LIVER TUMOR PROMOTING ACTIVITY OF BUTYLATED HYDROXYTOLUENE IN C-MYC TRANSGENIC MOUSE MODEL OF LIVER CANCER

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ABSTRACT

Liver tumor promoting activity of butylated hydroxytoluene (BHT) is controversial. butylated hydroxytoluene (BHT) had previously reported to have a promoting and protective roles in occurrence of tumour formation but strain differences, the effect upon various carcinogens, paradoxical dose responses and mechanisms of action remain major questions in the toxicology of BHT. Here, we investigate the promoter activity of BHT in c-myc transgenic mouse model of liver cancer. It was found that BHT increase liver to body weight ratio especially at age of 4 month immediately after treatment when compared with non- transgenic control. Moreover increase the mitotic figures and proliferation of hepatocyte either clarified in histopathology or immunohistochemistrly labeling index which consider evidence of promoting activity of BHT at early stage. Additionally, cytochrome p450 members played an important role in metabolism of BHT especially at early stage of 4 month agree with the increase of gene expression of these members while its expression decreased at 8.5 month. Notably, BHT treatment increase gene expression of cellular metabolism especially protein metabolism and drug detoxification like Cyp1a2, Cyp7b1 and Cyp2c40 was down regulation at age 8.5 month. While Cyp4a14, Cyp1a2 and Cyp4a29 were increased at 4 month immediately after treatment, Moreover ascorbate and aldarate metabolism was unchanged in BHT treatment.

INTRODUCTION

BHT is one of the most commonly used antioxidants in foods containing fats and oils and in food packaging and other food contact applications, cosmetics, drugs, and animal feeds to prevent oxygen-induced lipid peroxidation. **(FDA, 1973 and NIH, 1979, U.S. International Trade Commission, 1977)**. Because of the lack of reported toxic effects to people since its wide use in 1954, BHT was given a GRAS (Generally Recognized as Safe) status by the FDA at a level not to exceed 0.5 mg/kg body wt/day or 0.02 ppm in foods. According to this regulation, companies adding BHT to foods and drugs would be compelled to perform further studies on certain toxic effects of BHT **(FASEB, 1977)**.

The co-mutagenic and co-carcinogenic properties of BHT have been demonstrated in

tests ranging from the Ames test to cell transformation procedures to in vivo assays. These effects are probably mediated by metabolites of BHT, rather than by BHT itself **(Malkinson, 1983)**. While there were a few studies on the effects of BHT to humans, with most of these studies just identifying the metabolic products of BHT **(Babich, 1982)**.

Liver tumor promoting activity of butylated hydroxytoluene (BHT) is controversial. Witschi, (1981, 1986) also suggested that butylated hydroxytoluene (BHT) had both promoting and protective roles in occurrence of tumour formation but strain differences, the effect upon various carcinogens, paradoxical dose responses and mechanisms of action remain major questions in the toxicology of BHT.

More over, **Shirai et al. (1982)** investigated the carcinogenicity of butylated hydroxytoluene on long-term administration to B6C3F1 mice. Females mice given 1000 or 5000 ppm BHT and males given 5000 ppm showed reduced weight gain. No significant changes attributable to BHT treatment. Tumours were found in many organs; especially the lungs, liver, lymph nodes and spleen, in both the experimental and control groups, but none were related to BHT treatment. Inconsistent, butylated hydroxytoluene (BHT) found to be failed to induce biologically significant increases in cellular proliferation in the liver, urinary bladder and thyroid gland on feeding to young adult Wistar rats. Nevertheless, it has been reported to enhance the yield of liver tumors when fed to rats or mice that developed an appreciable background incidence of these tumors without treatment **(Lok et al., 1995). In contrast, Williams et al. (1991)** found that BHT at different doses promoted liver cancer at subsequent time points of 24, 36, and 48 weeks. the number of foci progressively increased, and at the end of the study, the incidence of liver neoplasms was 100% in mice treated with hydroxytoluene after exposure to 2-acetylaminofluorene (AAF). Additionally, Decade studies suggest that BHA, and perhaps BHT, were carcinogenic to rodents. Notably, the neoplastic effects observed at very high dietary levels of BHA and BHT occur only after effective biological defense mechanisms were overloaded **(Iverson, 1995)**.

Sun et al. (1996) studied that the induction of isozymes of drug-metabolizing enzymes by butylated hydroxytoluene (BHT) in the male ddY mouse. In mice given 0.05 and 0.15% BHT in the diet for 14 days cytochrome P-450 contents and the activities of uridine diphosphate-glucuronyl transferase (UDP-GT) and pentoxyresorufin O-dealkylase were markedly increased, while in those fed 0.15% BHT testosterone 6α, 16α and 16β hydroxylases were greatly increased, which indicated induction of cytochrome P-4.50 isozymes of the CYP2B family.

Vanyushin et al. (1998) found that a single intraperitoneal injection of butylated hydroxytoluene (BHT, 60 mg/kg body mass) resulted within a few hours in a strong increase in nuclear DNA (cytosine-5)-methyl transferase (methyl transferase) activity in the liver, kidneys, heart, spleen, brain and lungs of male rats.

In a dose-response study, the bromodeoxyuridine-labeling index (LI)LI was increased dramatically at first, then effects gradually

diminished with further exposure, and finally six BHT treatments failed to induce cell proliferation. In a two-stage model using urethane as the initiator, although up to five consecutive doses of BHT were able to exert continued enhancing effects in terms of adenoma yield, no increment was evident with further treatments. The data overall indicate that a rasH2/BHT model with five weekly administrations of BHT at a dose of 400 mg/kg is most efficacious **(Umemura et al. 2002)**.

Shibutani et al. (2002) investigated genes that were steadily up-regulated during the promotion stage in carcinogenesis. Rats received a single injection of diethylnitrosamine (DEN; 200 mg/kg body wt, i.p.), and then after 2 weeks rats were administered 600 p.p.m. of phenobarbital (PB) in the drinking water for up to 64 weeks. Also, animals were fed 1 p.p.m. ethinylestradiol (EE) or 3000 p.p.m. butylated hydroxytoluene (BHT) in the diet at promotion stage. Rats were exposed to partial hepatectomy (PH) at week 3. Both PB and EE treatments showed up-regulation of 16 genes in the livers at week 8 of promotion with the levels being three times or more than the basal expression of unpromoted liver. The majority of these genes were also up-regulated at week 8 by BHT treatment. Additionally, **Stierum et al. (2008)** reported that (BHT) induced changes in the expression of 10 genes, including phase I (CYP2B1/2; CYP3A9; CYP2C6) and phase II metabolism (GST 12). The CYP2B1/2 and GST expression findings were confirmed by real time RT-PCR, Western blotting, and enzyme activity in male Sprague-Dawley rats, 7-weeks old, fed a diet containing BHT for 28 days.

The rational of this study to investigation the tumor promting activity of butylated hydroxytoluene in a c-myc transgenic mouse model of liver cancer

MATERIAL AND METHODS

1. Laboratory animals, transgenicity and treatment :

The att-myc transgenic line was described earlier by **Dalemans et al. (1990)**. Transgenic mice strain was C57/Blex 6 background. PCR was carried out with hot star Tag DNA polymerase (Qiagen). Annealing temperature and the number of cycles are indicated in brackets after each primer pair. The transgene was verified by PCR of DNA extracted from tail biopsies **(Hogan et al., 1994)** and the following forward primer (fp) and reverse primer (rp) pair was used for a transgene-specific amplification: forward primer: 50- TCCTGTACCTCGT-CCGATTC-30; reverse primer : 50GTTGTGCTGGTGAG-TGGAGA-30 (60C, 31 cycles). 24 transgenic mice of both sex at 2 month age treated with BHT at dose of 300 mg/ kg once per week for 8 weeks while another 24 transgenic mice or non transgenic treated by corn oil as a vehicle control **(Witschi et al., 1981, 1986)**. At four different time points - i.e. at the age of 4, 5.5, 7 and 8.5 months - in vivo μCT and μPET imaging was employed; animals were sacrificed afterwards for histopathology.

2. In vivo imaging of transgenic animals by μCT and μPET

All imaging procedures were performed under inhalation anesthesia with isoflurane (Isoba vet., Essex Pharma, Germany) at a concentration of 4% for induction of anesthesia and

1-2% for maintenance. Mice were placed on a temperature controlled bed at 39°C (T/Pump, Gaymar, Orchard Park, NY, USA) and isoflurane was supplied via a nose cone. The animal respiration was spontaneous (Summit Anesthesia Solutions, Bend, OR, USA) and the breathing was monitored continuously using a small pressure transducer (Biovet, m2m imaging, Newark, NJ, USA). Breathing was maintained at a rate between 60 and 100 per minute. After image data acquisition the recovery time of the animlas from anaesthesia was usually less than five minutes. Overall the procedures were well tolerated.

Specifically, sequential μCT and ^{18}F-FDG μPET imaging was carried out with a total of 24 animals. Mice were imaged at the age of 4 (1st sacrifice), 5.5 (2nd sacrifice), 7 (3rd sacrifice) and 8.5 months (4th sacrifice). The transgenic control animals were examined at the age of 8.5 months only (4th sacrifice), although some explorative contrast-enhanced imaging was carried out at the age of 2, 5 and 7 months. All animals were sacrificed 2 days after the PET imaging. At this time the radioactive tracer declined to below the level of detection.

2.1 Contrast enhanced μCT imaging :

Three hours prior to CT scans anaesthetized mice were given an intravenous injection of a liver-specific iodinated contrast agent (DHOG, Fenestra LC, ART Inc., Saint-Laurent, Canada) at an approximately volume of 200-300 μl (10 μl/g bodyweight) into the tail vein. The image data acquisition was as recommended by the manufacturer and previously published protocols **(Von et al., 2009 and Weber et al., 2004)**.

The μCT scan was done with a high-resolution small animal computed tomography scanner (eXplore Locus, GE Healthcare, Chalfont St. Giles, UK). The scan parameters were set as follow: tube voltage 80 kVp, tube current 450 μA, number of acquisition 360, number of views 720, exposure time 100 ms, one average per frame, axial field-of-view 33 mm. Scans were recorded without respiratory gating. Total scan duration was about 12 minutes.

Image data was reconstructed using a cone-beam algorithm on an 8-node linux cluster. The resulting voxel size of the isotropic dataset was 45 μm. Arbitrary attenuation values were converted to the Hounsfield scale using a calibration phantom with water, air and bone inserts.

2.3 μPET imaging :

All animals were fasted prior to imaging for about 6 hours. Anaesthetized mice were given an intraperitoneal injection of 10 MBq (^{18}F)-2-fluoro-2-deoxyglucose (^{18}F-FDG, Department of Nuclear Medicine, Hannover Medical School, Germany) in a total volume of 50-100 μl sterile isotonic saline solution. PET imaging was in the same position as for CT scans and done on the same day. Static images were acquired 45 minutes after injection of the tracer using a high-resolution small animal PET camera (eXplore Vista, GE Healthcare, Chalfont St. Giles, UK). Total acquisition time was 30 minutes for a single bed position. Images were corrected for random events and scatter prior to reconstruction with a 3D-FORE/2D-OSEM iterative algorithm. No attenuation correction was used.

2.4 Image analysis :

μCT datasets were visualized and analyzed using the software packages Microview 2.2 (GE Healthcare, Chalfont St. Diles, UK), MeVisLab 2.0 (MeVis Medical Solutions AG, Bremen, Germany) and OsiriX (v.3.7.1 32-bit, Pixmeo Sarl). Total liver volume was calculated using the LiveWireMacro module (MeVisLab 2.0) which utilizes a contour-based semiautomatic segmentation method. Focal liver lesions were counted and quantified by 2D-measurement of the largest diameter. The diameter (d) of the lesions was used to estimated the tumor volume by the following formula: tumor volume (V) = $1/6 \times n \times d^3$. The volumes of all liver lesions were added to determine the total tumor volume. The tumor percentage of the liver was calculated as the ratio of the total tumor volume (ml) and the total liver volume (ml).

Rigid registration of PET and CT datasets was based on anatomical landmarks and used to generate fused datasets. Regions-of-interest (ROI) were manually defined for focal liver lesions of a diameter above 5 mm as detected in μCT and ^{18}F-FDG μPET imaging. The background (non-tumor) signal was determined by placing a ROI in the tumor-free liver parenchyma and the maximum count per volume was determined for each ROI to estimate the tumor-to-non-tumor ratios.

3. Sample collection and preparation

6 Mice were anaesthetized by an overdose of CO2 and sacrificed at the age of 4, 5.5, 7 and 8.5 months. The thorax was opened by standard surgical procedures and the liver was explanted and rinsed with PBS. All organs weight was recorded. The tumors were inspected macroscopically and separated from the liver. Upon anatomical preparation liver tissue was in preserved in buffered formalin 4% or frozen immediately in liquid nitrogen.

4. Histology

Tissue of transgenic treated animals, as well as liver from control animals, were fixed in 4% formaldehyde in PBS and embedded in paraffin by standard operating procedures. Paraffin blocks were sectioned into 3-5 mm thick slices and stained with haematoxylin and eosin (H and E).

5. Array hybridization and scanning

Total RNA was isolated from frozen liver tissues of transgenic mice at age of 4 and 8.5 months by using QIAGEN's RNeasy, total RNA isolation procedure. A second cleanup of isolated RNA was performed using the same RNA isolation kit. In all, 10 μg of total RNA was used for the synthesis of double-stranded cDNA with Superscript II RT and other reagents from Invitrogen Life Technologies. HPLC-purified T7-(dT)24 (GenSet SA) was used as a primer. After cleanup, double-stranded cDNA was used for the synthesis of biotin-labelled cRNA (Enzos BioArray High Yield RNA Transcript Labeling Kit, Affymetrix). cRNA purified with RNeasy spin columns from Qiagen was cleaved into fragments of 35–200 bases by metal induced hydrolysis. A measure of 10 μg of biotinylated fragmented cRNA washybridized onto the Murine Genome U74Av2 Array (MG-U74Av2). The array consists of 12 488 probe sets that represent RefSeq annotated sequences (B6000) in the Mouse UniGene database, as well as B6000 EST clones. The hybridized, washed and

coloured arrays were scanned using the Agilent Gene Arrays Scanner. Scanned image files were visually inspected for artifacts and then analysed, each image being scaled to an all probe set intensity of 150 for comparison between chips. The Affymetrixs Microarray Suite (version 5.0) was used to control the fluidics station and the scanner, to capture probe array data and to analyse hybridization intensity data. Default parameters provided in the Affymetrix data analysis software package were applied in running of analyses array track and student test. The array track and student test were used for data analysis for gene expression comparison between liver tumour of att-myc transgenic mice and normal liver of non- transgenic mice.

6. Immunohistochmeistry

Fixed liver tissue was embedded in paraffin and then sectioned at 4-5 mm. The sections were deparaffinized, rehydrated, and heated (using a microwave oven) to boiling 0.01 M citrate buffer (pH 6.0). Upon boiling, the sections were subsequently heated (low heat setting) for an additional 15 min. The sections were then blocked in 1.5% normal serum for 10 min at room temperature. The following antisera (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) ; anti- PCNA and anti - Brdu antisera were used. The liver sections were incubated with antisera overnight at room temperature using the following dilutions for 1:200. Immunoreactivity (as identified by brown color) to the respective protein was then visualized using the DAKO Staining System following the manufacturer's protocol. The negative control sections for were incubated with normal rabbit or goat IgG instead of primary antibody.. Sections were counter-stained with Mayer's hematoxylin. The sections were then photographed using an axiovision light microscope and Nikon DXM1200F digital camera.

7. Statistical analysis

The in vivo imaged liver volume and the ex vivo measured liver weight was recorded and the liver volume and the tumor volume were computed and defined as the tumor percentage. The statistical analysis of individual gene expression data are based on the Student's t-test.

RESULTS

The gene construct for the production of AAT-c-Myc mice and PCR analysis of c-myc from tail biopsies to identify transgenic mice were shown in figure 1a, b. By use of the alpha1-antitrypsin promoter targeted expression of c-Myc to the liver was achieved as originally described by Dalemans et al., 1990. C-myc was expressed in transgenic mice treated by BHT by 7 folds at age of 4 month while at 8.5 month was 12 folds in comparison to non-transgenic mice.

Liver lesions were detected by MicroCT fig (2a, b) with a lesion size varying between 0.9 mm and 11 mm. After intravenous injection of the liver-specific iodinated contrast agent DHOG the lesions were well defined and presented hypodens as compared to the normal liver parenchyma. Lesions of a size of > 1 mm could be identified with certainty. Importantly, and with the exception of one animal c-Myc transgenic animals treated with BHT showned very small lesions less than 5mm detected by Mico-CT. Mean of tunmor lesions was 4 mm for BHT and 3 mm for the corn oil

transgenic control, respectively. There is no change in liver volume (fig 2b) detected by MicroCT as there is no detected HCC that responsible of liver volume increament. Additionally, lungs were normal although BHT previously reported to increase lung tumor.

Furthermore, the glucose metabolism in liver lesions was determined by in vivo ^{18}F-FDG μPET imaging. To allow for accurate anatomical localization of focal ^{18}F-FDG uptake, registration and fusion of μCT and μPET datasets was done prior to quantitative analysis of PET data. The ^{18}F-FDG-uptake of BHT treated mice was mainly homogenously and similar to normal parenchymas of liver of control one.

Liver weight and liver weight to body weight ratio (fig 2b) was increased in transgenic mice treated by BHT especially at 4 month, immediately after treatment. While at successfully 5.5 to 8.5 month, there was no difference in liver weight or liver weight to body weight ratio.

The promoting activity of BHT in c-myc transgenic mice was examined histopathologically, by an increase in mitotic figures (2n bearing cells) and hyperplasia of hepatocyte (fig 4,5 and 6). Similar results recorded by **Clapp et al., (1973)** who found hyperplasia of hepatic bile ducts in mice following long-term administration of butylated hydroxytoluene. Moreover, there was a big macrocytic dysplastic nodules detected in liver of treated transgenic mice at age 8.5 month resemble hepatocellualar adenoma. While transgenic animal treated only with corn oil show only dysphasia that previously reported at this age in without any evidence of tumor growth. There was no record for hepatocellular cellular carcinoma in transgenic mice treated by BHT or its control.

Additionally, increased the proliferation of hepatocyte was also recorded by increase labeling index either by proliferating cellular antigen (PCNA) or bromodeoxyuridine-labeling (Brdu) (fig 7).

The most significant gene expression of BHT treated transgenic mice (fig 8) at 4 month was 28 while at 8.5 was 42 compared to non-transgenic mice. The most commen gene function pathway (KEGG) was 81 for BHT while the significant KEGG was 17 pathways at 8.5 month compared to non-transgenic mice.

BHT didn't regulated any gene related to cell cycle, DNA replication, p53 signaling pathway, mismatch repair, retinol metabolism, pyrimidine metabolism, arachidonic acid metabolism, whereas BHT were mainly regulated genes related to cellular metabolism and drug detoxification like Cyp1a2, Cyp7b1 and Cyp2c40 was down regulation at age 8.5 month. While Cyp4a14, Cyp1a2 and Cyp4a29 were increased at 4 month immediately after treatment. Moreover ascorbate and aldarate metabolism (KEGG) was unchanged in BHT treatment.

DISCUSSION

Because of the lack of reported toxic effects to people since its wide use in 1954, BHT was given a GRAS (Generally Recognized as Safe) status by the FDA at a level not to exceed 0.5 mg/kg body wt/day or 0.02 ppm in foods. Ac-

cording to this regulation, companies adding BHT to foods and drugs would be compelled to perform further studies on certain toxic effects of BHT **(FASEB, 1977)**.

Till now, there is no detected liver tumor promoting agent induce alone liver tumor in human. While there were a few studies on the effects of BHT to humans with most of these studies just identifying the metabolic products of BHT **(Babich, 1982)**. In the present study, we investigated tumor promoter activity of BHT in c-myc transgenic mice by histopathology, in-vivo modalities and gene expression.

Liver weight and liver weight to body weight ratio was only significantly increased due to effect of BHT toxicity at first sacrifice immediately after treatment when compared to non-transgenic control mice while there was no difference at successful time point at dose under experiment.

Indeed, contrast-enhanced μCT and 18F-FDG μPET metabolic imaging are facile methods for detection of liver lesions and can be considered as robust in vivo imaging techniques to quantify lesions and to characterize tumor morphology **(Henning et al., 2008 Von; Falck et al., 2009)**. In the present study, liver and tumor volume were quantified based on semiautomatic segmentation of CT scans **(Martiniova et al., 2010)**. There is no change in liver volume detected by MicroCT as there is no detected HCC that responsible of liver volume increament.

Notably, the promoting activity of BHT is controversial as BHT can behave as tumor promoting or act against tumor. This depends on overload of defense of liver detoxification mechanisms **(Imaida et al., 1983)**. In c-myc transgenic mice was examined histopathologically, by an increase in mitotic figures (2n bearing cells) that considered as precarcinogenic and increase proliferative cellular antigen detected by immunohistochemistry especially at 4 and 5.5 month. Moreover, there was a big macrocytic dysplastic nodules detected in liver of treated transgenic mice at age 8.5 month resemble hepatocellualar adenoma and suggested for expansion in later time for HCC.

Interestingly, cytochrom p450 played important role in metabolism of BHT espcially at age of 4 moth immediatlly after treatment **(Stierum et al., 2008; Shibutani et al., 2002)**. while some members of cytochrome p450 was down regulated at 8.5 month which enable starting appearance of precarcinogenic evidance like mitotic figures, hyperplasia and large macrocytic dysplastic nodules that may be expand later to HCC..

CONCLUSIONS

In the present study BHT as a tumor promoting agent and for such non-genotoxic carcinogens had a threshold has been established. Possible BHT accelerates tumor growth in the c-Myc transgenic model at later time points and / or higher doses but histopathology defined for this group mainly dysplastic nodules even at the last time point of sacrifice, e.g. 8.5 month. Cytochrome p450 suggested playing an important role in liver tumor promoting activity of BHT.

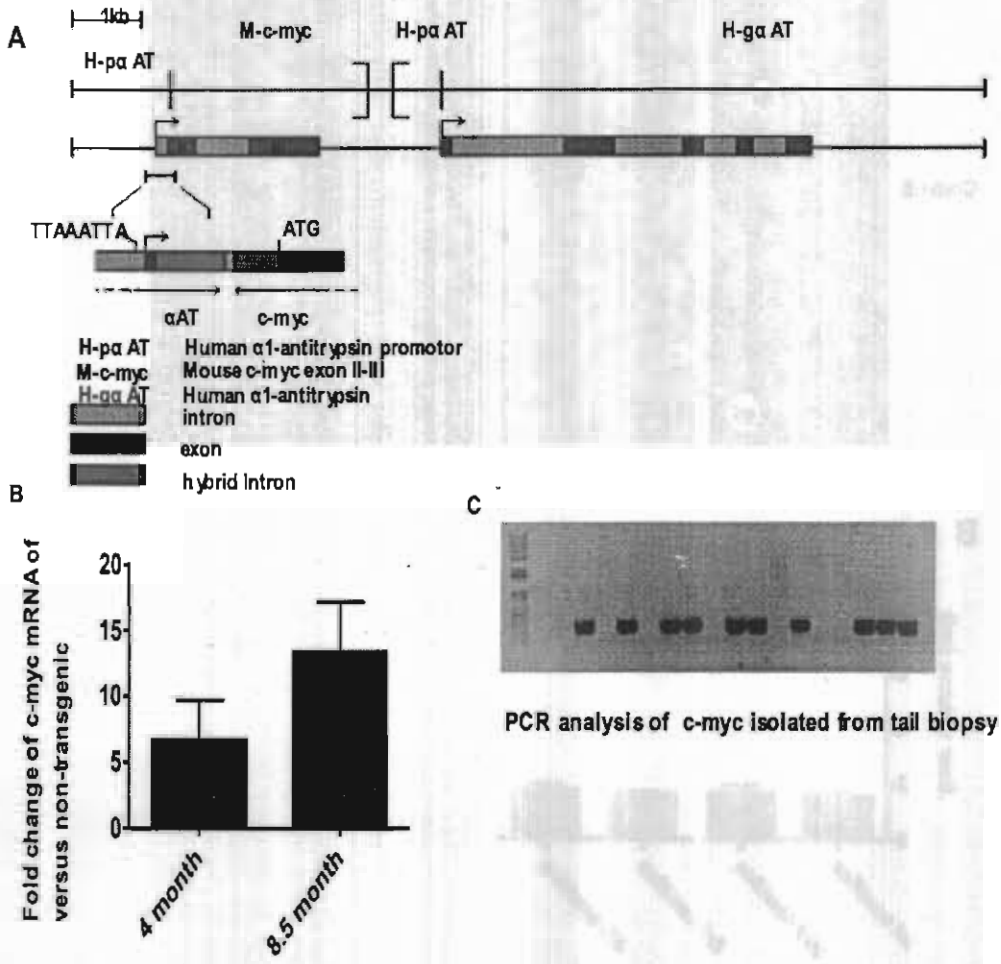

Fig (1 a, b) : show the gene construct for the production of AAT-c-Myc mice .

BHT previously reported to increase lung tumor.

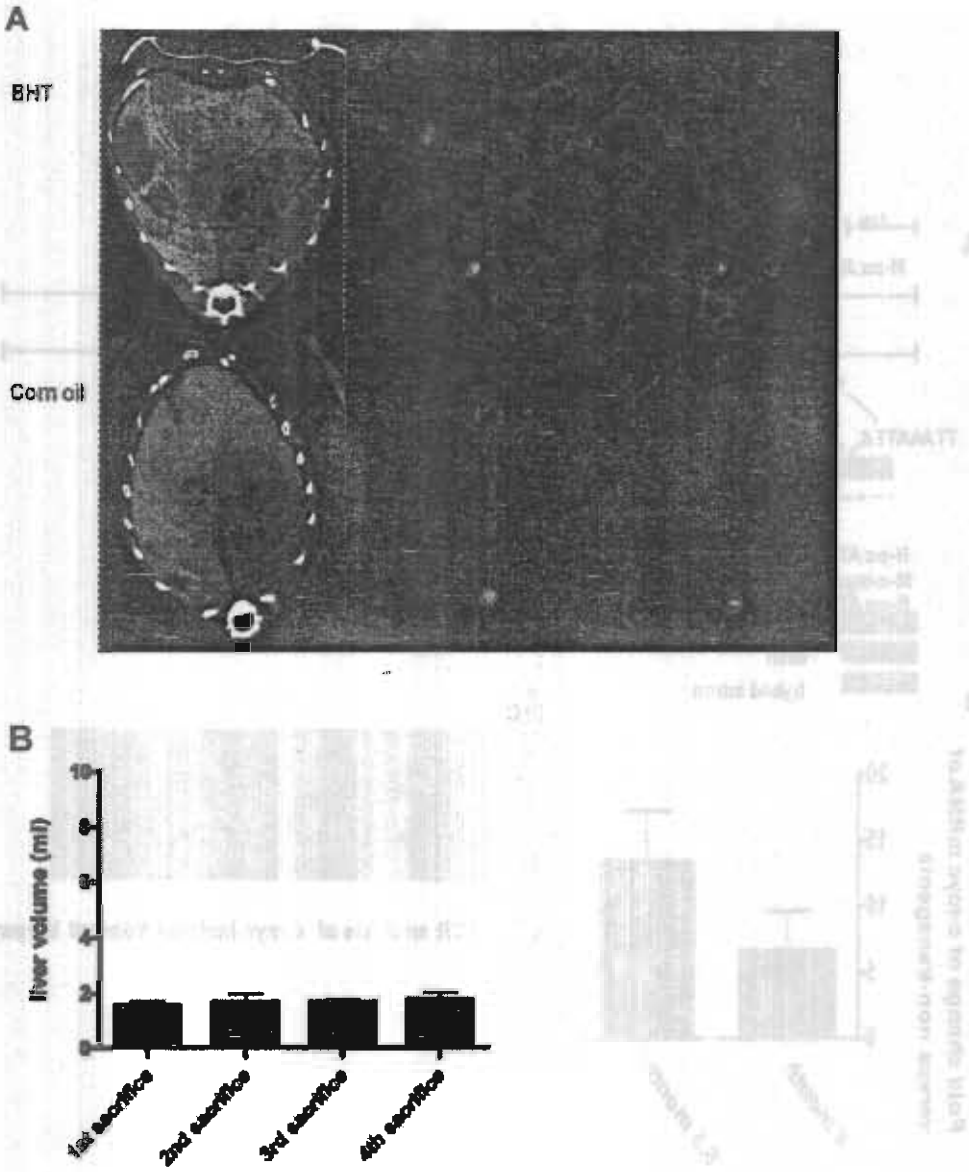

Fig (2) : show fused microCT and microPet scanning (a) of both BHT and corn oil. A transgenic treated by BHT show increase tumor load with 10 mm tumor lesions and show a very little glucose uptake detected by micro pet imaging. Note there was no change in liver volume at different time point of sacrifice (b) .

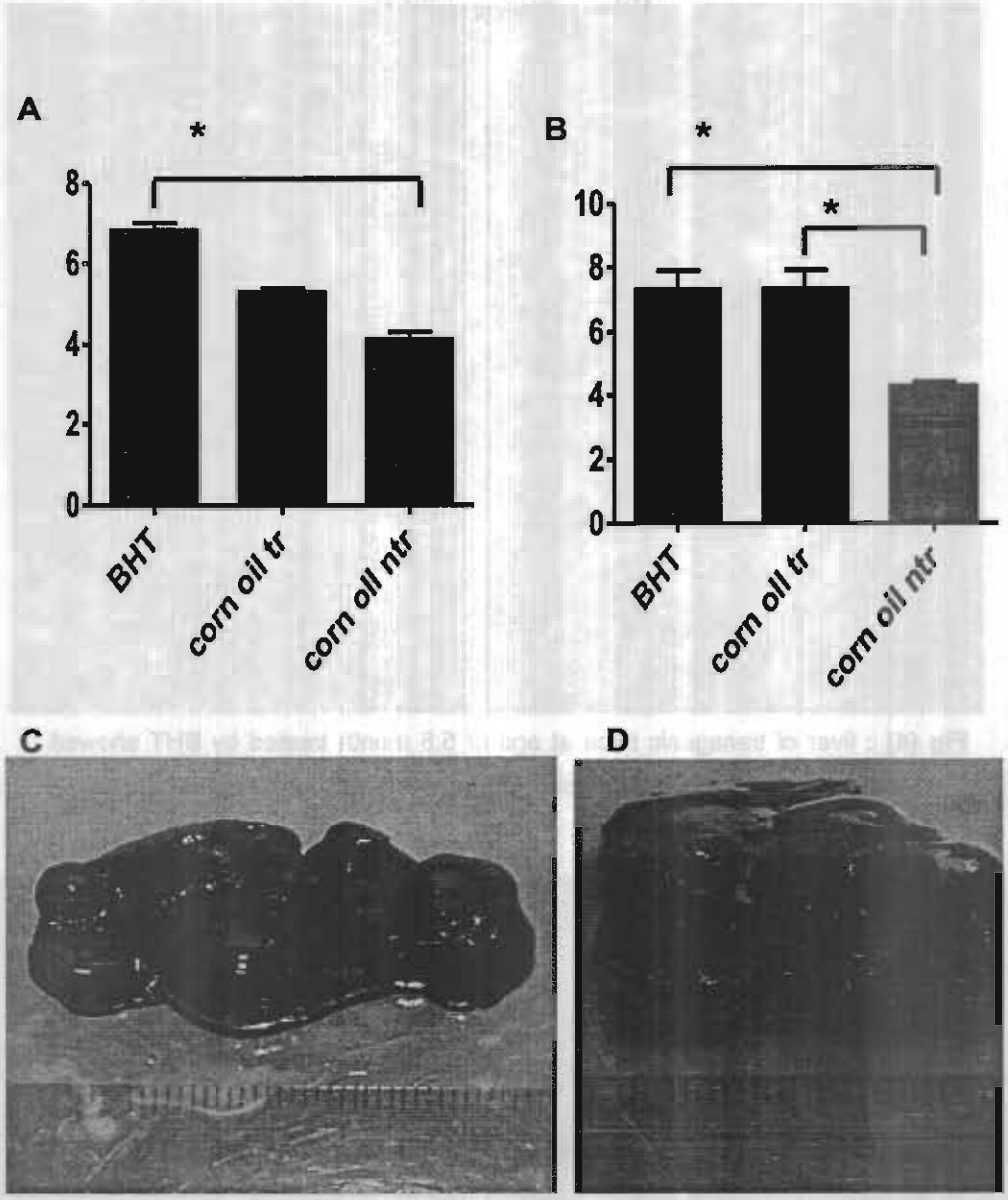

Fig (3) : show liver to body weight ratio of BHT treated transgenic mice significantly increase when compared to non transgenic control at both 4(a) and 8.5 (b) month but when compared to transgenic mice only increased at 4 months. Also showed gross picture of liver of transgenic mice treated by BHT show tumor while control transgenic one showed normal liver.

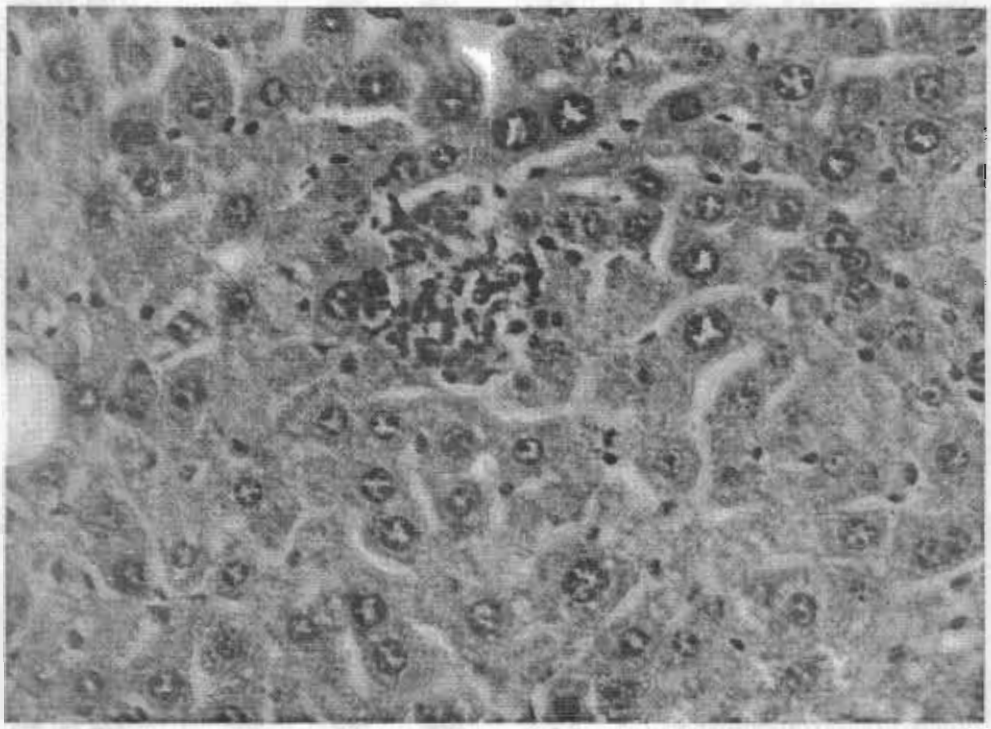

Fig (4) : liver of transgenic mice at age of 5.5 month treated by BHT showed dysphasia, increase mitotic figures, dysplastic nodules and apoptosis stained by (H&E) 250X .

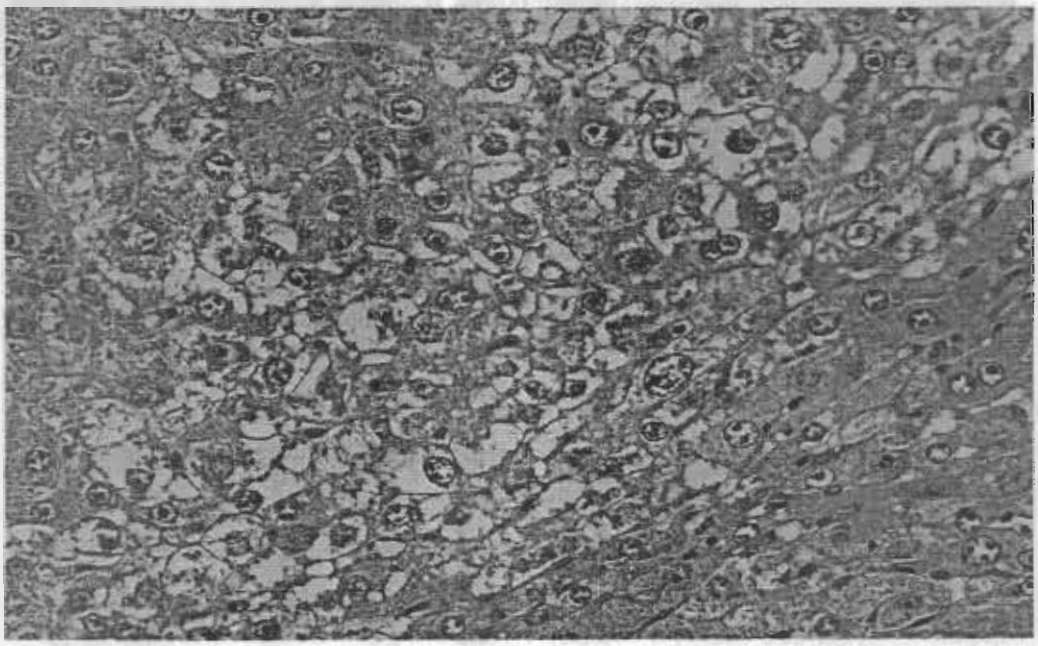

Fig (5) : liver of transgenic mice treated by BHT show large dysplatsic nodules at age of 8.5 month stained by (H&E) 250X.

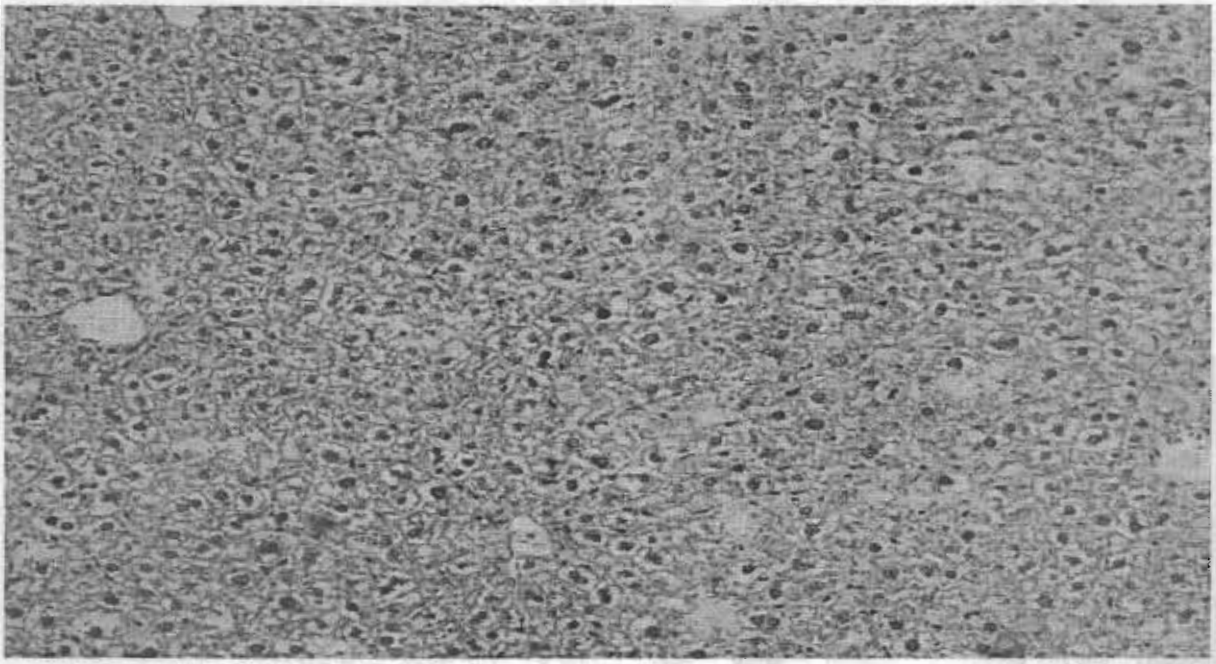

Fig (6) : normal liver of non-transgenic mice treated with con oil at age of 8.5 month stained (H&E) 100X

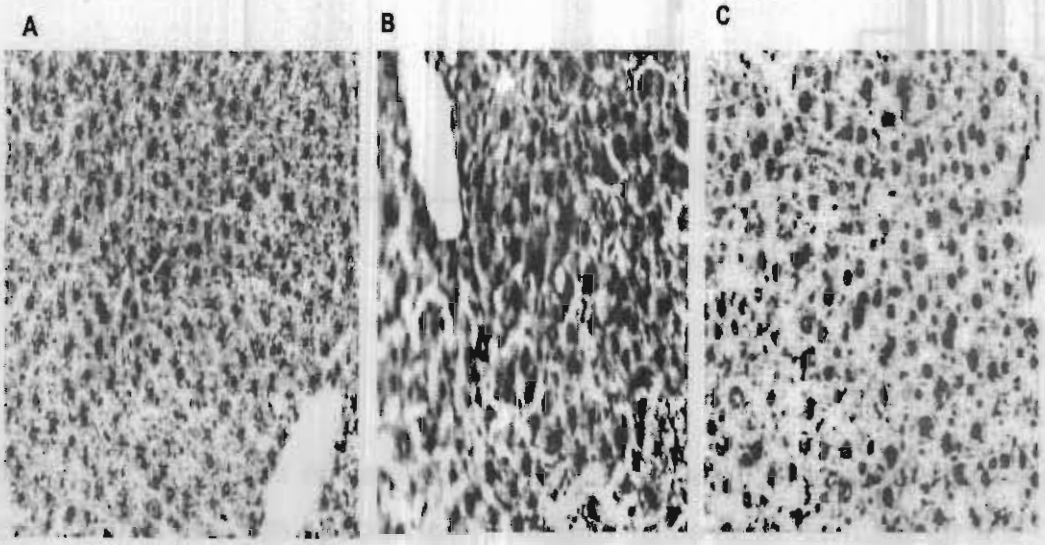

Fig (7) : increase labeling index Pcna (a) Brdu (b) non stained control liver section of transgenic animal treated by BHT 100x

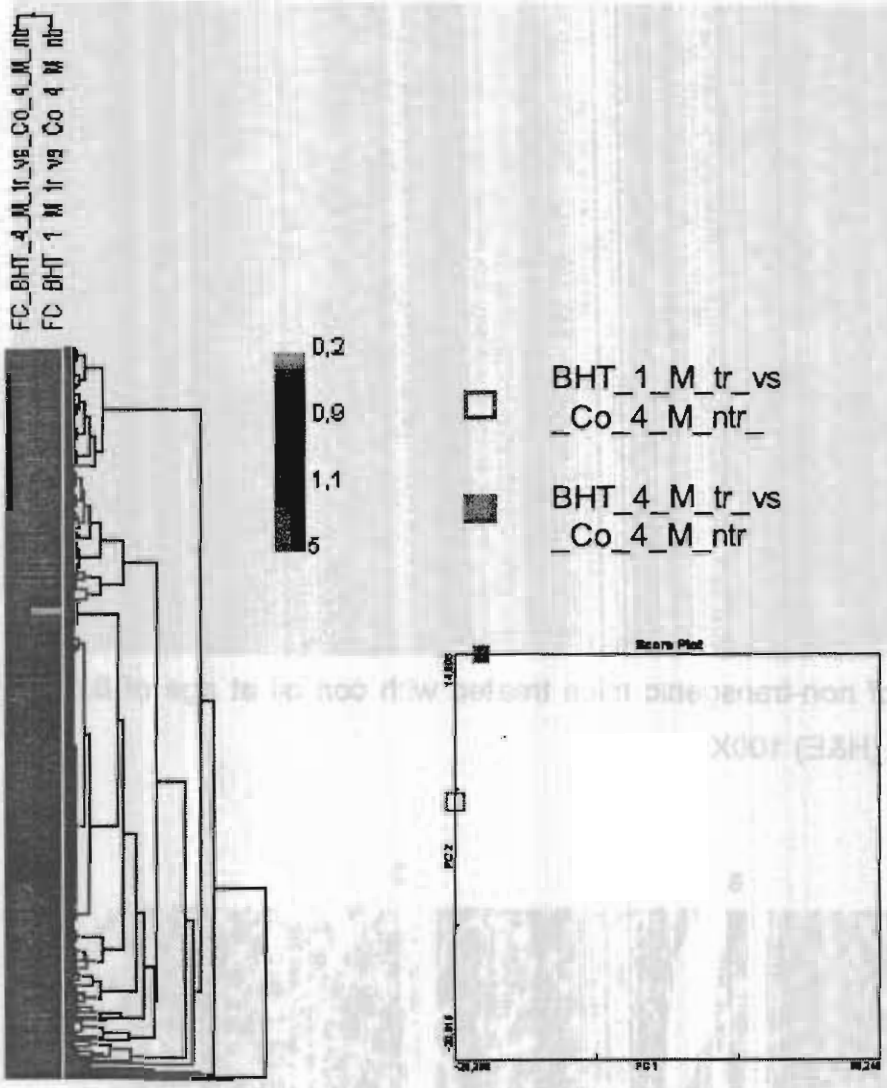

Fig (8) : show HCA and PCA analysis of gene expression in the liver of ATT-cmyc transgenic mice induced by BHT at 1st sacrifice (4 months) and 4th sacrifice (8.5 months) compared to the non transgenic control mice treated with vehicle.

Table (1) : show The most common gene function pathway (KEGG) for BHT compared to non-transgenic mice

Title	BHT_4_vs_Co_4_144	Gene Direction_BHT_4_vs_Co_4_144
Glycine, serine and threonine metabolism(KEGG)	Agxt(Agxt)	Up
Glycine, serine and threonine metabolism(KEGG)	Gldc(Gldc)	Up
Glycine, serine and threonine metabolism (KEGG)	Maob(Maob)	Up
Glycine, serine and threonine metabolism (KEGG)	Pipox(Pipox)	Up
Glycine, serine and threonine metabolism (KEGG)	Shmt1(Shmt1)	Up
Histidine metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up
Histidine metabolism(KEGG)	Hal(Hal)	Up
Histidine metabolism(KEGG)	Maob(Maob)	Up
Histidine metabolism(KEGG)	Uroc1(Uroc1)	Up
Biosynthesis of unsaturated fatty acids(KEGG)	Elovl2(Elovl2)	Up
Biosynthesis of unsaturated fatty acids(KEGG)	Elovl6(Elovl6)	Up
Biosynthesis of unsaturated fatty acids(KEGG)	Fads1(Fads1)	Up
Biosynthesis of unsaturated fatty acids(KEGG)	Fads2(Fads2)	Up
Tryptophan metabolism(KEGG)	Afmid(Afmid)	Up
Tryptophan metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up
Tryptophan metabolism(KEGG)	Kynu(Kynu)	Up
Tryptophan metabolism(KEGG)	Maob(Maob)	Up
One carbon pool by folate(KEGG)	Dhfr(Dhfr)	Up
One carbon pool by folate(KEGG)	Mthfd1(Mthfd1)	Up
One carbon pool by folate(KEGG)	Shmt1(Shmt1)	Up
Arginine and proline metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up
Arginine and proline metabolism(KEGG)	Maob(Maob)	Up
Arginine and proline metabolism(KEGG)	Otc(Otc)	Up
Arginine and proline metabolism(KEGG)	Srm(Srm)	Down
Sulfur metabolism(KEGG)	Papss2(Papss2)	Up
Sulfur metabolism(KEGG)	Sult1a1(Sult1a1)	Up
Folate biosynthesis(KEGG)	Dhfr(Dhfr)	Up
Folate biosynthesis(KEGG)	Gch1(Gch1)	Up
Lysine degradation(KEGG)	Aldh1b1(Aldh1b1)	Up
Lysine degradation(KEGG)	Bbox1(Bbox1)	Up
Lysine degradation(KEGG)	Pipox(Pipox)	Up
Glyoxylate and dicarboxylate metabolism (KEGG)	Afmid(Afmid)	Up
Glyoxylate and dicarboxylate metabolism(KEGG)	Mthfd1(Mthfd1)	Up
Steroid biosynthesis(KEGG)	Fdft1(Fdft1)	Up
Steroid biosynthesis(KEGG)	Sc5d(Sc5d)	Up
beta-Alanine metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up
beta-Alanine metabolism(KEGG)	Srm(Srm)	Down
Alanine, aspartate and glutamate metabolism (KEGG)	Agxt(Agxt)	Up
Alanine, aspartate and glutamate metabolism (KEGG)	Gpt1(Gpt)	Up
Propanoate metabolism(KEGG)	Acss2(Acss2)	Up
Propanoate metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up
Butanoate metabolism(KEGG)	Acsm3(Acsm3)	Up
Butanoate metabolism(KEGG)	Aldh1b1(Aldh1b1)	Up

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الملخص العربى

زيادة نشاط ماده البيتوليد هيدروكسي تولوين في احداث سرطان الكبد في الجرزان المعدله ورثيا والحاملة للجين ميك

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إن زيادة سرطان الكبد الناتج عن ماده البيتوليد هيدروكسي تولوين مثيره للجدل علي الرغم من انه قد ذكر ذلك في دراسات سابقه. ولكن هناك عوامل مؤثره في ذلك مثل سلاله الجرذان والجرعة المستخدمة وكذلك طريقه عمله داخل الكائن الحي. لقد تم استخدام الجرزان المعدله وراثيا والحامله للجين ميك لدراسة مدي زيادة السرطان في خلايا الكبد المهيئه جينيا لحدوثه ولكن عند عمر محدد. ولقد وجد في هذه التجربة ان ماده البيوتيليد هيدروكيس تولوين تزيد من وزن الكبد بالنسبة للوزن العام للجرذان وخاصه في عمر أربعه اشهر وبعد المعالجة مباشره وذلك مقارنه بالمجموعة الضابطة. وعلاوة على ذلك زيادة في الخلايا المنقسمة سواء بطريقه الهيستوياثولوجي او امينوهيستوكميستري. وهذا يعتبر مؤشرا علي تعزيز نشاط ماده البيوتيليد هيدروكسي تولوين في إحداث سرطان الكبد في هذه الجرزان المعدله وراثيا. بالإضافة الي ان بعض أعضاء السيتوكروم جين لعبت دورا هام في استقلاب هذه الماده وخاصه عند عمر أشهر مثل Cyp4a14, CYP1A2, Cyp4a29 وفي حين انخفض بعضها عند عمر 8.5 شهر مثل CYP1A2، Cyp7b1، Cyp2c40 وعلاوة على ذلك أسكوربات والديرات جين لم يتغير نتيجة المعاملة بماده البيتوليد هيدروكسي تولوين.