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Curcumin restores hepatic epigenetic changes in propylthiouracil(PTU) −Induced hypothyroid male rats: A study on DNMTs, MBDs, GADD45a, C/EBP-β and PCNA

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ABSTRACT

6-n-propyl-2-thiouracil (PTU), a thioamide drug, is used as an effective anti-thyroid agent to treat hyperthyroidism and Graves' disease. However, acute liver oxidative damage is an important side effect of the drug. In the present study, we report that PTU administration to rat induces hepatic epigenetic changes by upregulating expression of DNMT1, DNMT3a, DNMT3b, MBD4, MeCP2, p53 and Gadd45a and down-regulation of PCNA and C/EBP-β. This is accompanied by decrease in the cell population and augmentation of cellular lipid peroxidation, an index of oxidative stress, in liver. On the other hand, co-administration of curcumin, a polyphenol extract from the rhizome of *Curcuma longa* L, along with PTU ameliorates PTU- induced oxidative stress and epigenetic parameters except for the expression of MBD4. Also, co-administration of curcumin with PTU resulted in restoration of hepatic cell population and histoarchitecture. The protective effect of curcumin to PTU-induced hepatotoxicity is attributed to its antioxidative properties.

1. Introduction

Liver is the second largest organ of the body. It plays a critical role in maintaining whole-body metabolic homeostasis by carrying out a varied range of biochemical functions (Fabbrini et al., 2010). It is also responsible for metabolizing thyroid hormones and regulating their endocrine effects (Malik and Hodgson, 2002). Thyroid hormones (TH), thyroxine (T_4) and tri-iodothyronine (T_3) are essential for the development, growth, metabolism and oxygen consumption of almost all tissues of vertebrates including liver (Muller and Heuer, 2014; Yen, 2001). In the past, a number of studies have demonstrated that the expression of several hepatic genes associated with carbohydrate and lipid metabolism in liver are considerably influenced by thyroid hormones (Feng et al., 2015; Oppenheimer et al., 1987; Sinha et al., 2014; Venditti and Meo, 2006).

DNA methylation is considered as a mode of epigenetic control of gene regulation in eukaryotes (Attwood et al., 2002). Two protein families, DNA methyltransferases (DNMTs) and methyl CpG binding domain proteins (MBDs), have major roles in the DNA methylation of the genome. The DNMT family has four members: DNMT1, DNMT3a, DNMT3b and DNMT2. DNMT1 maintains the methylation pattern of DNA during replication whereas both DNMT3a and DNMT3b are responsible for *de novo* methylation of cytosine residues. The biological role of DNMT2 is still ambiguous (Hermann et al., 2004; Turek-Plewa and Jagodziński, 2005). On the other hand, the family of MBD consists of five proteins (Bogdanović and Veenstra, 2009; Hendrich and Bird, 1998). Four of them (MBD1, MBD2, MBD3 and MeCP2) are responsible for methylation-dependent repression of genes which involves binding of above proteins to the methylated CpG sites and alteration of chromatin structure (Clouaire and Stancheva, 2008; Esfandiari, 2003; Jones et al., 1998; Squillaro et al., 2010). The fifth (MBD4; Uralic DNA glycosidase) contributes to DNA repair. Recent findings suggest that MBD4 plays a critical role in apoptosis and transcriptional repression (Defossez and Stancheva, 2011; Screaton et al., 2003). DNA

Abbreviations: BSA, Bovine serum albumin; C/EBP-β, CCAAT/ enhancer-binding protein β; cDNA, complementary deoxyribonucleic acid; CPCSEA, Committee for the purpose of Control and Supervision of Experiments on Animals; DNA, Deoxyribonucleic acid; DNMT, DNA methyltransferases; Gadd45a, Growth Arrest and DNA Damage-inducible; IAEC, Institutional Animal Ethics Committee; LPx, lipid peroxidation; MBD, methyl binding domain; MeCP2, methyl-CpG-binding protein 2; PCNA, proliferating cell nuclear antigen; PTU, 6-n- Propyl-2- thiouracil; RNA, Ribonucleic acid; ROS, Reactive oxygen species; T3, triiodothyronine; T4, thyroxine; TBARS, thiobarbituric acid-reactive substances; TH, thyroid hormone; TSH, thyroid stimulating hormone

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methylation has, therefore, been suggested as an interface between genome and the environment and any deviation from its normal pattern may be attributed to the pathophysiological state as a consequence of exposure to xenobiotics (Feil and Fraga, 2012). Another two proteins play an important role in genome stability. They are Gadd45a and p53. The Gadd45a gene, a member of family of stress response genes, is involved in genomic stability, DNA repair, cell cycle checkpoints and suppression of cell growth (Hollander and Fornace, 2002; Zhan, 2005) besides DNA demethylation (Barreto et al., 2007). Similarly, p53 protein is known to maintain genome stability in response to diverse stress signals by coordinating specific cellular responses that include cellular activities in cell cycle, DNA repair, and apoptosis (Bieging et al., 2014).

6-n-propyl-2-thiouracil (PTU), a thioamide drug, is used as an effective anti-thyroid agent to treat hyperthyroidism and Graves' disease (Astwood, 1984; Cooper, 2005; Landau, 1984). Another anti-thyroid drug used to treat hyperthyroidism is methimazole. Nevertheless, controversy still exists regarding their doses and toxicities (Hackmon et al., 2012; Nakamura et al., 2007). Although both the drugs exhibit hepatotoxicity, PTU is recommended to hyperthyroid patients when they are allergic to methimazole (Cooper, 2005). Although PTU and methimazole are the only two available drugs which are recommended for treatment of hyperthyroidism, both the drugs are linked to hepatotoxicity (Akmal and Kung, 2014; Vitug and Goldman, 1985).

Earlier, we have reported that mitochondrial respiratory functions, as well as expression of antioxidant defence enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, were considerably up-regulated in liver of adult male rats by PTU (Das and Chainy, 2001; Subudhi et al., 2009; Subudhi and Chainy, 2010). Recently, we have reported that persistent neonatal exposure of rats to PTU leads to induction of oxidative stress in adult liver with considerable changes in the expression of DNA methylating enzymes, DNA binding proteins and genome stabilizing proteins as well as expression of transcriptional factors (Bunker et al., 2017, 2016; Martelli et al., 1995).

Curcumin is a yellow-coloured polyphenol compound present in turmeric (*Curcuma longa*) which is widely used as a common spice by Indians as well as South Asians. Curcumin has been shown to possess many medicinal properties including, immunomodulatory, antioxidant, antimutagenic and anticarcinogenic (Gupta et al., 2012; Pulido-Moran et al., 2016). In addition, curcumin has been reported to modify expression of enzymes and binding proteins involved in the epigenetic process (Boyanapalli and Kong, 2015; Huang et al., 2016; Miceli et al., 2014; Mirza et al., 2013). Curcumin has been reported to inhibit oxidative stress induced in rat liver by endotoxin (Kaur et al., 2006), ethanol (Samuhasaneeto et al., 2009), iron overload (Badria et al., 2015), arsenite-induced stress (El-Demerdash et al., 2009), in diabetic condition (Palma et al., 2013) and in PTU-induced hypothyroid rats (Subudhi and Chainy, 2012). Several studies have shown that a strong link exists between oxidative stress and the epigenetic process in various pathophysiological states (Arita and Costa, 2014) including aging and age-related diseases (Cencioni et al., 2013; Guillaumet-Adkins et al., 2017) and exposure to various xenobiotics (Baccarelli and Bollati, 2009). Since oxidative stress is involved in hepatic pathogenesis of PTU induced liver injury (Heidari et al., 2015), it will be of interest to know whether PTU affects epigenetic process in liver or not and, if so, whether curcumin can be used as a protective agent against PTU-induced hepatotoxicity.

With this background, the present study has been carried out with two major objectives: first, to determine the impact of PTU on the expression of rat hepatic proteins involved in the epigenetic process, genome stabilization and cell proliferation during adulthood; secondly, to assess the efficacy of curcumin on PTU-induced above hepatic changes. The epigenetic changes in the liver have been ascertained by studying the expression of various proteins associated with DNA methylation (DNMT1a, DNMT3a, DNMT3b, MBD4, MeCP2), genome stabilization (Gaad 45a, p53) and cell proliferation (PCNA). Besides, effects of PTU and curcumin on the expression of CCAAT/enhancer-binding protein β (C/EBP-β), an important transcriptional factor that plays decisive role in regulating expression of multiple hepatocyte-specific genes (Lekstrom-Himes and Xanthopoulos, 1998) and proliferative cell nuclear antigen (PCNA), a protein involved in DNA replication, repair, chromatin remodelling and the epigenetic process (Moldovan et al., 2007) were investigated.

2. Materials and methods

2.1. Chemicals

Agarose, bovine serum albumin (BSA), thiobarbituric acid (TBA), curcumin, Coomassie brilliant blue G-250, 6-propyl 2-thiouracil (PTU), sodium-dodecyl- sulfate (SDS) and Trizma base were obtained from Sigma (St Louis, MO, USA). Ribonuclease inhibitor, DNase I, proteinase K, RNase A, prestained protein ladder, Revert Aid H-Minus first strand cDNA synthesis Kit, M-MuLv reverse transcriptase and Taq DNA polymerase were purchased from Fermentas (Thermo Fisher Scientific Inc., MA, USA). TRIzol reagent and SYBR Green were obtained from Invitrogen (Thermo Fisher Scientific Inc., MA, USA). Chemiluminescence luminol reagent, rabbit polyclonal anti-G3PDH, C/EBP-β, PCNA, biotin goat anti-rabbit IgG and HRP-conjugated anti-rabbit goat IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), whereas streptavidin-HRP conjugate was purchased from Genei, India. PVDF membrane was procured from PALL Life Sciences (Pall India Pvt. Ltd., Mumbai, India). Pure paraffin of melting temperature (58–60 °C) was obtained from s.d fine chem-Ltd. (Mumbai, India). Acrylamide, N, N bis-acrylamide, ammonium persulphate (APS), N,N,N′,N′-tetramethylethane-1,2-diamine (TEMED), phenol, chloroform and isoamyl alcohol of molecular biology grade were procured from SISCO Research Laboratory (Mumbai, India). ELISA kits for T_3, T_4, and TSH were purchased from Monobind, Inc. (CA, USA). All other chemicals used were of the highest purified grade.

2.2. Animals

Adult male rats of Wistar strain (*Rattus norvegicus*) were obtained from the National Institute of Nutrition (Hyderabad, India). Rats were housed and bred in the animal room of the Department of Zoology and Biotechnology, Utkal University. Animal room was maintained at 25 ± 2 °C with 12 h artificial light (6 h–18 h) followed by 12 h (18 h–6 h) darkness. Rats were fed daily with freshly cooked food containing flour of Bengal gram (*Cicer arietinum*, 20%), wheat (*Triticum sativum*, 40%), rice (*Oryza sativa*, 30%), egg (1%) and common salt (1%) in the morning and water soaked whole Bengal gram in the afternoon. Tap water was supplied *ad libitum*. Animal care and maintenance were supervised by Animal Ethics Committee (IAEC) of the Department of Zoology, Utkal University, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Experiments were performed as per their guidelines.

2.3. Experimental design

Adult male rats of 330 ± 10 days old were divided into five groups each containing 5 animals:

Group I: Euthyroid (Control) (Drinking water)
Group II: Vehicle control (Drinking water + Olive oil)
Group III: Hypothyroid (0.05% PTU in drinking water)
Group IV: Curcumin (Drinking water + Curcumin in olive oil)
Group V: Hypothyroid + Curcumin (0.05% PTU in drinking water + Curcumin in olive oil)

Hypothyroidism was induced in rats by administering 0.05% PTU in

Table 1
Accession No., gene specific primer sequences, annealing temperature and product size of G3PDH, C/EBP-β, DNMT1, DNMT3a, DNMT3b, MBD4, MeCP2, Gadd45a, p53 and PCNA.

Primers	Accession no.	Primer Sequences (5′-3′)	Annealing Temp.(^{0}C)	Product Size (bp)
G3PDH	NM_ 017008.4	**S**-ATGGAGAAGGCTGGGGCTCACCT **As**-AGCCCTTCCACGATGCCAAAGTTGT	60	209
C/EBP-β	NM_024125.5	**S**-GACAAGCTGAGCGACGAG **As**-AGCTGCTCCACCACCTTCTTCTG	60	158
DNMT1	NM_ 053354.3	**S**-ACCTACCACGCCGACAT **As**-AGGTCCTCTCCGTACTCCA	60	104
DNMT3a	NM_ 001003957.2	**S**-CAGCAAAGTGAGGACCATTA **As**-AACACCCTTTCCATTTCAG	60	123
DNMT3b	NM_ 001003959.1	**S**-GAATTTGAGCAGCCCAGGTTG **AS**-AAGAAGAGCCTTCCTGTGCC	60	310
MBD4	XM_002726448.3	**S**-CCTACCGGATCTTTTGTGTCA **As**-GATTTTCCCAAAGCCAGTCAT	60	120
MeCP2	NM_022673.2	**S**-ACTTCTCGTCAAGATGCCTTTCC **As**-TTTTCGCTTTCTGCCAAGGG	60	112
Gadd45a	NM_024127.2	**S**-ATTCGTGCTTTCTGTTGC **As**-GCTCTTGTCGTTCTCCAGTA	60	96
p53	NM_030989.3	**S**-CTACTAAGGTCGTGAGACGCTGCC **As**-TCAGCATACAGGTTTCCTTCCACC	60	106
PCNA	NM_022381.3	**S**-TAAGGGCTGAAGATAATGCTGAT **As**-CCTGTTCTGGGATTCCAAGTT	60	126

drinking water for 30 days (Ladenson et al., 1986; Subudhi and Chainy, 2012). Group IV and Group V rats were supplemented with 30 mg/kg body weight of curcumin (Reddy and Lokesh, 1996; Subudhi and Chainy, 2012). Curcumin was dissolved in olive oil (vehicle solution). Group II rats were given olive oil as vehicle control by gavage during the experiments. On the 31st day, after recording the body weights, the animals of all groups were sacrificed between 10 a.m. and 11 a.m. by decapitation under chloroform anesthesia, and trunk blood was collected and allowed to clot at room temperature. Serum was obtained by centrifugation at 1000 × *g* for 10 min and stored in aliquots at −20 °C till further use. Liver was dissected out, cleaned in cold normal saline (0.9%, w/v), pat-dried on filter paper, weighed and kept at −80 °C till further use.

2.4. Measurement of T_3, T_4 and TSH

T_3, T_4 and TSH contents in serum samples were measured using commercially available ELISA kits (Monobind Inc, USA). The prepared ELISA plates were read in a Microplate Reader (550, BIO-RAD).

2.5. Histological analyses

Liver tissue was fixed in freshly prepared sublimate formol and dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin wax. Tissues were sectioned (5 μm) and stained with hematoxylin and eosin. Photographs of the sections were captured in Olympus BX 53 microscope attached with MicroPublisher 5.0 CCD cooled camera, by Q-capture pro7 software for qualitative and quantitative characterization of hepatocyte.

2.6. Counting of hepatocytes

Photographs of rat liver sections were taken at X400 magnification by Olympus BX 53 microscope by Q-capture pro7 software from three different focus areas from one section. For counting of number of nuclei, we have used the method described earlier (Shi et al., 1998). For each rat, the total tissue area, approximately 2 mm^2 (9 fields. 0.223mm^2/fields), was counted from a random sample of three sections. The hepatocytes that exhibited distinct nuclei and cell boundary were considered for counting and number of nuclei/mm^2 was quantified.

2.7. Tissue processing and cellular fractionation

Liver tissue was minced properly on an ice box and a 20% (w/v) homogenate was prepared in 50 mM phosphate buffer, pH 7.4 with a Potter-Elvejhem type motor-driven glass teflon homogenizer in a cold room (Blue Star, India) at 4 °C. A part of the above crude homogenate was treated with triton X-100 (final concentration 0.1%, v/v) for 15 min and centrifuged at 10000 × *g* for 15 min at 4 °C. The supernatant was used for western blotting. The remaining part of the crude homogenate was centrifuged at 1000 × *g* for 15 min at 4 °C to pellet down the cell debris and the resulting supernatant was used to determine lipid peroxidation.

2.8. Protein estimation

The protein content of samples was measured by the method of Bradford (1976) using BSA as standard. Protein content was expressed as mg/g wet weight of tissue.

2.9. Estimation of lipid peroxides

Lipid peroxidation in samples was determined by monitoring the formation of thiobarbituric acid-reactive substances (TBARS) according to the method of Ohkawa et al. (1979). TBARS concentration was calculated from its extinction coefficient $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Wills, 1969). Results were expressed as nmol/TBARS formed/mg protein.

2.10. Tissue preparation, western blotting, RNA isolation and cDNA preparation

Tissue preparation, total RNA isolation, cDNA synthesis and real-time PCR analysis with gene-specific primer using SYBR Green PCR master mix have been described earlier (Bunker et al., 2016). The primer sequences are presented in Table 1. All results were normalized with G3PDH gene (Endogenous control). PCR was repeated in three replicates in 5 animals. The expression levels were calculated by ΔΔCT method (Livak and Schmittgen, 2001). 25–50 μg of protein sample was resolved in 12% SDS-PAGE and transferred to PVDF membrane (0.4 μm, PALL Life Sciences) at 23 mA current for 1 h. The membrane was blocked by incubating it with 5% blocking solution for 1 h at room temperature. The blot was incubated with anti-catalase (1: 5000) for 1 h at room temperature. Blots of C/EBP-β, p53 and PCNA were incubated

with anti-rabbit polyclonal antibodies (1: 500) for overnight at 4 °C, respectively. The blots were washed 3 times (each 10 min) with washing solution. Blots of G3PDH (1:7500), DNMT1, DNMT3a, DNMT3b, MBD4, MeCP2, Gadd45a and C/EBP-β, p53 and PCNA (1:5000) were incubated with HRP-conjugated anti-rabbit goat IgG for 1 h at room temperature, respectively. After incubation, blot was washed 3 times (each 10 min) with washing solution and finally incubated with TBS for 5 min and specific immunoreactive proteins were detected with ECL kit on X-ray films. The band intensity was quantified through densitometry. Rabbit polyclonal anti-G3PDH (1:1000) was used as internal control.

2.11. DNA fragmentation assay and detection of DNA damage by alkaline comet assay

DNA fragmentation assay and DNA alkaline comet assay were carried out according to the methods of Perandones et al. (1993) and Singh et al. (2003), respectively.

2.12. Statistical analysis

Quantification of PCR products and western blot bands were performed using computer-assisted densitometry ImageJ, Image Analysis Software, NIH, USA. Relative integrated densities were expressed as the ratio of band intensities of proteins/G3PDH. The data are presented as mean ± standard deviation of five animals. The level of significance was determined by one-way ANOVA, followed by Tukey HSD test. A difference was considered statistically significant at $p < 0.05$.

3. Results

3.1. Body weight, relative liver weight and relative heart weight

Body weight, relative liver and heart weights of rats of control and experimental groups are presented in Table 2. The final body weight of group III rats was significantly ($p < 0.05$) reduced by 13% in comparison with group I rats. The final body weight of group II, IV and V rats did not change in comparison with that of group I rats. Similarly, the relative weights of liver and heart did not change in group II, III, IV, and V rats when compared with group I rats.

3.2. Serum T_3, T_4 and TSH levels

T_3, T_4 and TSH levels in serum samples of different groups of rats are depicted in Table 3. The levels of T_3 and T_4 in serum sample of group III rats were significantly ($p < 0.05$) lower by 41% and 84% in comparison with group I rats, respectively. Similarly, the levels of T_3 and T_4 were significantly ($p < 0.05$) lower by 36% and 59% in group V rats when compared with group I rats. The levels of T_3 and T_4 did not change in group II and group IV rats in comparison with group I rats. Whereas, level of TSH in serum samples of group III and group V rats were considerably higher than that of group I rats. Its level did not change in group II and group IV when compared with group I rats.

3.3. Liver histology, hepatocytes nuclei counting and lipid peroxidation

Histological analysis of liver samples of group I and II rats exhibited normal morphology of hepatocyte sheaths and adjoining sinusoid spaces. A decrease in hepatocyte nuclei number (17%) was observed in group III rats along with congestion of hepatocytes sheaths and reduction in sinusoid spaces in comparison with group I. The number of hepatocytes in liver sections of Group II, IV and V rats did not change in comparison with those of group I rats. Hepatocyte nuclei number significantly increased along with increase in sinusoid space in liver sample of group V rats in comparison with group III rats (Table 4; Fig. 1). Effects of PTU and curcumin on level of lipid peroxidation in liver of adult male rats are presented in Table 4. Lipid peroxidation level was significantly higher by 39% in liver of group III rats in comparison with group I. Although lipid peroxidation level in liver of group V rats did not change in comparison with group I and group II, a 23% decrease in its level was recorded in group IV rats compared to group I and group II.

3.4. DNA damage

No significant difference in DNA fragmentation values of liver tissues was detected among rats of group I, II, III, IV, and V. Similarly, no significant differences in comet assay parameters of liver cells of rats of group I, II, III, IV and V were noticed (Table 5; Fig. 2).

3.5. Effects on expression of C/EBP-β

Levels of transcripts and translated products of C/EBP-β of different groups are presented in Fig. 3A and B. Transcript level of C/EBP-β was significantly lower in group III (~2 fold) and group V (1.35 fold) rats in comparison with group I (Fig. 3B). Level of translated products of C/EBP-β was significantly lower in group III (45%) and group V (28%) rats than that of group I (Fig. 3B). Transcript level and translated products of C/EBP-β did not change significantly in group II and IV rats compared with group I (Fig. 3A and B).

3.6. Effects on expression of Gadd45a

Levels of transcripts and translated products of Gadd45a of different groups are presented in Fig. 3C and D. Transcript level of Gadd45a was significantly higher in group III (4.1 fold) rats in comparison with group I (Fig. 3C). Level of translated products of Gadd45a was significantly higher in group III (55%) rats than that of group I (Fig. 3D). However,

Table 2
Effects of PTU and curcumin on Body and Organ weights(g) of adult male rats.

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
Initial Body weight(g) (%)	326 ± 5.48[a] (100 ± .68[a])	329 ± 7.42[a] (101 ± 2.28[a])	328 ± 19.23[a] (101 ± 5.92[a])	333 ± 16.05[a] (102 ± 4.91[a])	329 ± 15.17[a] (101 ± 4.66[a])
Final body weight(g) (%)	358 ± 5.71[a] (100 ± 1.59[a])	357 ± 2.74[a] (99.70 ± .76[a])	310 ± 20.32[b] (87 ± 5.70[b])	346 ± 16.73[a] (97 ± 4.70[a])	347 ± 12.55[a] (97 ± 3.50[a])
Relative liver weight(g) (%)	2.99 ± 0.03[a] (100 ± 1.0[a])	3.03 ± 0.02[a] (101 ± 0.66[a])	3.07 ± 0.09[a] (103 ± 0.02[a])	3.07 ± 0.07[a] (103 ± 2.35[a])	3.05 ± 0.07[a] (102 ± 2.35[a])
Relative heart weight(g) (%)	0.28 ± 0.005[a] (100 ± 1.79[a])	0.29 ± 0.003[a] (103 ± 1.06[a])	0.3 ± 0.009[a] (107 ± 3.21[a])	0.3 ± 0.009[a] (107 ± 3.21[a])	0.3 ± 0.005[a] (107 ± 1.78[a])

Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Data are expressed as mean ± S.D. of five animals. Parentheses contain % of changes. Data having different superscripts (a-d) in the same row differ significantly from each other.

Table 3
Effects of PTU and curcumin on serum levels of T3, T4 and TSH of adult male rats.

Parameters	Groups				
Serum Hormones	Group I	Group II	Group III	Group IV	Group V
Total T_3 (ng/dl) (%)	126 ± 9.80[a] (100 ± 8.0[a])	128 ± 14.80[a] (102 ± 11.80[a])	74 ± 7.62[b] (59 ± 6.08[b])	134 ± 16.12[a] (106 ± 13[a])	80 ± 9.0[c] (64 ± 7.2[c])
Total T_4 (%)	6.26 ± 0.90[a] (100 ± 14.37[a])	6.61 ± 0.78[a] (105 ± 12.40[a])	0.96 ± 0.20[b] (15.3 ± 2.83[b])	5.90 ± 0.62[a] (94 ± 9.9[a])	2.55 ± 0.2[c] (41 ± 3.22[c])
TSH (%)	0.22 ± 0.05[a] (100 ± 23[a])	0.29 ± 0.04[a] (132 ± 18.2[a])	4.33 ± 0.42[b] (1969 ± 191[b])	0.18 ± 0.08[a] (82 ± 36[a])	3.87 ± 0.26[c] (1759 ± 119[c])

Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Data are expressed as mean ± S.D. of five animals. Parentheses contain % of changes. Data having different superscripts (a-d) in the same row differ significantly from each other.

Table 4
Effects of PTU and curcumin on number of nuclei/mm^2 and lipid peroxidation in the liver of adult male rats.

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
No of nuclei/mm^2 (%)	106 ± 5.13[a] (100 ± .84[a])	108 ± 2.64[a] (102 ± .50[a])	88 ± 6.08[b] (83 ± 5.74[b])	109 ± 5.13[a] (103 ± .85[a])	103 ± 7.64[a] (97 ± .20[a])
LPx (nmol TBARS/mg protein) %	1.20 ± 0.05[a] (100 ± 4.20[a])	1.17 ± 0.03[a] (98 ± 2.51[a])	1.66 ± 0.07[b] (139 ± 5.86[b])	0.92 ± 0.08[c] (77 ± 6.69[c])	1.19 ± 0.08[ad] (99 ± 6.72[ad])

Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Data are expressed as mean ± S.D. of five animals. Parentheses contain % of changes. Data having different superscripts (a-d) in the same row differ significantly from each other.

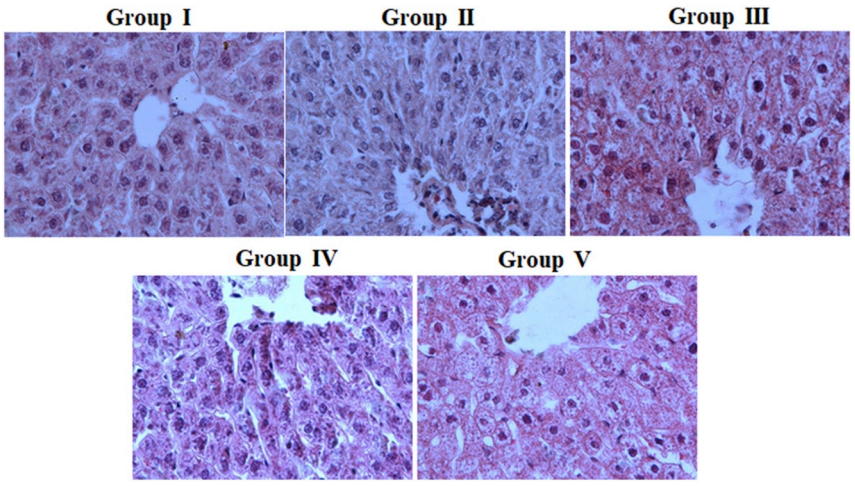

Fig. 1. Effects of PTU and curcumin on histo-architecture of liver (transverse section; hematoxylin and eosin staining) in PTU and curcumin treated adult male rats. Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Photomicrographs are given with 100 μm scale bars and 400x magnification.

Table 5
Effects of PTU and curcumin on DNA fragmentation and comet assay parameters in the liver of adult male rats.

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
DNA fragmentation (%)	2.85 ± 0.31[a] (100 ± 11.0[a])	2.92 ± 0.32[a] (103 ± 11.30[a])	2.89 ± 0.35[a] (102 ± 13.0[a])	2.81 ± 0.40[a] (98 ± 14.0[a])	2.97 ± 0.37[a] (105 ± 13.0[a])
Tail length (pixel) (%)	3.03 ± 0.48[a] (100 ± 16.0[a])	2.86 ± 0.53[a] (95 ± 18.0[a])	3.15 ± 0.37[a] (104 ± 13.0[a])	3.32 ± 0.53[a] (109 ± 17[a])	3.22 ± 0.51[a] (98 ± 15.0[a])
% DNA in tail (%)	1.59 ± 0.35[a] (100 ± 22.0[a])	1.72 ± 0.35[a] (108 ± 22.0[a])	1.64 ± 0.31[a] (104 ± 20.0[a])	1.69 ± 0.28[a] (107 ± 18.0[a])	1.76 ± 0.21[a] (111 ± 14.0[a])
Tail moment (%)	0.05 ± 0.01[a] (100 ± 20.0[a])	0.048 ± 0.01[a] (96 ± 20.0[a])	0.05 ± 0.01[a] (100 ± 20.0[a])	0.06 ± 0.015[a] (120 ± 30.0[a])	0.06 ± 0.01[a] (120 ± 20.0[a])

Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: curcumin treated rats and Group V: PTU and curcumin treated rats. Data are expressed in percentage (%) given in parentheses. Data are expressed as mean ± S.D. of five animals. Bars having superscript of same letter (a) do not differ significantly from each other.

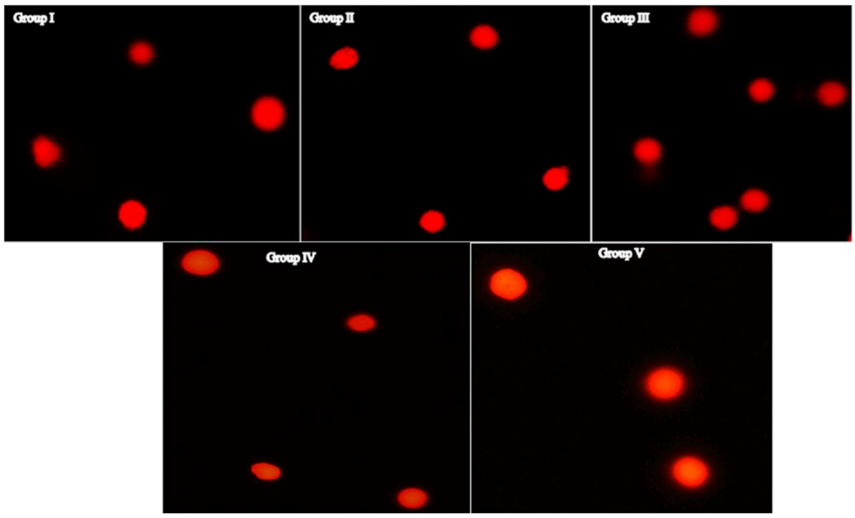

Fig. 2. DNA damage analysis through single cell electrophoresis (comet assay) in the hepatocytes of PTU and curcumin treated male rats. Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: curcumin treated rats and Group V: PTU and curcumin treated rats.

levels of transcripts and translated products did not change in group II, IV and V rats than group I (Fig. 3C and D).

3.7. Effects on expression of p53

Transcript level of p53 was significantly higher in group III (1.95 fold) rats in comparison with group I (Fig. 3E). However, its level was significantly decreased in group IV (1.33 fold) rats than group I. Transcript level of p53 did not change in group II and V rats than group I. Level of translated products of p53 was significantly higher in group III (25%) rats than that of group I. Its level did not change in group II, IV and V rats in comparison with group I (Fig. 3F).

3.8. Effects on expression of PCNA

Levels of transcripts and translated products of PCNA of different groups are presented in Fig. 3G and H. Transcript level of PCNA was significantly lower by 1.6 fold in group III rats in comparison with group I (Fig. 3G). Level of translated products of PCNA was significantly lower by 30% in group III rats than group I (Fig. 3H). Levels of transcripts and translated products of PCNA did not change in group II, IV and V rats in comparison with group I (Fig. 3G and H).

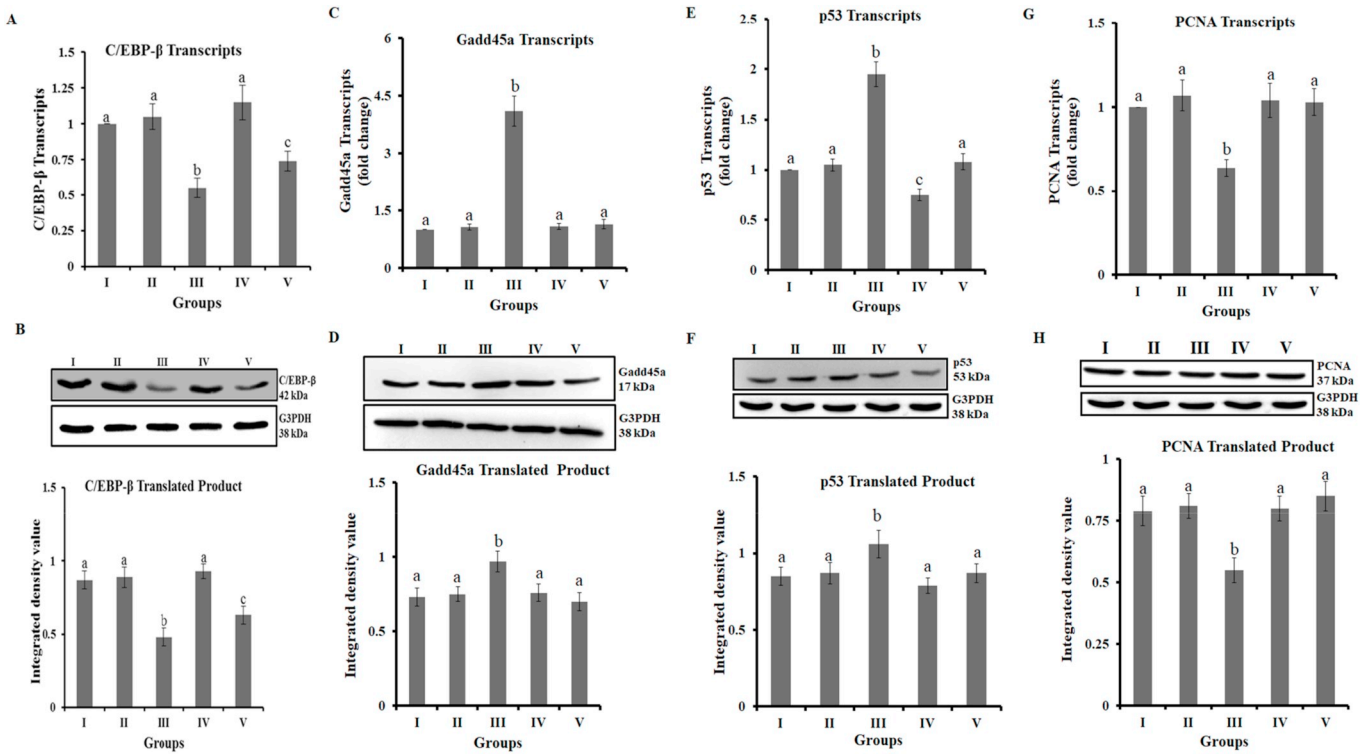

Fig. 3. Effects of PTU and curcumin on transcripts of (A, C, E and G) and translated products (B, D, F and H) of C/EBP- β, Gadd45a, p53 and PCNA in the liver of adult male rats. Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Transcript levels are expressed in fold change relative to G3PDH transcript (endogenous control). Data are expressed as mean ± S.D. of five animals. Bars having superscripts of different letters differ significantly from each other.

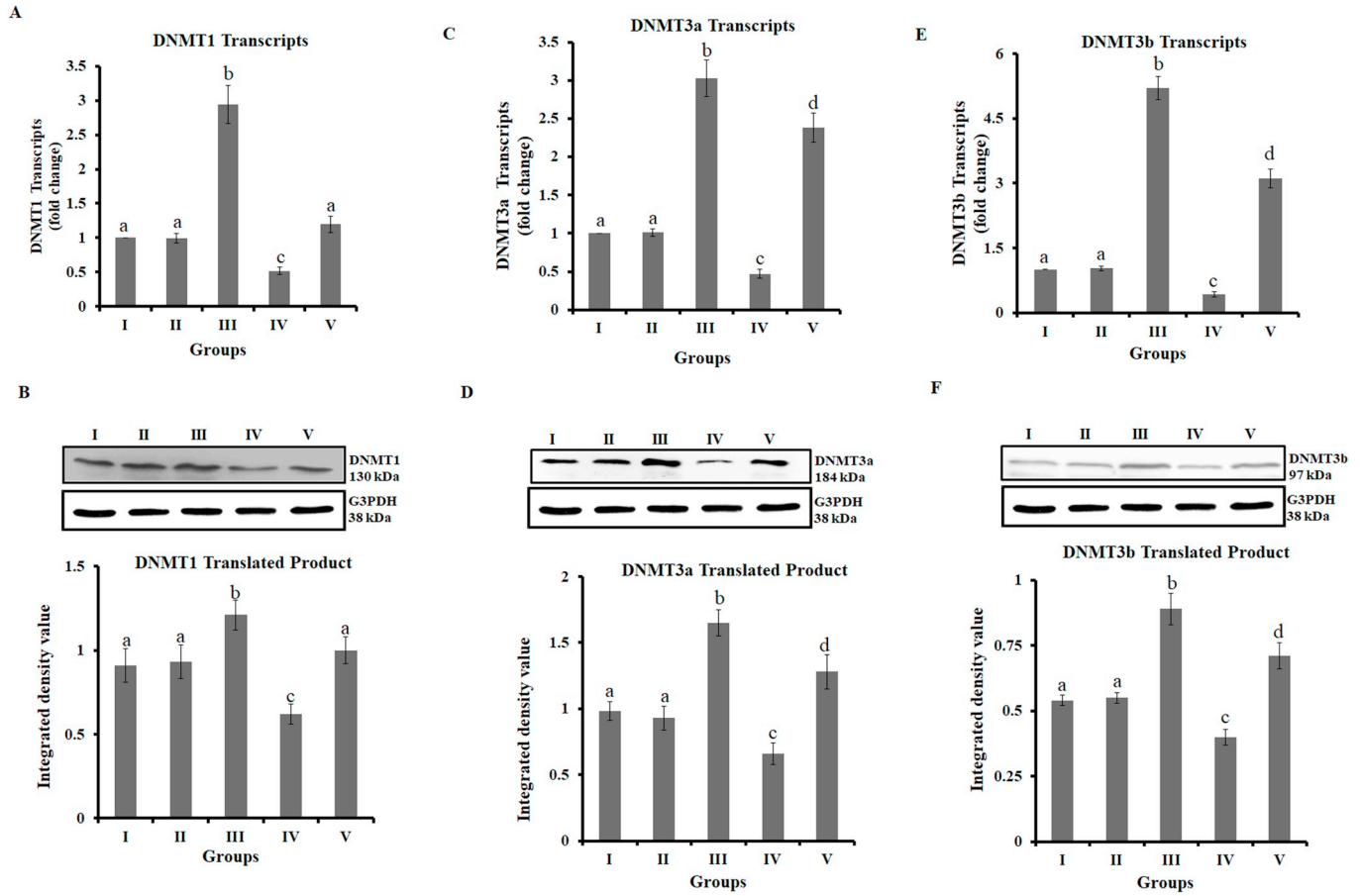

Fig. 4. Effects of PTU and curcumin on transcripts and translated products of DNMT1 (A and B), DNMT3a (C and D) and DNMT3b (E and F) in the liver of adult male rats. Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Transcript levels are expressed in fold change relative to G3PDH transcript (endogenous control). Data are expressed as mean ± S.D. of five animals. Bars having superscripts of different letters differ significantly from each other.

3.9. Effects on expression of DNMT1, DNMT3a and DNMT3b

Levels of transcripts and translated products hepatic DNMTs of different groups are presented in Fig. 4. Transcript level of DNMT1was significantly higher by ~3 fold in group III rats in comparison with group I (Fig. 4A). In contrast, its level was significantly decreased (~2 fold) in group IV rats than that of group I (Fig. 4A). Level of translated products of DNMT1 was significantly higher by 33% in group III rats in comparison with group I (Fig. 4B). In contrast, its level was significantly decreased (32%) in group IV rats than group I rats (Fig. 4B). The transcripts and translated products of DNMT1 did not change in group II and V rats in comparison with group I rats (Fig. 4A and B).

The transcript levels of DNMT3a were significantly increased in group III (~3.1fold) and group V (~2.44 fold) rats than group I (Fig. 4C). In contrast, its level was significantly decreased in group IV (~2 fold) rats when compared with group I (Fig. 4C). Translated products of DNMT3a was significantly higher in group III (69%) and group V (41%) rats in comparison with group I, respectively (Fig. 4D). Level of translated products of DNMT3a was significantly lower by 33% in group IV rats when compared with group I rats (Fig. 4D). The transcript and translated products of DNMT3a did not change in group II rats in comparison with group I (Fig. 4C and D).

The transcript level of DNMT3b was significantly higher in group III (~5.2 fold) and group V (~3.1 fold) rats than that of group I, respectively (Fig. 4E). Transcript level of DNMT3b was significantly lower by ~2 fold in group IV when compared with group I rats (Fig. 4E). Level of translated products of DNMT3b was significantly higher in Group III (64%) and group V (31%) rats in comparison with group I rats, respectively (Fig. 4F). Level of translated products of DNMT3a was significantly lower in group IV rats when compared with group I rats (Fig. 4F). Levels of transcripts and translated product of DNMT3b did not change in group II rats when compared with group I (Fig. 4E and F).

3.10. Effects on expression of MBD4 and MeCP2

Levels of transcripts and translated products of MBD4 and MeCP2 of different groups are presented in Fig. 5. Transcript level of MBD4 was significantly higher in group III (~4.55 fold) and group V (4.45 fold) in comparison with group I rats, respectively (Fig. 5A). However, its level did not change in group II and IV rats when compared with group I rats (Fig. 5A). Levels of translated products of MBD4 were significantly higher by 67% and 70% in group III and V rats in comparison with group I rats, respectively (Fig. 5B). Transcript and translated levels of MBD4 was did not change in group II and IV rats in comparison with group I rats (Fig. 5A and B).

Transcript level of MeCP2 was significantly higher in group III (4.34 fold) rats in comparison with group I rats (Fig. 5C). Whereas, its level was decreased in group IV (~2.5 fold) rats than group I rats (Fig. 5C). Level of translated products of MeCP2 was significantly higher in group III (37%) rats in comparison with group I (Fig. 5D). However, its level was significantly lower in group IV (33%) rats than group I (Fig. 5D). Transcript and translated levels did not change in group II and group V rats in comparison with group I rats (Fig. 5C and D).

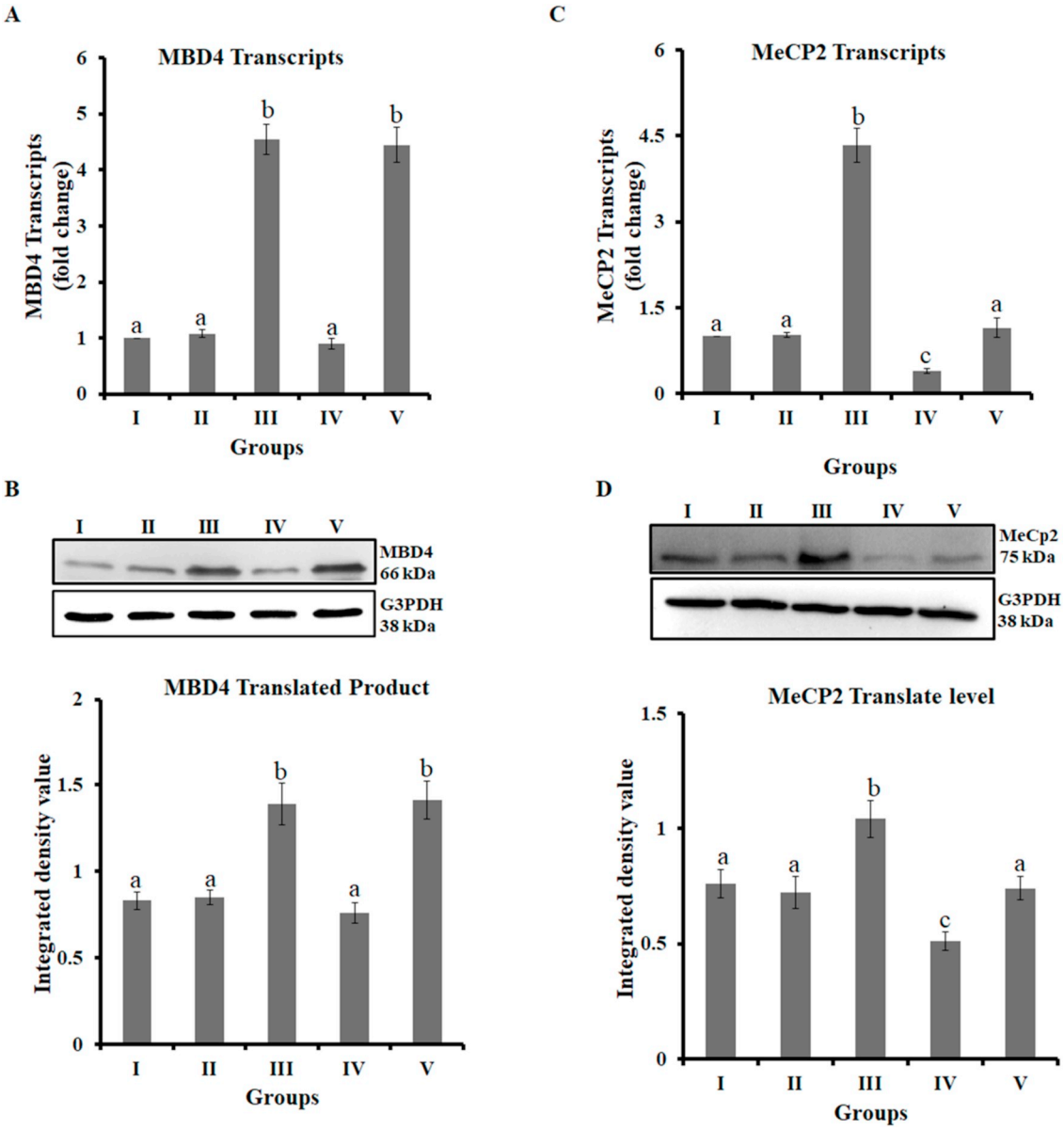

Fig. 5. Effects of PTU and curcumin on transcripts (A and C) and translated product (B and D) of MBD4 and MeCP2 in the liver of adult male rats. Group I: Control rats; Group II: Vehicle treated rats; Group III: PTU treated rats; Group IV: Curcumin treated rats and Group V: PTU and Curcumin treated rats. Transcript levels are expressed in fold change relative to G3PDH transcript (endogenous control). Data are expressed as mean ± S.D. of five animals. Bars having superscripts of different letters differ significantly from each other.

4. Discussion

PTU is a well-known thiouracil-derived anti-thyroid drug that inhibits synthesis of thyroid hormones by interfering with thyroperoxidase and peripheral deiodinase (Zoeller and Crofton, 2005). Significant reduction in serum T_3 and T_4 and elevation in TSH levels along with a decrease in body weight and a change in histo-architecture of liver confirmed the induction of hypothyroidism by PTU. It also confirmed the hepatotoxic nature of the drug. In the present study, we failed to record hepatic DNA damage as evident from DNA fragmentation and comet assays and these results are in agreement with earlier reports (Bunker et al., 2017; Martelli et al., 1995).

A dynamic equilibrium exists among p53, Gadd45a, and PCNA in cells under normal physiological state. It seems PTU disturbs such equilibrium state in the liver by disrupting physiological titers of thyroid hormones or by augmenting oxidative stress or by both (Das and Chainy, 2001; Subudhi et al., 2009). It is reported that the induction of Gadd45a, a nuclear protein that binds itself to multiple important cellular proteins such as PCNA, p21, and core histone proteins, is regulated by p53 (Jin et al., 2002). Overexpression of p53 and Gadd 45a by PTU may be an adaptation to prevent replication of damaged DNA. The p53 is known to regulate expression of several genes that are associated with various p53-dependent cellular functions including cell cycle arrest (Liu and Xu, 2011). In this context, members of Gadd45 family deserve special attention because of their important role in stress-signaling pathways, controlling cell cycle arrest, DNA repair and cell survival/apoptosis (Liebermann and Hoffman, 2008; Vairapandi et al., 2002). It may be mentioned here that induction of expression of Gadd45a has been recorded in several mammalian cells in response to DNA damaging agents (Liebermann and Hoffman, 2008; Vairapandi et al., 2002). It is suggested that overexpression of p53 and Gadd45a in liver of adult rats in response to PTU may be an adaptive response to prevent DNA damage. The proliferative activity of eukaryotic cells is commonly assayed by determining the expression of

PCNA, the auxiliary protein of DNA polymerase δ which is required for DNA synthesis during replication (Connolly and Bogdanffy, 1993; Strzalka and Ziemienowicz, 2011). PCNA exhibits several functions depending on the environmental condition of cells (Mailand et al., 2013). Besides DNA replication, in conjunction with p53, PCNA causes cell cycle arrest and repair of damaged DNA (Paunesku et al., 2001). Decrease in PCNA expression by PTU suggests the entrance of hepatocytes to the S phase which may be due to exhaustion of proliferative capability of liver cells. Our results are in agreement with previous data where PTU-induced hypothyroidism resulted in down-regulation of PCNA expression by western blotting in rat liver (Alisi et al., 2005). It has been reported that the destiny of a cell can vary from temporary arrest to death depending on the level of oxidative stress and the extent of its DNA damage (Macip et al., 2006). Further, the results of the present study suggest that PTU-induced hypothyroidism might have resulted in the inhibition of cell cycle progression of hepatocytes along with induction of quiescent state by depressing the expression of PCNA and elevating expression of p53 and Gadd45a. Itahana et al. (2002) have suggested that p53 has a significant contribution in induction and retaining the quiescent state of normal cells. At present, it is difficult to understand the mechanism(s) through which PTU resulted in up-regulation of p53 and Gadd45a in liver, because functions of both the proteins are tightly regulated through several biochemical processes (Kruse and Gu, 2009; Tamura et al., 2012).

It is apparent from the present study that supplementation of curcumin along with PTU was unable to restore serum levels of T_3, T_4 and TSH. Nevertheless, curcumin treatment along with PTU restores the number of cells and normal histological features of liver. Results of the present study are in agreement with our earlier report (Subudhi et al., 2009). Both PCNA and p53 are closely associated with cell cycle and their expression is important for the successful completion and continuation of cell cycle (Paunesku et al., 2001).

It is interesting to note that PTU-induced decrease in transcripts and translated products of PCNA and increase in transcripts and translated products of p53 and Gadd45a are restored to normal level by curcumin. The decrease in LPX level and restoration of expression of above proteins to that of control values by curcumin in PTU-treated rats suggest the possible involvement of ROS and oxidative stress. It is reported that curcumin inhibits lipid peroxidation by scavenging superoxide anions and hydroxyl radicals (Ruby et al., 1995). Recently, we have demonstrated that exposure to PTU resulted in upsetting antioxidant defenses (Sahoo et al., 2015) and pro-apoptotic and anti-apoptotic signaling pathways (Sahoo et al., 2017) in developing rat testis. It is worthy to mention here that paracetamol-induced alterations in oxidative stress parameters and pro-apoptotic and anti-apoptotic signaling pathways in liver of mice were ameliorated by curcumin (Bulku et al., 2012). Curcumin is an established antioxidant, anti-inflammatory and anti-cancerous drug (Noorafshan and Ashkani-Esfahani, 2013).

CCAAT enhancer binding protein (C/EBP α/β) is a transcription factor mainly expressed in liver and intestinal tissues, which plays a pivotal role in energy metabolism (Birkenmeier et al., 1989; Crosson et al., 1997; Pedersen et al., 2007; Schrem, 2004). Our results relating to the expression of C/EBP-β agree with our earlier report (Bunker et al., 2016), where we demonstrated that expression of C/EBP-β decreased in response to PTU treatment. However, treatments of curcumin to PTU-treated rats restore its expression to the normal value possibly due to anti-oxidative capacity of curcumin (Menon and Sudheer, 2007).

One of the goals of the present study was to identify the signature genes responsible for the toxicity induced by PTU in rat liver and to find out the possibilities of their reversion to normal levels by antioxidant therapy. Analyses of transcript and translated products of DNMT1, DNMT3a, DNMT3b, MBD4, MeCP2 and Gadd45a revealed upregulation of expressions of above genes in rat liver in response to PTU exposure. The exact molecular mechanism(s) by which PTU upregulated expression of proteins is difficult to explain at present. It is possible that augmentation of their expression may be due to induction of oxidative stress (OS) by PTU (Das and Chainy, 2001; Heidari et al., 2015; Subudhi et al., 2009; Subudhi and Chainy, 2012). Several studies have demonstrated a link between OS and epigenetic alterations in cancer (Venza et al., 2015), diabetes (Feng et al., 2013), aging (Cencioni et al., 2013) and exposure to toxic agents (Baccarelli and Bollati, 2009). Further, it has been reported that reduction in the expression of glutathione S-transferase P1 gene, one of the principal antioxidant enzymes, in cells occurred due to methylation of its promoter as a consequence of up-regulation of MBDs, HDAC and DNMT complex in response to oxidative stress (Donkena et al., 2010; Miller et al., 1999).

Up-regulation of expression of above proteins in liver by PTU is of particular importance in view of their putative roles in regulation of gene expression, maintaining genome stabilization and repair. It is possible that all the three DNMTs act in concert to repress target genes due to hypermethylation of their respective promoters causing either down-regulation or silencing of genes in response to PTU. Increased expressions of DNMTs by PTU might cause enhanced methylation amount in various genes. It is a well-established fact that expression of a gene has a reciprocal relationship with the amount of methylation in it (Phillips, 2008). The increase and changes in methylation pattern of liver catalase promoter in response to neonatal PTU treatment to rats support the above hypothesis (Bunker et al., 2016). Changes in the expression or activity of MeCP2 and MBD4 by PTU treatment may therefore provide mechanisms by which PTU might have prevented binding of transcription inhibitors to promoter regions. MBD4 is likely to be responsible for DNA mismatch repair (Jaenisch and Bird, 2003). The protein also demonstrates strong DNA glycosylase activity and corrects G: T mismatches which occur due to deamination of 5-methylcytosine residues in symmetrically methylated CpG base pairs (Hendrich et al., 1999). Elevated MBD4 in PTU treated rats probably facilitates the elimination of CpG:TpG mismatches in DNA caused by PTU due to generation of oxidative stress (Das and Chainy, 2001; Heidari et al., 2015; Subudhi et al., 2009; Subudhi and Chainy, 2012). MBD1, MBD2, MBD3 and MeCP2 are thought to inhibit the binding of transcriptional factors to promoter regions and thus are proposed as an alternative mechanism of transcription inhibition by hypermethylation (Clouaire and Stancheva, 2008; Fujita et al., 1999). Among them, MeCP2 is the most abundantly expressed protein under physiological conditions and plays a central role in recruiting transcriptional repressors (Im et al., 2010; Nan et al., 1997; Tudor et al., 2002). Recent studies indicate that MeCP2 can act as an activator or a repressor for expression of genes depending on its specific post-translational modifications (Chahrour et al., 2008; Horvath and Monteggia, 2018). Two important observations were made regarding the effects of curcumin on expression of DNMTs, MBD4, MeCP2 and Gadd45a in liver of rats. Upregulation of above genes in liver by PTU were down-regulated in response to curcumin treatment. Antioxidant properties of curcumin are well established (Menon and Sudheer, 2007; Noorafshan and Ashkani-Esfahani, 2013). Therefore, the ameliorating effects of curcumin on PTU-induced changes in expression of proteins in rat liver may be attributed to its antioxidative properties. It may be noted that expressions of DNMT1, DNMT3a, DNMT3b, MeCP2 and Gadd45a are also down-regulated in liver of control rats by curcumin. It is possible that steady levels of reactive oxygen species may be responsible for expression of above genes under normal physiological state and their down-regulation in liver of control rats by curcumin may be due to their quenching by curcumin. The possible involvement of reactive oxygen species, particularly superoxide radicals, in regulating epigenetic process that strongly modulate expression of different genes is reported (Campos et al., 2007; Creppy et al., 2002; Mikhed et al., 2015; Valinluck, 2004). It is reported that only mRNA and protein of DNMT3b was reduced by curcumin in liver tissues of carbon tetrachloride (CCl_4) exposed rats. However, the authors did not report the effect of curcumin on the expression of DNMTs in rats that were not treated with CCl_4. Very little information is available in literature on the physiological significance of

MeCP2 in organs other than brain (Amir et al., 1999; Chahrour et al., 2008; Horvath and Monteggia, 2018). Recently, it has been shown that MeCP2 in association with NcoR- HDAC3 corepressor complex coordinate the biosynthetic pathways of triglyceride and cholesterol in liver tissue with an augmentation of hepatic cholesterol content (Kyle et al., 2016). Therefore, the possibility of reduction in cholesterol level in rat liver due to disturbance of cholesterol biosynthesis as a consequence of down regulation of MeCP2 expression in response to curcumin is expected. In fact, an observed decrease in hepatic and serum cholesterol content (Rao et al., 1970) in rats and a decrease in serum cholesterol level in hypothyroid rats (Subudhi et al., 2009) in response to curcumin corroborate the findings.

In silico analysis demonstrated that curcumin has the potential to inhibit the catalytic function of DNMT1 by blocking the thiolate group of cysteine residue at 1226 position (Liu et al., 2009; Yoo and Medina-Franco, 2011). It is reported that curcumin decreased mRNA as well as proteins level of DNMT1 in breast cancer cell lines (Du et al., 2012). Therefore, the functional capacity of DNMT1 in liver of curcumin-treated rats may be reduced due to decline in its catalytic function along with its expression. The significance of different DNMT isoforms in tissues during normal physiological and disease state is not well understood (Lyko, 2017). Therefore, the decline in the expression of DNMT isoforms in liver by curcumin and its physiological relevance warrants further study.

The results of the present study indicate that elevations of MBD4, both at transcription and translational levels in liver by PTU, were not affected by curcumin treatment. This suggests that upregulation of MBD4 by PTU may not be mediated through PTU-induced OS. This may be due to some permanent changes in its regulatory mechanism(s) in response to PTU treatment. The elevation in MBD4 levels may be due to decrease in levels of THs in response to PTU treatment to rats. Our observations are in agreement with the findings of Peng et al. (2011) where authors have noticed upregulation of MBD4 expression in skeletal muscles of thyroidectomized adult rats.

In conclusion, the present study provides overall evidence for the hepatoprotective effects of curcumin against PTU-induced liver injury in male rats. Curcumin improved histopathological and epigenetic changes noticed in PTU-induced liver injury, which may be linked to the recovery of the antioxidant defense system and amelioration of oxidative stress. Present data are encouraging and bring to light the immense therapeutic potential of curcumin in combating PTU-induced hepatotoxicity.

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References

Akmal, A., Kung, J., 2014. Propylthiouracil, and methimazole, and carbimazole-related hepatotoxicity. Expet Opin. Drug Saf. 13, 1397–1406. https://doi.org/10.1517/14740338.2014.953796.

Alisi, A., Demori, I., Spagnuolo, S., Pierantozzi, E., Fugassa, E., Leoni, S., 2005. Thyroid status affects rat liver regeneration after partial hepatectomy by regulating cell cycle and apoptosis. Cell. Physiol. Biochem. 15, 069–076. https://doi.org/10.1159/000083639.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., Zoghbi, H.Y., 1999. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat. Genet. 23, 185–188. https://doi.org/10.1038/13810.

Arita, A., Costa, m, 2014. Oxidative stress and the epigenome in human disease. J. Genetics Genome Res. 1, 010.

Astwood, E.B., 1984. Landmark article May 8, 1943: treatment of hyperthyroidism with thiourea and thiouracil. By E.B. Astwood. J. Am. Med. Assoc. 251, 1743–1746.

Attwood, J.T., Yung, R.L., Richardson, B.C., 2002. DNA methylation and the regulation of gene transcription. Cell. Mol. Life Sci. 59, 241–257.

Baccarelli, A., Bollati, V., 2009. Epigenetics and environmental chemicals. Curr. Opin. Pediatr. 21, 243–251.

Badria, F.A., Ibrahim, A.S., Badria, A.F., Elmarakby, A.A., 2015. Curcumin attenuates iron accumulation and oxidative stress in the liver and spleen of chronic iron-overloaded rats. PloS One 10, e0134156. https://doi.org/10.1371/journal.pone.0134156.

Barreto, G., Schäfer, A., Marhold, J., Stach, D., Swaminathan, S.K., Handa, V., Döderlein, G., Maltry, N., Wu, W., Lyko, F., Niehrs, C., 2007. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445, 671–675. https://doi.org/10.1038/nature05515.

Bieging, K.T., Mello, S.S., Attardi, L.D., 2014. Unravelling mechanisms of p53-mediated tumour suppression. Nat. Rev. Canc. 14, 359–370. https://doi.org/10.1038/nrc3711.

Birkenmeier, E.H., Gwynn, B., Howard, S., Jerry, J., Gordon, J.I., Landschulz, W.H., McKnight, S.L., 1989. Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. Genes Dev. 3, 1146–1156.

Bogdanović, O., Veenstra, G.J.C., 2009. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. Chromosoma 118, 549–565. https://doi.org/10.1007/s00412-009-0221-9.

Boyanapalli, S.S.S., Kong, A.-N.T., 2015. "Curcumin, the king of spices": epigenetic regulatory mechanisms in the prevention of cancer, neurological, and inflammatory diseases. Curr. Pharmacol. Reports 1, 129–139. https://doi.org/10.1007/s40495-015-0018-x.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bulku, E., Stohs, S.J., Cicero, L., Brooks, T., Halley, H., Ray, S.D., 2012. Curcumin exposure modulates multiple pro-apoptotic and anti-apoptotic signaling pathways to antagonize acetaminophen-induced toxicity. Curr. Neurovascular Res. 9, 58–71. https://doi.org/10.2174/156720212799297083.

Bunker, S.K., Dandapat, J., Chainy, G.B.N., Sahoo, S.K., Nayak, P.K., 2017. Neonatal exposure to 6-n-Propyl-Thiouracil, an anti-thyroid drug, alters expression of hepatic DNA methyltransferases, methyl CpG-binding proteins, Gadd45a, p53, and PCNA in adult male rats. Europ. Thyroid J. 6, 281–291. https://doi.org/10.1159/000479681.

Bunker, S.K., Dandapat, J., Sahoo, S.K., Roy, A., Chainy, G.B.N., 2016. Neonatal persistent exposure to 6-Propyl-2-thiouracil, a thyroid-disrupting chemical, differentially modulates expression of hepatic catalase and C/EBP-β in adult rats: PTU modulates catalase and C/EBP-β expressions. J. Biochem. Mol. Toxicol. 30, 80–90. https://doi.org/10.1002/jbt.21766.

Campos, A.C.E., Molognoni, F., Melo, F.H.M., Galdieri, L.C., Carneiro, C.R.W., D'Almeida, V., Correa, M., Jasiulionis, M.G., 2007. Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. Neoplasia 9, 1111–1121.

Cencioni, C., Spallotta, F., Martelli, F., Valente, S., Mai, A., Zeiher, A., Gaetano, C., 2013. Oxidative stress and epigenetic regulation in ageing and age-related diseases. Int. J. Mol. Sci. 14, 17643–17663. https://doi.org/10.3390/ijms140917643.

Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., Zoghbi, H.Y., 2008. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320, 1224–1229. https://doi.org/10.1126/science.1153252.

Clouaire, T., Stancheva, I., 2008. Methyl-CpG binding proteins: specialized transcriptional repressors or structural components of chromatin? Cell. Mol. Life Sci. 65, 1509–1522. https://doi.org/10.1007/s00018-008-7324-y.

Connolly, K.M., Bogdanffy, M.S., 1993. Evaluation of proliferating cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation in rat liver: a dual-stain comparison with 5-bromo-2'-deoxyuridine. J. Histochem. Cytochem. 41, 1–6. https://doi.org/10.1177/41.1.7678022.

Cooper, D.S., 2005. Antithyroid drugs. N. Engl. J. Med. 352, 905–917. https://doi.org/10.1056/NEJMra042972.

Creppy, E.E., Traoré, A., Baudrimont, I., Cascante, M., Carratú, M.-R., 2002. Recent advances in the study of epigenetic effects induced by the phycotoxin okadaic acid. Toxicology 181–182, 433–439.

Crosson, S.M., Davies, G.F., Roesler, W.J., 1997. Hepatic expression of CCAAT/enhancer binding protein alpha: hormonal and metabolic regulation in rats. Diabetologia 40, 1117–1124.

Das, K., Chainy, G.B., 2001. Modulation of rat liver mitochondrial antioxidant defence system by thyroid hormone. Biochim. Biophys. Acta 1537, 1–13.

Defossez, P.-A., Stancheva, I., 2011. Biological functions of methyl-CpG-binding proteins. In: Progress in Molecular Biology and Translational Science. Elsevier, pp. 377–398. https://doi.org/10.1016/B978-0-12-387685-0.00012-3.

Donkena, K.V., Young, C.Y.F., Tindall, D.J., 2010. Oxidative stress and DNA methylation in prostate cancer. Obstet. Gynecol. Int. 2010, 1–14. https://doi.org/10.1155/2010/302051.

Du, J., Zhou, N., Liu, H., Jiang, F., Wang, Y., Hu, C., Qi, H., Zhong, C., Wang, X., Li, Z., 2012. Arsenic Induces Functional Re-Expression of Estrogen Receptor α by

Demethylation of DNA in Estrogen Receptor-Negative Human Breast Cancer. PloS One 7, e35957. https://doi.org/10.1371/journal.pone.0035957.
El-Demerdash, F.M., Yousef, M.I., Radwan, F.M.E., 2009. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. Food Chem. Toxicol. 47, 249–254. https://doi.org/10.1016/j.fct.2008.11.013.
Esfandiari, F., 2003. Methyl deficiency causes reduction of the methyl-CpG-binding protein, MeCP2, in rat liver. Carcinogenesis 24, 1935–1940. https://doi.org/10.1093/carcin/bgg163.
Fabbrini, E., Sullivan, S., Klein, S., 2010. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 51, 679–689. https://doi.org/10.1002/hep.23280.
Feil, R., Fraga, M.F., 2012. Epigenetics and the environment: emerging patterns and implications. Nat. Rev. Genet. 13, 97–109. https://doi.org/10.1038/nrg3142.
Feng, B., Ruiz, M.A., Chakrabarti, S., 2013. Oxidative-stress-induced epigenetic changes in chronic diabetic complications. Can. J. Physiol. Pharmacol. 91, 213–220. https://doi.org/10.1139/cjpp-2012-0251.
Feng, H.-W., Jiang, T., Zhang, H.-P., Wang, Z., Zhang, H.-L., Zhang, H., Chen, X.-M., Fan, X.-L., Tian, Y.-D., Jia, T., 2015. Comparisons of thyroid hormone, intelligence, attention, and quality of Life in children with obstructive sleep apnea hypopnea syndrome before and after endoscopic adenoidectomy. BioMed Res. Int. 2015, 1–6. https://doi.org/10.1155/2015/523716.
Fujita, N., Takebayashi, S., Okumura, K., Kudo, S., Chiba, T., Saya, H., Nakao, M., 1999. Methylation-mediated transcriptional silencing in euchromatin by methyl-CpG binding protein MBD1 isoforms. Mol. Cell Biol. 19, 6415–6426.
Guillaumet-Adkins, A., Yañez, Y., Peris-Diaz, M.D., Calabria, I., Palanca-Ballester, C., Sandoval, J., 2017. Epigenetics and oxidative stress in aging. Oxid. Med. Cellular Longevity 2017, 1–8. https://doi.org/10.1155/2017/9175806.
Gupta, S.C., Patchva, S., Koh, W., Aggarwal, B.B., 2012. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clin. Exp. Pharmacol. Physiol. 39, 283–299. https://doi.org/10.1111/j.1440-1681.2011.05648.x.
Hackmon, R., Blichowski, M., Koren, G., 2012. Motherisk rounds. J. Obstet. Gynaecol. Can. 34, 1077–1086. https://doi.org/10.1016/S1701-2163(16)35438-X.
Heidari, R., Niknahad, H., Jamshidzadeh, A., Eghbal, M.A., Abdoli, N., 2015. An Overview on the Proposed Mechanisms of Antithyroid Drugs-induced Liver Injury. Advanced Pharmaceutical Bulletin eISSN 2251-7308. https://doi.org/10.5681/apb.2015.001.
Hendrich, B., Bird, A., 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol. Cell Biol. 18, 6538–6547.
Hendrich, B., Hardeland, U., Ng, H.-H., Jiricny, J., Bird, A., 1999. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 401, 301–304. https://doi.org/10.1038/45843.
Hermann, A., Gowher, H., Jeltsch, A., 2004. Biochemistry and biology of mammalian DNA methyltransferases. Cell. Mol. Life Sci. 61, 2571–2587. https://doi.org/10.1007/s00018-004-4201-1.
Hollander, M.C., Fornace, A.J., 2002. Genomic instability, centrosome amplification, cell cycle checkpoints and Gadd45a. Oncogene 21, 6228–6233. https://doi.org/10.1038/sj.onc.1205774.
Horvath, P.M., Monteggia, L.M., 2018. MeCP2 as an activator of gene expression. Trends Neurosci. 41, 72–74. https://doi.org/10.1016/j.tins.2017.11.005.
Huang, H.-C., Zheng, B.-W., Guo, Y., Zhao, J., Zhao, J.-Y., Ma, X.-W., Jiang, Z.-F., 2016. Antioxidative and neuroprotective effects of curcumin in an alzheimer's disease rat model Co-treated with intracerebroventricular streptozotocin and subcutaneous D-galactose. J. Alzheim. Dis. 52, 899–911. https://doi.org/10.3233/JAD-150872.
Im, H.-I., Hollander, J.A., Bali, P., Kenny, P.J., 2010. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat. Neurosci. 13, 1120–1127. https://doi.org/10.1038/nn.2615.
Itahana, K., Dimri, G.P., Hara, E., Itahana, Y., Zou, Y., Desprez, P.-Y., Campisi, J., 2002. A role for p53 in maintaining and establishing the quiescence growth arrest in human cells. J. Biol. Chem. 277, 18206–18214. https://doi.org/10.1074/jbc.M201028200.
Jaenisch, R., Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat. Genet. 33, 245–254. https://doi.org/10.1038/ng1089.
Jin, S., Tong, T., Fan, W., Fan, F., Antinore, M.J., Zhu, X., Mazzacurati, L., Li, X., Petrik, K.L., Rajasekaran, B., Wu, M., Zhan, Q., 2002. GADD45-induced cell cycle G2-M arrest associates with altered subcellular distribution of cyclin B1 and is independent of p38 kinase activity. Oncogene 21, 8696–8704. https://doi.org/10.1038/sj.onc.1206034.
Jones, P.L., Jan Veenstra, G.C., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J., Wolffe, A.P., 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat. Genet. 19, 187–191. https://doi.org/10.1038/561.
Kaur, G., Tirkey, N., Bharrhan, S., Chanana, V., Rishi, P., Chopra, K., 2006. Inhibition of oxidative stress and cytokine activity by curcumin in amelioration of endotoxin-induced experimental hepatoxicity in rodents. Clin. Exp. Immunol. 145, 313–321. https://doi.org/10.1111/j.1365-2249.2006.03108.x.
Kruse, J.-P., Gu, W., 2009. Modes of p53 regulation. Cell 137, 609–622. https://doi.org/10.1016/j.cell.2009.04.050.
Kyle, S.M., Saha, P.K., Brown, H.M., Chan, L.C., Justice, M.J., 2016. MeCP2 co-ordinates liver lipid metabolism with the NCoR1/HDAC3 corepressor complex. Hum. Mol. Genet ddw156. https://doi.org/10.1093/hmg/ddw156.
Ladenson, P.W., Kieffer, J.D., Farwell, A.P., Ridgway, E.C., 1986. Modulation of myocardial L-triiodothyronine receptors in normal, hypothyroid, and hyperthyroid rats. Metab. Clin. Exp. 35, 5–12.
Landau, R.L., 1984. Landmark perspective: treatment of hyperthyroidism. J. Am. Med. Assoc. 251, 1747–1748.
Lekstrom-Himes, J., Xanthopoulos, K.G., 1998. Biological role of the CCAAT/Enhancer-binding protein family of transcription factors. J. Biol. Chem. 273, 28545–28548. https://doi.org/10.1074/jbc.273.44.28545.
Liebermann, D.A., Hoffman, B., 2008. Gadd45 in stress signaling. J. Mol. Signal. 3, 15. https://doi.org/10.1186/1750-2187-3-15.
Liu, D., Xu, Y., 2011. p53, oxidative stress, and aging. Antioxidants Redox Signal. 15, 1669–1678. https://doi.org/10.1089/ars.2010.3644.
Liu, Z., Xie, Z., Jones, W., Pavlovicz, R.E., Liu, S., Yu, J., Li, P., Lin, J., Fuchs, J.R., Marcucci, G., Li, C., Chan, K.K., 2009. Curcumin is a potent DNA hypomethylation agent. Bioorg. Med. Chem. Lett 19, 706–709. https://doi.org/10.1016/j.bmcl.2008.12.041.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Lyko, F., 2017. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat. Rev. Genet. 19, 81–92. https://doi.org/10.1038/nrg.2017.80.
Macip, S., Kosoy, A., Lee, S.W., O'Connell, M.J., Aaronson, S.A., 2006. Oxidative stress induces a prolonged but reversible arrest in p53-null cancer cells, involving a Chk1-dependent G2 checkpoint. Oncogene 25, 6037–6047. https://doi.org/10.1038/sj.onc.1209629.
Mailand, N., Gibbs-Seymour, I., Bekker-Jensen, S., 2013. Regulation of PCNA–protein interactions for genome stability. Nat. Rev. Mol. Cell Biol. 14, 269–282. https://doi.org/10.1038/nrm3562.
Malik, R., Hodgson, H., 2002. The relationship between the thyroid gland and the liver. QJM 95, 559–569.
Martelli, A., Mattioli, F., Fazio, S., Andrae, U., Brambilla, G., 1995. DNA repair synthesis and DNA fragmentation in primary cultures of human and rat hepatocytes exposed to cyproterone acetate. Carcinogenesis 16, 1265–1269.
Menon, V.P., Sudheer, A.R., 2007. Antioxidant and anti-inflammatory properties of curcumin. In: Aggarwal, B.B., Surh, Y.-J., Shishodia, S. (Eds.), The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Springer US, Boston, MA, pp. 105–125. https://doi.org/10.1007/978-0-387-46401-5_3.
Miceli, M., Bontempo, P., Nebbioso, A., Altucci, L., 2014. Natural compounds in epigenetics: a current view. Food Chem. Toxicol. 73, 71–83. https://doi.org/10.1016/j.fct.2014.08.005.
Mikhed, Y., Görlach, A., Knaus, U.G., Daiber, A., 2015. Redox regulation of genome stability by effects on gene expression, epigenetic pathways and DNA damage/repair. Redox Biology 5, 275–289. https://doi.org/10.1016/j.redox.2015.05.008.
Miller, D., Briggs, D., Snowden, H., Hamlington, J., Rollinson, S., Lilford, R., Krawetz, S.A., 1999. A complex population of RNAs exists in human ejaculate spermatozoa: implications for understanding molecular aspects of spermiogenesis. Gene 237, 385–392.
Mirza, S., Sharma, G., Parshad, R., Gupta, S.D., Pandya, P., Ralhan, R., 2013. Expression of DNA methyltransferases in breast cancer patients and to analyze the effect of natural compounds on DNA methyltransferases and associated proteins. J. Breast Cancer 16, 23. https://doi.org/10.4048/jbc.2013.16.1.23.
Moldovan, G.-L., Pfander, B., Jentsch, S., 2007. PCNA, the Maestro of the replication fork. Cell 129, 665–679. https://doi.org/10.1016/j.cell.2007.05.003.
Muller, J., Heuer, H., 2014. Expression pattern of thyroid hormone transporters in the postnatal mouse brain. Front. Endocrinol. 5. https://doi.org/10.3389/fendo.2014.00092.
Nakamura, H., Noh, J.Y., Itoh, K., Fukata, S., Miyauchi, A., Hamada, N., 2007. Comparison of methimazole and propylthiouracil in patients with hyperthyroidism caused by Graves' disease. J. Clin. Endocrinol. Metabol. 92, 2157–2162. https://doi.org/10.1210/jc.2006-2135.
Nan, X., Campoy, F.J., Bird, A., 1997. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88, 471–481.
Noorafshan, A., Ashkani-Esfahani, S., 2013. A review of therapeutic effects of curcumin. Curr. Pharmaceut. Des. 19, 2032–2046.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Oppenheimer, J.H., Schwartz, H.L., Mariash, C.N., Kinlaw, W.B., Wong, N.C.W., Freake, H.C., 1987. Advances in our understanding of thyroid hormone action at the cellular level*. Endocr. Rev. 8, 288–308. https://doi.org/10.1210/edrv-8-3-288.
Palma, C.C.S.S.V., Pavesi, M., Nogueira, V., Clemente, E.L., Vasconcellos, M. de F.B.M.P., Pereira, L., Pacheco, F., Braga, T., Bello, L., Soares, J., dos Santos, S.C., Campos, V.P.L.C., Gomes, M., 2013. Prevalence of thyroid dysfunction in patients with diabetes mellitus. Diabetol. Metab. Syndrome 5, 58. https://doi.org/10.1186/1758-5996-5-58.
Paunesku, T., Mittal, S., Protić, M., Oryhon, J., Korolev, S.V., Joachimiak, A., Woloschak, G.E., 2001. Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. Int. J. Radiat. Biol. 77, 1007–1021. https://doi.org/10.1080/09553000110069335.
Pedersen, T.Å., Bereshchenko, O., Garcia-Silva, S., Ermakova, O., Kurz, E., Mandrup, S., Porse, B.T., Nerlov, C., 2007. Distinct C/EBPα motifs regulate lipogenic and gluconeogenic gene expression in vivo. EMBO J. 26, 1081–1093. https://doi.org/10.1038/sj.emboj.7601563.
Peng, S., Xu, N., Huang, L., Gao, X., Hu, W., Yu, R., Liu, H., 2011. The gene expressions of DNA methylation/demethylation enzymes and cytochrome c oxidase subunit 4 in skeletal muscle of thyroidectomized rats. Afr. J. Biotechnol. 10, 730–733.
Perandones, C.E., Illera, V.A., Peckham, D., Stunz, L.L., Ashman, R.F., 1993. Regulation of apoptosis in vitro in mature murine spleen T cells. J. Immunol. 151, 3521–3529.
Phillips, T., 2008. The role of methylation in gene expression. Nat. Educ. 1, 116.
Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., Ramirez-Tortosa, Mc, 2016. Curcumin and health. Molecules 21, 264. https://doi.org/10.3390/molecules21030264.
Rao, D.S., Sekhara, N.C., Satyanarayana, M.N., Srinivasan, M., 1970. Effect of curcumin

on serum and liver cholesterol levels in the rat. J. Nutr. 100, 1307–1315. https://doi.org/10.1093/jn/100.11.1307.
Reddy, A.C., Lokesh, B.R., 1996. Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. Toxicology 107, 39–45.
Ruby, A.J., Kuttan, G., Babu, K.D., Rajasekharan, K.N., Kuttan, R., 1995. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett. 94, 79–83.
Sahoo, S.K., Chainy, G.B.N., Dandapat, J., 2015. Neonatal hypothyroidism alters expression of antioxidant enzymes and redox status in adult rat seminiferous tubule cells. Curr. Trends Biotechnol. Pharm. 9, 117–131.
Sahoo, S.K., Dandpat, J., Chainy, G.B.N., 2017. Differential expression of apoptotic proteins in seminiferous tubule cells of adult rats by neonatal exposure to 6-n-propyl-2-thiouracil (PTU), a thyroid disrupting chemical. Indian J. Exp. Biol. 55, 634–641.
Samuhasaneeto, S., Thong-Ngam, D., Kulaputana, O., Suyasunanont, D., Klaikeaw, N., 2009. Curcumin decreased oxidative stress, inhibited NF- κ B activation, and improved liver pathology in ethanol-induced liver injury in rats. J. Biomed. Biotechnol. 2009, 1–8. https://doi.org/10.1155/2009/981963.
Schrem, H., 2004. Liver-enriched transcription factors in liver function and development. Part II: the C/EBPs and D site-binding protein in cell cycle control, carcinogenesis, circadian gene regulation, liver regeneration, apoptosis, and liver-specific gene regulation. Pharmacol. Rev. 56, 291–330. https://doi.org/10.1124/pr.56.2.5.
Screaton, R.A., Kiessling, S., Sansom, O.J., Millar, C.B., Maddison, K., Bird, A., Clarke, A.R., Frisch, S.M., 2003. Fas-associated death domain protein interacts with methyl-CpG binding domain protein 4: a potential link between genome surveillance and apoptosis. Proc. Natl. Acad. Sci. Unit. States Am. 100, 5211–5216. https://doi.org/10.1073/pnas.0431215100.
Shi, J., Aisaki, K., Ikawa, Y., Wake, K., 1998. Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. Am. J. Pathol. 153, 515–525. https://doi.org/10.1016/S0002-9440(10)65594-0.
Singh, N.P., Muller, C.H., Berger, R.E., 2003. Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil. Steril. 80, 1420–1430.
Sinha, R.A., Singh, B.K., Yen, P.M., 2014. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. Trends Endocrinol. Metabol. 25, 538–545. https://doi.org/10.1016/j.tem.2014.07.001.
Squillaro, T., Alessio, N., Cipollaro, M., Renieri, A., Giordano, A., Galderisi, U., 2010. Partial silencing of methyl cytosine protein binding 2 (*MECP2*) in mesenchymal stem cells induces senescence with an increase in damaged DNA. Faseb. J. 24, 1593–1603. https://doi.org/10.1096/fj.09-143057.
Strzalka, W., Ziemienowicz, A., 2011. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. Ann. Bot. 107, 1127–1140. https://doi.org/10.1093/aob/mcq243.
Subudhi, U., Chainy, G.B.N., 2012. Curcumin and vitamin E modulate hepatic antioxidant gene expression in PTU-induced hypothyroid rats. Mol. Biol. Rep. 39, 9849–9861. https://doi.org/10.1007/s11033-012-1851-1.
Subudhi, U., Chainy, G.B.N., 2010. Expression of hepatic antioxidant genes in l-thyroxine-induced hyperthyroid rats: regulation by vitamin E and curcumin. Chem. Biol. Interact. 183, 304–316. https://doi.org/10.1016/j.cbi.2009.11.004.
Subudhi, U., Das, K., Paital, B., Bhanja, S., Chainy, G.B.N., 2009. Supplementation of curcumin and vitamin E enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats. Life Sci. 84, 372–379. https://doi.org/10.1016/j.lfs.2008.12.024.
Tamura, R.E., de Vasconcellos, J.F., Sarkar, D., Libermann, T.A., Fisher, P.B., Zerbini, L.F., 2012. GADD45 proteins: central players in tumorigenesis. Curr. Mol. Med. 12, 634–651.
Tudor, M., Akbarian, S., Chen, R.Z., Jaenisch, R., 2002. Nonlinear partial differential equations and applications: transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc. Natl. Acad. Sci. Unit. States Am. 99, 15536–15541. https://doi.org/10.1073/pnas.242566899.
Turek-Plewa, J., Jagodziński, P.P., 2005. The role of mammalian DNA methyltransferases in the regulation of gene expression. Cell. Mol. Biol. Lett. 10, 631–647.
Vairapandi, M., Balliet, A.G., Hoffman, B., Liebermann, D.A., 2002. GADD45b and GADD45g are cdc2/cyclinB1 kinase inhibitors with a role in S and G2/M cell cycle checkpoints induced by genotoxic stress. J. Cell. Physiol. 192, 327–338. https://doi.org/10.1002/jcp.10140.
Valinluck, V., 2004. Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). Nucleic Acids Res. 32, 4100–4108. https://doi.org/10.1093/nar/gkh739.
Venditti, P., Meo, S.D., 2006. Thyroid hormone-induced oxidative stress. Cell. Mol. Life Sci. 63, 414–434. https://doi.org/10.1007/s00018-005-5457-9.
Venza, M., Visalli, M., Beninati, C., De Gaetano, G.V., Teti, D., Venza, I., 2015. Cellular mechanisms of oxidative stress and action in Melanoma. Oxid. Med. Cellular Longevity 2015, 1–11. https://doi.org/10.1155/2015/481782.
Vitug, A.C., Goldman, J.M., 1985. Hepatotoxicity from antithyroid drugs. Horm. Res. 21, 229–234. https://doi.org/10.1159/000180054.
Wills, E.D., 1969. Lipid peroxide formation in microsomes. Relationship of hydroxylation to lipid peroxide formation. Biochem. J. 113, 333–341.
Yen, P.M., 2001. Physiological and molecular basis of thyroid hormone action. Physiol. Rev. 81, 1097–1142. https://doi.org/10.1152/physrev.2001.81.3.1097.
Yoo, J., Medina-Franco, J.L., 2011. Homology modeling, docking and structure-based pharmacophore of inhibitors of DNA methyltransferase. J. Comput. Aided Mol. Des. 25, 555–567. https://doi.org/10.1007/s10822-011-9441-1.
Zhan, Q., 2005. Gadd45a, a p53- and BRCA1-regulated stress protein, in cellular response to DNA damage. Mutat. Res. Fund Mol. Mech. Mutagen 569, 133–143. https://doi.org/10.1016/j.mrfmmm.2004.06.055.
Zoeller, R.T., Crofton, K.M., 2005. Mode of action: developmental thyroid hormone insufficiency–neurological abnormalities resulting from exposure to propylthiouracil. Crit. Rev. Toxicol. 35, 771–781.