LJ. Mutagenicity in *Escherichia coli* of the major DNA adduct derived from the endogenous mutagen malondialdehyde. *Proc Natl Acad Sci USA* 1997; **94**: 8652–8657.
24. Eckl PM. Genotoxicity of HNE. *Mol Aspects Med* 2003; **24**: 161–165.
25. Nair J, Sone H, Nagao M, Barbin A, Barsch H. Copper-dependent formation of miscoding etheno-DNA adducts in liver of Long Evans Cinnamon (LEC) rats developing hereditary hepatitis and hepatocellular carcinoma. *Cancer Res* 1996; **56**: 1267–1271.
26. Nair J, Strand S, Frank N *et al*. Apoptosis and age-dependent induction of nuclear and mitochondrial etheno-DNA adducts in Long-Evans Cinnamon (LEC) rats: enhanced DNA damage by dietary curcumin upon copper accumulation. *Carcinogenesis* 2005; **26**: 1307–1315.
27. Choudhury S, Zhang R, Frenkel K, Kawamori T, Chung FL, Roy R. Evidence of alterations in base excision repair of oxidative DNA damage during spontaneous hepatocarcinogenesis in Long Evans Cinnamon rats. *Cancer Res* 2003; **63**: 7704–7707.
28. Feng Z, Hu W, Tang MS. Trans-4-hydroxy-2-nonenal inhibits nucleotide excision repair in human cells: a possible mechanism for lipid peroxidation-induced carcinogenesis. *Proc Natl Acad Sci USA* 2004; **101**: 8598–8602.
29. Jia G, Tohyama C, Sone H. DNA damage triggers imbalance of proliferation and apoptosis during development of preneoplastic foci in the liver of Long-Evans Cinnamon rats. *Int J Oncol* 2002; **21**: 755–761.
30. Ba Y, Tonoki H, Tada M, Nakata D, Hamada J, Moriuchi T. Transcriptional slippage of p53 gene enhanced by cellular damage in rat liver: monitoring the slippage by a yeast functional assay. *Mutat Res* 2000; **447**: 209–220.
31. Kita Y, Masaki T, Funakoshi F *et al*. Expression of G1 phase-related cell cycle molecules in naturally developing hepatocellular carcinoma of Long-Evans Cinnamon rats. *Int J Oncol* 2004; **24**: 1205–1211.
32. Shimada H, Takahashi M, Shimada A *et al*. Protection from spontaneous hepatocellular damage by N-benzyl-D-glucamine dithiocarbamate in Long-Evans Cinnamon rats, an animal model of Wilson's disease. *Toxicol Appl Pharmacol* 2005; **202**: 59–67.
33. Klein D, Lichtmannegger J, Heinzmann U, Summer KH. Dissolution of copper-rich granules in hepatic lysosomes by D-penicillamine prevents the development of fulminant hepatitis in Long-Evans Cinnamon rats. *J Hepatol* 2000; **32**: 193–201.
34. Kitamura Y, Nishikawa A, Nakamura H *et al*. Effects of N-acetylcysteine, quercetin, and phytic acid on spontaneous hepatic and renal lesions in LEC rats. *Toxicol Pathol* 2005; **33**: 584–592.
35. Hawkins RL, Mori M, Inoue M, Torii K. Proline, ascorbic acid, or thioredoxin affect jaundice and mortality in Long Evans Cinnamon rats. *Pharmacol Biochem Behav* 1995; **52**: 509–515.
36. Frank N, Knauft J, Amelung F, Nair J, Wesch H, Bartsch H. No prevention of liver and kidney tumors in Long-Evans Cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. *Mutat Res* 2003; **523/524**: 127–135.
37. Chang B, Nishikawa M, Nishiguchi S, Inoue M. L-Carnitine inhibits hepatocarcinogenesis via protection of mitochondria. *Int J Cancer* 2005; **113**: 719–729.
38. Yamamoto H, Watanabe T, Mizuno H *et al*. The antioxidant effect of DL-alpha-lipoic acid on copper-induced acute hepatitis in Long-Evans Cinnamon (LEC) rats. *Free Radic Res* 2001; **34**: 69–80.
39. Du C, Fujii Y, Ito M *et al*. Dietary polyunsaturated fatty acids suppress acute hepatitis, alter gene expression and prolong survival of female Long-Evans Cinnamon rats, a model of Wilson disease. *J Nutr Biochem* 2004; **15**: 273–280.