Original Article

Alteration of gut microbial community after N,N-Dimethylformamide exposure

Man Zhang, Min Zheng, Zhijun Wu, Mingyue Guan, Shuai Liu, Wenjin Zhao
and Juan Cheng

*National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention,
Beijing 100050, China*

(Received May 23, 2016; Accepted January 20, 2017)

ABSTRACT — N,N-Dimethylformamide (DMF), a solvent commonly used in factories, can induce liver toxicities, including hepatitis, fibrosis, cirrhosis and hepatoma. It is well known that the gut microbial community plays a role in the metabolism of many toxic substance and in liver regeneration. However, the effect of DMF on rat gut microbial community is poorly understood. The gut microbiotas in control rats and rats exposed to DMF were characterized by high-throughput sequencing of the bacterial 16S rRNA gene. The levels of biochemical parameters in the serum of rats, including cholesterol, bile acid, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were evaluated. The weight was lower in the DMF exposure group than in the control group. DMF exposure led to changes in gut microbiotas that were reflected in a decreased abundance of Prevotellaceae, Lactobacillaceae, and increased abundance of S24-7, Bacteroidaceae, Rikenellaceae and Peptostreptococcaceae. Compared with control group, the cholesterol level was substantially reduced in the DMF exposure group ($p < 0.05$), while the concentration of bile acid was significantly increased in the DMF exposure group ($p < 0.05$). The present data established that the gut microbiotasy were changed after DMF exposure, and it revealed the relationship between DMF and gut microbiotas for the first time.

Key words: N,N-Dimethylformamide, DMF, High-throughput sequencing, Liver damage,
Gut microbial community

INTRODUCTION

N,N-Dimethylformamide (DMF), commonly used in factories handling polyurethane materials and acrylic fibers and in the processes of the production of chlordimeform, synthetic leathers, films and fibers, can lead to liver toxicities, including hepatitis, fibrosis, cirrhosis and hepatoma (He *et al.*, 2015; Kim and Chung, 2013). Exposure in the occupational environment may occur when working in leather manufacturing factories. To date, there are more than 2000 such factories in the east-southern areas of China (Zhang *et al.*, 2014) with possibly thousands of product lines, which could produce above 400 million meters of leather annually. The absorption of DMF through the lungs, skin and digestive tract could stimulate local reactions. Symptoms of DMF exposure include fatigue, nausea, vomiting and dizziness and other adverse reactions. Serious toxic liver injury may also

occur in humans after acute poisoning of DMF, with abnormal changes of serum parameters (Hamada *et al.*, 2009).

Despite considerable attention drawn to studying toxic effects of occupational exposure to DMF, mechanisms of DMF-induced liver toxicity is still unclear. Emerging evidence suggests that human gut microbes, which have coevolved with humans, play a key role in many host metabolic, nutritional, physiological, immunological and pathological processes, and perturbations in the gut microbial community impact human health (Gangarapu *et al.*, 2014). Although highly stable over time, gut microbiota may be influenced by a number of factors, including environmental stimulus, disease, diet, and age (Goel *et al.*, 2014). A recent study demonstrates that intestinal microbiota is apparently altered during liver regeneration, and bile acid, which plays a significant role in liver regeneration, interacted with microbes (Liu

et al., 2016). Evidence also suggests that different pathological condition of liver diseases, including viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease, may alter gut microbiota differently (Brenner *et al.*, 2015). DMF exposure results in liver toxicity and may also lead to altered gut microbiota. However, whether and how DMF influenced the gut microbial community is poorly understood. The objective of this study was to evaluate the effect of DMF on the gut microbial community in Wistar rats. This is the first study to demonstrate the shift of hepatic parameters and body weight gains in relation to gut microbiota in DMF-mediated liver damage.

MATERIALS AND METHODS

Animals and feces sample collection

The 15 male Wistar rats (180–200 g) used in this study were purchased from Beijing Vital River Laboratory Animal Technology Company and all procedures involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee of the Chinese Centre for Disease Control and Prevention (CDC). Rats were fed with standard rodent chow, had water available *ad libitum*, and were housed at the room temperature at 23°C with a 12-hr light-dark cycle. The rats were dosed orally with either 150 mg per body weight (mg/kg·bw) DMF (D4551, Sigma-Aldrich, St. Louis, MO, USA) ($n = 10$) or Millipore® water ($n = 5$) for 28 days. Their body weight was measured weekly. Feces were collected on the 28th day of exposure to DMF.

Sample preparation (DNA extraction, library construction & sequencing)

Feces DNA was extracted with TIANGEN® TIANamp Stool DNA kit (TIANGEN Biotech Co., Ltd, Beijing, China). The concentration and purity of the DNA were determined by spectrophotometry. Sequencing libraries were constructed using PCR primers (Klindworth *et al.*, 2013) designed to amplify the V4 hypervariable region of the 16S rRNA gene. The PCR-based library was constructed with 2 PCR cycles. The first PCR cycle was performed in a 25 µL solution containing 1 × KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Woburn, MA, USA), 0.25 µM each of forward primer and reverse primer, and 10ng template DNA. 2 × 300 bp sequencing, image processing and base calling was performed on the MiSeq.

Data processing (Quality filtering and picking of OTUs)

According to Mothur methods (Schloss *et al.*, 2009), optimal sequences were obtained by trimming the score less than 20, contained N bases, or homopolymer more than 10 bp from sequence reads, and by discarding mismatched (≥ 4 bp) sequences, less than 300 bp or more than 500 bp sequences. In addition, chimeras were removed by using UCHIME software (Edgar *et al.*, 2011).

We calculated the richness and α diversity of the samples at operational taxonomic unit (OTU) cutoffs of 0.03 distance units by using the numbers of OTU sequences, the numbers of observed OTUs, Chao, ACE estimated minimum number of OTUs, Good coverage, Shannon's index, nonparametric Shannon index and Simpson's index. Rarefaction curves were calculated for all the samples. We aligned the OTU sequences with SILVA database by using PyNAST (Caporaso *et al.*, 2010), built the phylogenetic tree with FastTree software (Price *et al.*, 2009), and generated the distance matrix between communities with UniFrac software (Lozupone and Knight, 2005). The clustering heatmap, principal component analysis (PCA) and nonmetric multi-dimensional scaling (NMDS) analysis were performed based on the distance matrix data.

Liver biochemical parameters assays

At the end of the study, on day 28, 2.0 mL of blood was drawn into ethylenediamine tetraacetic acid (EDTA)-treated tubes. All the blood samples were centrifuged at $3000 \times g$ for 10 min to separate plasma. Serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHO), bile acid (BA), triglyceride (TG) and lactic dehydrogenase (LDH), were analyzed using a biochemical analyzer (AU480; Beckman Coulter, Orange, FL, USA).

Statistical analysis

The values obtained from the samples collected from the control group were compared to those from the DMF exposure group. Student's t-test was performed for continuous variables with normal distribution and Wilcoxon rank test was used for continuous variables with skewed distribution. The Metastats statistical software was used to test the differences of species abundance between control group and DMF exposure group, and a cladogram was built by using GraPhlAn software. Body weight and liver biochemical parameters data were presented as mean $\pm$ S.D. and analyzed with GraphPad Prism version 6.01 (GraphPad Software Inc., La Jolla, CA, USA) P-values of < 0.05 were considered statistically significant.

RESULTS

Previous studies showed that exposure to DMF could reduce weight gain in humans and animals (Kelich *et al.*, 1995). Thus, we monitored body weight during 4 weeks of exposure to DMF (150 mg/kg-bw). As expected, there was no significant difference in the initial body weight between the 2 groups (Fig. 1). However, at week one, the DMF exposure group weighed significantly less ($p < 0.01$) than the control group, and the differences between the two increased over the course of the study.

Alpha diversity includes community richness, community evenness, and community diversity. The index of alpha diversity was calculated using the Mothur method. After strict quality control, 571124 valid sequences reads with an average length of 411 bp were obtained from the 15 samples (Table 1). Chao 1 richness, Good coverage rate and Shannon's index in the DMF exposure group were always higher than those in the control group, but the total bacterial richness estimated by Chao1 and the Good coverage rate did not significantly differ between the control and DMF groups (Fig. 2). We aligned OTU sequences with SILVA database by using PyNASt, constructed phylogenetic trees with FastTree (Table 2 and Fig. 3). Beta diversity data showed differences in bacterial community composition between control samples and DMF exposure samples, with certain OTUs demonstrating significant changes in relative abundance.

The most abundant phyla consisted of Bacteroidetes and Firmicutes, which accounted for $\geq 90\%$ of all

sequences (Figs. 4A and 4B). Bacteroidetes decreased while Firmicutes increased after 28 days' exposure to 150 mg/kg-bw DMF. Gut microbiota on the phylum level did not change much, whereas on the family level there were significant changes. At lower taxonomic levels, Lactobacillaceae, Christensenellaceae, Clostridiaceae, Lachnospiraceae, Peptostreptococcaceae, and Erysipelotrichaceae were the most abundant taxa within the Firmicutes phylum (Figs. 4C and 4D). Prevotellaceae, S24-7, Bacteroidaceae, Porphyromonadaceae, Rikenellaceae were the most abundant taxa within the Bacteroidetes phylum. Overall, There were 30 genera significantly changed after DMF exposure. Revotellaceae decreased dramatically in the control group, whereas Lactobacillaceae and Porphyromonadaceae decreased and S24-7 increased in the DMF exposure group. In addition, 2 unclassified taxa in the DMF exposure group were shifted: one, in Bacteroidales increased, while the other one in Bactobacillales decreased. These changes are depicted in a cladogram in Fig. 5. Details of each taxon, including name, means, SD and P value, are provided in Table 3.

The effects of DMF on serum AST, ALT, CHO, TG, bile acid, and LDH levels are represented in Fig. 6. The levels of CHO, and bile acid in serum were significantly increased in the DMF exposure group (2.16 ± 0.36 , 41.55 ± 11.09), as compared to the control group (1.66 ± 0.25 , 16.90 ± 7.37). After DMF exposure, the levels of AST, TG and LDH were significantly reduced (112.3 ± 3.27 ,

Fig. 1. Weight gain in control group and DMF exposure group during whole experiment period ($n = 5$ in control group, $n = 10$ in DMF exposure group). After exposure to 150 mg/kg-bw DMF, the body weight gain was slower than that of control group ($P < 0.01$). Values are shown as mean $\pm$ S.D.

Table 1. Numbers of reads in Control and DMF-exposure groups.

Name	total pairs	clean pairs	filter pairs
Control 1	42463	41102	39265
Control 2	42536	41252	39986
Control 3	52762	51238	48496
Control 4	44587	43383	40830
Control 5	51618	50147	46392
DMF 1	44612	43252	41321
DMF 2	32922	31886	30586
DMF 3	36710	35464	34053
DMF 4	45682	44360	42301
DMF 5	43692	42463	40162
DMF 6	97023	93841	89770
DMF 7	37835	36387	34771
DMF 8	65074	62592	60013
DMF 9	82326	79738	76560
DMF 10	39390	38160	36858

Fig. 2. Results of Alpha diversity index which including chao curve (a), good coverage curve (b), rarefaction curve (c) and Shannon curve (d).

0.50 ± 0.06 , 609.7 ± 80.94), when compared with the control group (164.7 ± 16.07 , 1.08 ± 0.19 , 1187.0 ± 151.2).

DISCUSSION

Clinical and animal studies have demonstrated that DMF exposure could result in liver damage, gastrointestinal disorders, and altered serum biochemical parameters in both human and animal models. The present study shows that DMF can also influence the gut microbiota composition. The gut and the liver are the key organs in nutrient absorption and metabolism, and the gut microbiota plays a critical role in liver diseases, such as alcoholic fatty liver disease, non-alcoholic fatty liver disease, liver cirrhosis and liver regeneration (Liu *et al.*, 2016). However, whether DMF, a industrial chemical used in leather

production, changed gut microbiota composition or not is uncertain. In the present study, we investigated the effect of DMF on the gut microbiota composition, which could offer an clue to how DMF caused liver damage.

Our findings indicate that oral exposure of male rats to DMF for 4 weeks reduced weight gain, increased the levels of CHO and BA, decreased the levels of AST, TG and LDH, while the level of ALT had no obvious change. Moreover, the composition of gut microbiotas was significant different in the DMF exposure group when compared with control group, especially on the family level. Increased Prevotellaceae and decreased S24-7 at family level were observed.

Previous studies show that body weights in both humans and animals after DMF exposure were lower than controls (Xing *et al.*, 2014). Consistent with these stud-

DMF exposure alters gut microbial community

Table 2. Results of Alpha diversity index.

Name	nseqs	sobs	chao	ace	coverage	shannon	npshannon	simpson
Control 1	32439	376	411	405	0.998459	3.645458	3.655585	0.075093
Control 2	33956	394	430	430	0.99838	3.691743	3.702418	0.084777
Control 3	40215	407	447	439	0.998707	3.894781	3.902808	0.06158
Control 4	33495	384	420	414	0.998537	3.849218	3.858464	0.072748
Control 5	36461	402	459	436	0.998492	3.821466	3.830202	0.081864
DMF 1	33209	439	460	462	0.998645	4.26225	4.272222	0.039507
DMF 2	24569	382	423	421	0.997639	4.008117	1.022533	0.061966
DMF 3	27628	404	454	456	0.997394	3.936179	3.95254	0.042314
DMF 4	34011	444	508	477	0.998383	4.476484	4.485034	0.031408
DMF 5	33042	408	447	437	0.998517	4.297863	4.307083	0.028759
DMF 6	73238	433	459	454	0.999467	4.053617	4.057539	0.044811
DMF 7	27748	365	400	405	0.99791	3.579377	3.593729	0.085692
DMF 8	47926	451	483	480	0.998957	4.009295	4.016524	0.057884
DMF 9	62484	476	497	497	0.999328	4.068461	4.073703	0.052351
DMF 10	30703	407	443	438	0.998718	3.881596	3.89298	0.072558

nseqs: number of sequences; sobs: OTU number; chao: diversity calculated with Chao method; ace: diversity calculated with ACE method; coverage: Good coverage; shannon: Shannon's index; npshannon: nonparametric Shannon's index; simpson: Simpson's index.

Fig. 3. Results of Beta diversity index which including UniFrac Heatmap (a), UniFrac PCA 3D plot (b), UniFrac PC1 vs. PC2 (c) and UniFrac NMDS (d).

Fig. 4. DMF exposure changed gut microbiota composition. After DMF exposure, gut microbiota community had no obvious change at the phylum level when compared with control group (a and b), while DMF exposure significantly changed gut microbiota composition at the family level as compared with control (c and d). The changes in gut microbiotas were reflected in a decreased abundance of Prevotellaceae, Lactobacillaceae, and increased abundance of S24-7, Bacteroidaceae, Rikenellaceae and Lachnospiraceae.

Fig. 5. DMF exposure induced statistically significant differences in the gut microbiome. Thirty taxonomy were significantly changed after 28 days' DMF exposure ($P < 0.05$). The data which the average of abundance means in Control and DMF exposure group was greater than 0.0001 were displayed with color (Green: greater in DMF exposure group; Red: greater in Control group). The two innermost dots represent the highest level of taxonomy are the kingdoms Archaea and Bacteria, followed outwards by the lower levels: phylum, class, order, family, genus and species.

DMF exposure alters gut microbial community

Table 3. Meta-analysis results in gut microbiome of DMF exposure rats compared with control group rats.

Taxonomy	Mean (Con)	SD (Con)	Mean (DMF)	SD (DMF)	p-value
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); Prevotellaceae (100); Prevotella (100);	0.26748	0.02202	0.19086	0.01830	0.01848
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); S24-7 (100); unclassified (100);	0.07875	0.00820	0.13863	0.01178	0.00130
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); unclassified (100); unclassified (100);	0.05180	0.00205	0.07536	0.00466	0.00076
Bacteria (100); Firmicutes (100); Bacilli (100); Lactobacillales (100); Lactobacillaceae (100); Lactobacillus (100);	0.02259	0.00185	0.01447	0.00305	0.03892
Bacteria (100); Firmicutes (100); Bacilli (100); Lactobacillales (100); unclassified (100); unclassified (100);	0.00638	0.00046	0.00291	0.00046	0.00024
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); Bacteroidaceae (100); Bacteroides (100);	0.00622	0.00087	0.01667	0.00414	0.02728
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); Porphyromonadaceae (100); Parabacteroides (100);	0.00229	0.00029	0.00144	0.00022	0.03412
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); Rikenellaceae (100); Alistipes (100);	0.00195	0.00024	0.00371	0.00027	0.00061
Bacteria (100); Firmicutes (100); Clostridia (100); Clostridiales (100); Peptostreptococcaceae (100); unclassified (100);	0.00094	0.00052	0.00277	0.00065	0.04653
Bacteria (100); Proteobacteria (100); Deltaproteobacteria (100); Desulfovibrionales (100); unclassified (100); unclassified (100);	0.00041	0.00008	0.00019	0.00004	0.02764
Bacteria (100); Actinobacteria (100); Coriobacteriia (100); Coriobacteriales (100); Coriobacteriaceae (100); unclassified (100);	0.00030	0.00005	0.00013	0.00003	0.00826
Bacteria (100); Firmicutes (100); Clostridia (100); Clostridiales (100); Lachnospiraceae (100); Anaerosporebacter (100);	0.00024	0.00004	0.00095	0.00028	0.02453
Bacteria (100); Firmicutes (100); Clostridia (100); Clostridiales (100); Christensenellaceae (100); unclassified (100);	0.00022	0.00008	0.00080	0.00023	0.03304
Bacteria (100); Firmicutes (100); Erysipelotrichia (100); Erysipelotrichales (100); Erysipelotrichaceae (100); Turicibacter (100);	0.00016	0.00012	0.00092	0.00026	0.02003
Bacteria (100); Firmicutes (100); Erysipelotrichia (100); Erysipelotrichales (100); Erysipelotrichaceae (100); unclassified (100);	0.00012	0.00003	0.00025	0.00004	0.02744
Bacteria (100); Proteobacteria (100); Alphaproteobacteria (100); unclassified (100); unclassified (100); unclassified (100);	0.00009	0.00002	0.00034	0.00011	0.03527
Bacteria (100); Acidobacteria (100); Acidobacteria (100); Subgroup_6 (100); unclassified (100); unclassified (100);	0.00006	0.00002	0.00001	0.00001	0.01155
Bacteria (100); Proteobacteria (100); Deltaproteobacteria (100); Desulfovibrionales (100); Desulfovibrionaceae (100); Desulfovibrio (100);	0.00002	0.00001	0.00050	0.00018	0.01858

Table 3. (Continued).

Taxonomy	Mean (Con)	SD (Con)	Mean (DMF)	SD (DMF)	p-value
Bacteria (100); Bacteroidetes (100); Bacteroidia (100); Bacteroidales (100); Porphyromonadaceae (100); Odoribacter (100);	0.00002	0.00002	0.00018	0.00007	0.03404
Bacteria (100); Firmicutes (100); Erysipelotrichia (100); Erysipelotrichales (100); Erysipelotrichaceae (100); Allobaculum (100);	0.00002	0.00001	0.00043	0.00018	0.04233
Bacteria (100); Proteobacteria (100); Alphaproteobacteria (100); Rhizobiales (100); unclassified (100); unclassified (100);	0.00002	0.00001	0.00000	0.00000	0.03331
Bacteria (100); Proteobacteria (100); Gammaproteobacteria (100); Xanthomonadales (100); Xanthomonadaceae (100); unclassified (100);	0.00002	0.00001	0.00000	0.00000	0.03331
Bacteria (100); Bacteroidetes (100); Sphingobacteriia (100); Sphingobacteriales (100); Chitinophagaceae (100); unclassified (100);	0.00002	0.00001	0.00000	0.00000	0.00883
Bacteria (100); Actinobacteria (100); Actinobacteria (100); Micrococcales (100); Brevibacteriaceae (100); Brevibacterium (100);	0.00002	0.00001	0.00000	0.00000	0.03331
Bacteria (100); Bacteroidetes (100); Flavobacteriia (100); Flavobacteriales (100); Flavobacteriaceae (100); Flavobacterium (100);	0.00002	0.00001	0.00000	0.00000	0.02879
Bacteria (100); Proteobacteria (100); Gammaproteobacteria (100); Xanthomonadales (100); unclassified (100); unclassified (100);	0.00001	0.00001	0.00000	0.00000	0.02879
Bacteria (100); Actinobacteria (100); Actinobacteria (100); Micrococcales (100); Intrasporangiaceae (100); unclassified (100);	0.00001	0.00001	0.00000	0.00000	0.02879
Bacteria (100); Firmicutes (100); Clostridia (100); Clostridiales (100); Clostridiaceae_1 (100); Clostridium_sensu_stricto_1 (100);	0.00001	0.00001	0.00018	0.00007	0.02620
Bacteria (100); Actinobacteria (100); Actinobacteria (100); Bifidobacteriales (100); Bifidobacteriaceae (100); Bifidobacterium (100);	0.00000	0.00000	0.00005	0.00002	0.04515
Bacteria (100); Tenericutes (100); Mollicutes (100); RF9 (100); unclassified (100); unclassified (100);	0.00000	0.00000	0.00005	0.00002	0.00400

ies, we also found that the weight gain in DMF exposure group was lower. Concurrently, high levels of CHO and BA were observed. Bile acids, amphipathic detergent-like molecules, are end-products of hepatic cholesterol catabolism, which are critical for solubilization and absorption of CHO, dietary lipids, and liposoluble vitamins. Moreover, bile acids promote the secretion of CHO from the liver. Bile acids are nutrient signaling molecules by activating a bile acid-sensing nuclear receptor, farnesoid X receptor (FXR), which regulates lipid, CHO, and glucose metabolism. Elevated levels of CHO and BA in the DMF-exposed group might be one of the reasons of the lower weight gain.

There has been increasing focus on the role of gut microbiota to explore the pathogenic mechanisms of many diseases. In our study, the proportion of S24-7 fam-

ily was significantly increased while the Prevotellaceae family was significantly reduced. Prevotellaceae are Gram-negative anaerobic rods, which involved in the etiology of periodontitis and commonly found in the human oral cavity (Downes and Wade, 2011). Previous studies showed that the proliferation of Prevotellaceae was inhibited by bile acid, which was consistent with the findings in present study. A recent study demonstrates that the abundance of Prevotellaceae in feces of Parkinson's Disease (PD) patients was significantly reduced when compared with controls (Scheperjans *et al.*, 2015), which might explain the relationship between DMF and central nervous system damage. In addition, S24-7, a Gram-negative bacillus, in the Bacteroidetes phylum, was significantly increased after DMF exposure as compared with the control group. A previous study demonstrated that the

DMF exposure alters gut microbial community

Fig. 6. DMF (150 mg/kg·bw) affects AST, ALT, CHO, TG, bile acid, and LDH levels. The levels of (b) AST, (e) TG and (f) LDH were significantly reduced after exposure to DMF, as compared with control group, while the levels of (c) CHO and (d) bile acid were significantly increased in DMF exposure group when compared with control group. The level of (a) ALT in serum showed no obvious changes between these two groups. Values are shown as mean ± S.D. Asterisks indicate significant differences from the control group (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

level of S24-7 was significantly reduced after a sedentary high-fat diet as compared with other treatment groups (Evans *et al.*, 2014), which could link the lower body weight to the increased S24-7 level. In mouse models of parenteral nutrition, enteral antibiotic treatment markedly attenuated parenteral nutrition-associated liver injury with significant reduction of S24-7 and Xu's studies illustrated that *Lentinula edodes*-derived polysaccharide (L2), which could enhance systemic and mucosal immunity, reduced the S24-7 family (Xu and Zhang, 2015). In addition, some studies reported that S24-7, which could produce LPS, stimulated Toll-like receptor 4 (TLR4) to active NF- κ B signal pathway, and was correlated positively with Mdr expression (Lu *et al.*, 2008). These data showed that DMF resulted in increased S24-7 and decreased Prevotellaceae, and it might be one of the reasons that why DMF

induced liver and brain damage.

In animal studies, alcohol-exposed animals had lower proportions of *Lactobacillus*, introducing *Lactobacillus* could relieve alcohol-induced liver injury and restore gut microbiota in both animals and humans (Bull-Otterson *et al.*, 2013; Komatsuzaki and Shima, 2012). Consistent with these studies, we found that the proportion of *Lactobacillaceae* was reduced after DMF exposure (3.09%), as compared with the control group (5.25%).

In this study, we have shown that after orally administered with 150 mg/kg·bw DMF, Wistar male rats had a lower body weight, gut microbiota communication was shifted with an increased S24-7 family and a decreased prevotellaceas family, and the serum parameters were altered with decreased levels of TG or LDH,

and increased levels of CHO or bile acid. Our study suggests that gut microbiota community after DMF exposure was significantly shifted, and it might play a critical role in DMF-induced liver damage. Restoring the normal intestinal macrobiota may have clinical potential to prevent or rescue DMF-induced toxicity.

ACKNOWLEDGMENTS

This work was supported by National Nature Science Foundation of China [grant number 81372963].

Authors' contributions: Juan CHENG and Man ZHANG designed the research; Man ZHANG, Min ZHENG, Zhijun WU, Mingyue Guan, Shuai LIU and Wenjin Zhao performed the research; Man ZHANG and Juan CHENG analyzed the data; Man ZHANG and Juan CHENG wrote the paper.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

- Brenner, D.A., Paik, Y.H. and Schnabl, B. (2015): Role of gut microbiota in liver disease. *J. Clin. Gastroenterol.*, **49**, Suppl 1, S25-S27.
- Bull-Ottersson, L., Feng, W., Kirpich, I., Wang, Y., Qin, X., Liu, Y., Gobejishvili, L., Joshi-Barve, S., Ayvaz, T., Petrosino, J., Kong, M., Barker, D., McClain, C. and Barve, S. (2013): Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS One*, **8**, e53028.
- Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L. and Knight, R. (2010): PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, **26**, 266-267.
- Downes, J. and Wade, W.G. (2011): *Prevotella fusca* sp. nov. and *Prevotella scopos* sp. nov., isolated from the human oral cavity. *Int. J. Syst. Evol. Microbiol.*, **61** (Pt 4), 854-858.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011): UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194-2200.
- Evans, C.C., LePard, K.J., Kwak, J.W., Stancukas, M.C., Laskowski, S., Dougherty, J., Moulton, L., Glawe, A., Wang, Y., Leone, V., Antonopoulos, D.A., Smith, D., Chang, E.B. and Ciancio, M.J. (2014): Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *PLoS One*, **9**, e92193.
- Gangarapu, V., Yildiz, K., Ince, A.T. and Baysal, B. (2014): Role of gut microbiota: obesity and NAFLD. *Turk. J. Gastroenterol.*, **25**, 133-140.
- Goel, A., Gupta, M. and Aggarwal, R. (2014): Gut microbiota and liver disease. *J. Gastroenterol. Hepatol.*, **29**, 1139-1148.
- Hamada, M., Abe, M., Tokumoto, Y., Miyake, T., Murakami, H., Hiasa, Y., Matsuura, B., Sato, K. and Onji, M. (2009): Occupational liver injury due to N,N-dimethylformamide in the synthetics industry. *Intern. Med.*, **48**, 1647-1650.
- He, J., Liu, J., Kong, Y., Yang, W. and Zhang, Z. (2015): Serum activities of liver enzymes in workers exposed to sub-TLV levels of dimethylformamide. *Int. J. Occup. Med. Environ. Health*, **28**, 395-398.
- Kelich, S.L., Mercieca, M.D. and Pohland, R.C. (1995): Developmental toxicity of N-methylformamide administered by gavage to CD rats and New Zealand white rabbits. *Fundam. Appl. Toxicol.*, **27**, 239-246.
- Kim, K.W. and Chung, Y.H. (2013): Hepatotoxicity in rats treated with dimethylformamide or toluene or both. *Toxicol. Res.*, **29**, 187-193.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F.O. (2013): Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic. Acids. Res.*, **41**, e1.
- Komatsuzaki, N. and Shima, J. (2012): Effects of live *Lactobacillus paracasei* on plasma lipid concentration in rats fed an ethanol-containing diet. *Biosci. Biotechnol. Biochem.*, **76**, 232-237.
- Liu, H.X., Rocha, C.S., Dandekar, S. and Yvonne Wan, Y.J. (2016): Functional analysis of the relationship between intestinal microbiota and the expression of hepatic genes and pathways during the course of liver regeneration. *J. Hepatol.*, **64**, 641-650.
- Lozupone, C. and Knight, R. (2005): UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.*, **71**, 8228-8235.
- Lu, Y.C., Yeh, W.C. and Ohashi, P.S. (2008): LPS/TLR4 signal transduction pathway. *Cytokine*, **42**, 145-151.
- Price, M.N., Dehal, P.S. and Arkin, A.P. (2009): FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.*, **26**, 1641-1650.
- Scheperjans, F., Aho, V., Pereira, P.A., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola-Rautio, J., Pohja, M., Kinnunen, E., Murros, K. and Auvinen, P. (2015): Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov. Disord.*, **30**, 350-358.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J. and Weber, C.F. (2009): Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.*, **75**, 7537-7541.
- Xing, M., Chen, Q., Lou, X., Zhang, M., Cai, D. and Xia, Y. (2014): [Toxicity of intragastrically administered N, N-dimethylformamide in female Wistar rats]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*, **32**, 370-373.
- Xu, X. and Zhang, X. (2015): *Lentinula edodes*-derived polysaccharide alters the spatial structure of gut microbiota in mice. *PLoS One*, **10**, e0115037.
- Zhang, Q., Huang, C., Wei, Y., Zhu, Q., Tian, W. and Wang, C. (2014): Risk assessment of N,N-dimethylformamide on residents living near synthetic leather factories. *Environ. Sci. Pollut. Res. Int.*, **21**, 3534-3539.