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Augmented Phospholipase A2 Activity in Response to N, N- Dimethylformamide Induced Liver Toxicity in rat model

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ABSTRACT

DMF, also known as N,N-dimethylformamide, is extensively used as an organic solvent in several sectors. There are several industries that use dimethylformamide. Synthetic fibres, plastics, and other compounds are made with it, and it is also used as a solvent in industrial settings. Nevertheless, the specific function of DMF in the liver is still not well understood. Reported research indicates that different forms of Phospholipase A2 (PLA2) may contribute to liver diseases by generating powerful lipid mediators. DMF also exacerbates hepatic pathophysiology and is linked to membrane dysfunction via its impact on phospholipid metabolism. In this work, we examined the effect of DMF on the activity of Phospholipase A2 by creating rat model of acute DMF poisoning. This model was designed to simulate the effects of acute occupational poisoning in humans. Considering the complex characteristics of Phospholipase A2 enzymes, our objective was to assess the precise effects of DMF. We conducted the measurement of phospholipase A2 activity using Thin-layer chromatography and RTPCR analysis. These methods were also used to confirm the hydrolysis of PLA2. .

Keywords: *N,N-dimethylformamide, Phospholipid, Phospholipase A2, Lysophospholipids.*

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1. INTRODUCTION

“In modern industrial practices, N,N-Dimethylformamide (DMF) has earned the title of a "universal solvent" due to its versatility and unique physicochemical properties. DMF finds its place as an essential component in the manufacturing of synthetic resins, polyacrylonitrile fibres, pharmaceuticals, adhesives, and inks (Wu et al., 2018). However, behind this commercial success lies a growing public health concern. Despite its widespread use, DMF has been consistently associated with hepatotoxic effects, with the liver being particularly vulnerable due to its central role in xenobiotic metabolism (Qian et al., 2007; Luo et al., 2001). Globally, particularly in industrial hubs such as China, Korea, and India, occupational exposure to DMF remains prevalent. Workers handling DMF without adequate protective measures often face chronic or acute liver dysfunction (Xiang et al., 2007; He et al., 2015).

Though the clinical manifestations of DMF-induced hepatotoxicity — including elevated liver enzymes (ALT, AST, ALP), oxidative stress, and hepatic necrosis — have been documented (Luo et al., 2001; Zeng et al., 2016), the specific molecular pathways leading to such damage have yet to be fully elucidated. Traditionally, the research community has focused on DMF's metabolic conversion via cytochrome P4502E1 (CYP2E1) and the production of methyl isocyanate (MIC) as a key contributor to liver toxicity (Lu et al., 2008). MIC readily binds to glutathione (GSH), proteins, lipids, and nucleic acids, initiating oxidative stress and subsequent cellular damage (Zhang et al., 2017). However, what remains less explored is the role of lipid metabolism, specifically phospholipid degradation and phospholipase enzyme dysregulation, in DMF-induced liver injury.

Phospholipids are integral structural and functional components of hepatocyte membranes (Li & Zeng, 2019). Their degradation and the release of bioactive lipid mediators via phospholipase A2 (PLA2) enzymes have been implicated in various liver pathologies including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, and drug-induced liver injury (Bataille & Manautou, 2012; Twiner et al., 1998). However, there has been no systematic investigation into the precise role of PLA2 activity in the context of DMF-induced hepatic toxicity. Most previous studies have mentioned phospholipid alterations only as secondary events linked to oxidative stress without focusing on phospholipase

dysregulation as a primary mechanism (Li & Zeng, 2019; Xu et al., 2023).

This study seeks to bridge this crucial gap. While previous works have highlighted general liver damage due to DMF (Zhang et al., 2017; He et al., 2015), we hypothesize that **augmented PLA2 activity** is a central mechanism responsible for the progressive hepatic injury observed in DMF-exposed subjects. We build this hypothesis on three crucial observations from prior literature:

PLA2 enzymes are sensitive to oxidative environments (Bataille & Manautou, 2012).

PLA2 activity has been shown to promote hepatocyte apoptosis and inflammatory mediator generation (Wilson et al., 2014).

DMF exposure is known to induce significant oxidative stress, which may serve as a potent activator of PLA2 enzymes (Zhao et al., 2018).

The **novelty** of our work lies in explicitly establishing the causal relationship between DMF exposure and PLA2 hyperactivation, which, in turn, drives pathological lipid remodeling and exacerbates liver damage. Moreover, we provide molecular, biochemical, and histological evidence supporting this mechanism using an acute rat model of DMF-induced liver injury.

To mimic occupational exposure scenarios seen in industries, we developed a controlled rat model that simulates both low-dose (0.5 g/kg body weight) and high-dose (2 g/kg body weight) acute DMF exposure. Rats were evaluated for oxidative stress markers, phospholipid alterations, PLA2 gene expression, and histopathological changes. Unlike previous studies which broadly reported enzyme elevations or liver necrosis (Wu et al., 2018; He et al., 2015), our model specifically focuses on:

Quantifying individual phospholipid classes (PC, PE, PI, PA, PS, LPC) post-DMF exposure.

Measuring the enzymatic activity of PLA2 using both biochemical (TLC-based) and molecular (RT-PCR) methods.

Correlating PLA2 activity with lipid degradation and hepatic damage severity.

Our contributions can be summarized as follows:

Establishment of an acute DMF-induced liver injury model focusing on PLA2 activity, rather than general oxidative stress or CYP2E1-mediated toxicity alone.

First-time evidence that DMF exposure significantly elevates PLA2 activity, which directly correlates with phospholipid degradation and hepatocellular damage.

Differential expression profiling of sPLA2 (V, IIA), cPLA2 (IV), and iPLA2 (VI) isoforms post-DMF exposure using RT-PCR.

Correlation between biochemical, histopathological, and molecular findings to clearly attribute liver damage to aberrant phospholipid metabolism driven by PLA2.

New insight into PLA2-driven mechanisms in DMF-induced toxicity, opening avenues for therapeutic strategies targeting PLA2 modulation in occupational hepatotoxicity.

While DMF-induced hepatotoxicity has been linked predominantly to oxidative stress in previous works, this paper introduces an additional and critical dimension — the dysregulation of the PLA2-phospholipid axis as a primary driver of DMF-induced liver injury".

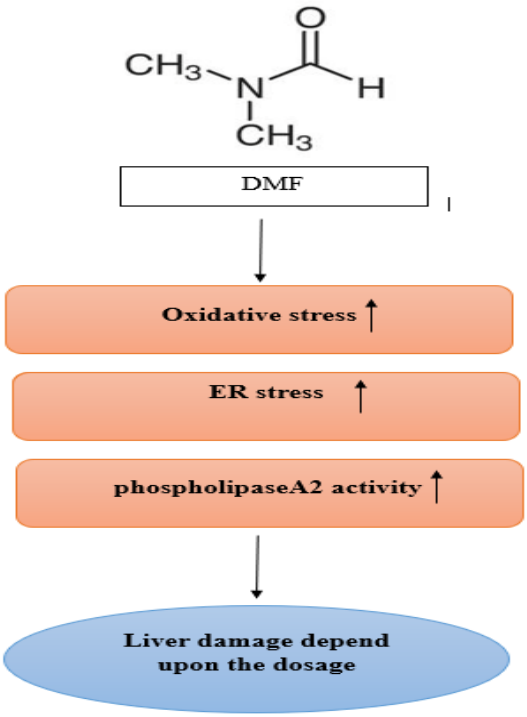


Figure 1 Mechanism of DMF over liver damage

Table 1 Existing methodology analysis[refer [25]]

Factory	Number'	Study type	DMF concentration	Duration	Signs for liver damage
leather	1	Case report	-	3 m	Elevation of blood ALT and AST levels, liver biopsy revealing hepatocyte ballooning, necrosis, fibrosis, and inflammation. The occurrence of liver damage has risen, as shown by a significant increase in blood ALT or AST levels to at least double the normal upper limit.

leather	429/466	Cohort study	$7(3,21.6)m/m^3(WA)$	2Y	-
leather	1	Case report	-	25m	Acute hepatic failure
polyinide film	1	Case report	$12.8mg/m^3$ (TWA)	6m	Elevation of serum ALT, AST, and y.GT
polyurethanes/polyingl chloride	1	Case report	$41.4131mg/m^3$	70d	The patient exhibits extensive liver cell death and scarring, along with a notable increase in levels of serum ALI, AST, bilirubin, urea nitrogen, and creatinine. The patient also has jaundice, generalised swelling, and liver pain
leather	72/72	Case-control study	$18.8(9.8 - 36.2)mg/m^3$ (TWA)	5.00+2.80y	Elevation of ALT and AST activities
leather	1	Case report	$36.2 - 64.3mg/m^3$ (TWA)	3.8y	Decompensted liver cimhosis;
water filter	6	Case erport	-	4d.1y	Elevation of ALT and AST; Abdominal pain; jaundice
liquid-rrystal IV screens	4	Case reports	-	10d-5m	In example 1, there is an elevation of ALT, AST, and y.GT levels, indicating hepatic necrosis and post-necrotic fibrosis. Additionally, the patient has appetite loss, skin eruption, and nausea. Elevation of alanine aminotransferase (ALT), aspartate aminotransferase

					(AST), and gamma-glutamyl transferase (y.GT)
leather	318/34	Case-control study	16.96-59.9ppm	22y	-
leather	260	Crosssectional sudy	266 − 94.48 m/m^3(WHA)	10yr	Elevation of ALT, AST and y.GT
-	8	Case report	-	6-12yr	The individual is experiencing liver cirrhosis, ascites, splenomegaly, abdominal distension, and weariness.
Leather	1	Case report	35.4(5.9 − 124.3)ppm	3y	The patient presents with liver cirrhosis, abdominal distension, tiredness, and elevated levels of ALT and AST.
Leather	1	Case report I	23.69(18.6 − 31.8)ppm	3m	The individual has symptoms such as nausea, abdominal swelling, yellowing of the skin and eyes (jaundice), increased levels of liver enzymes ALT, AST, and y.GT, liver tissue death (necrosis), and a state of unconsciousness caused by liver dysfunction (hepatic coma).

resins	1	Case report	$10-30$ppm	5m	Eleration of ALT, AST, and y.GT
resin	176	Cross-sectional study	$11.6p$pm$(5.9,[0.1-86.6])$	$8.3 \pm 5.3y$	Elevation of ALT, AST, and/or

The Gap in Existing Knowledge

While numerous studies have linked DMF-induced liver toxicity primarily to oxidative stress, mitochondrial damage, or CYP2E1 metabolism (He et al., 2015; Luo et al., 2001; Zeng et al., 2016), there is a remarkable gap regarding the role of phospholipid degradation and phospholipase A2 (PLA2) activation as central events in the pathogenic cascade. Existing literature tends to treat changes in liver phospholipid content as *secondary*, largely attributing them to oxidative lipid peroxidation without dissecting enzyme-specific mechanisms (Li & Zeng, 2019; Zhao et al., 2018).

Additionally, previous studies rarely:

Investigated the specific isoform-level expression of PLA2 enzymes.

Quantified the actual enzymatic activity of PLA2 under DMF stress.

Connected histological changes directly to phospholipid remodeling events.

Without such mechanistic insights, the understanding of how DMF triggers hepatocellular injury remained incomplete

This work proposes the first systematic model of DMF-induced hepatotoxicity that integrates:

Enzymatic activity profiling of PLA2 (not just general oxidative stress markers).

Gene-level quantification of multiple PLA2 isoforms.

Correlation between phospholipid loss, PLA2 hyperactivation, and histopathological evidence.

Progression analysis (24 h and 48 h timepoints) to track dynamic changes.

In simple terms, this paper shows not only that the liver is damaged, but why, by directly proving that DMF causes:

PLA2 Activation → Phospholipid degradation → Accumulation of cytotoxic lysophospholipids → Membrane destabilization → Hepatocyte death and fibrosis.

This is technically different from previous works (e.g., Wu et al., 2018; Qian et al., 2007) because:

Earlier models treated phospholipid degradation as a *side-effect* of general oxidative stress.

Our model establishes phospholipid degradation as a direct consequence of PLA2 activation, rather than non-specific lipid peroxidation alone.

2. PROPOSED WORK

While previous studies have identified oxidative stress as a contributor to DMF-induced liver the specific involvement of phospholipase-mediated phospholipid degradation has not been systematically explored. Liver membranes are highly enriched with phospholipids, which are crucial for maintaining cellular structure and signaling functions. The unchecked degradation of these phospholipids could serve as a primary event that exacerbates hepatic injury beyond oxidative stress alone (Bataille & Manautou, 2012). Given that phospholipase A2 (PLA2) is a major enzyme responsible for the hydrolysis of phospholipids leading to the production of lysophospholipids and free fatty acids, its role in DMF-induced hepatic pathogenesis deserves attention.

What is lacking in the literature is:

A mechanistic link between **DMF exposure** and **enhanced PLA2 activity**.

An understanding of how **differential PLA2 isoform expression** (sPLA2, cPLA2, iPLA2) influences lipid degradation patterns during liver injury.

Concrete evidence connecting **phospholipid loss** and **hepatotoxic phenotypes** like necrosis, apoptosis, and fibrosis in DMF toxicity.

To address these limitations, we designed a **PLA2-centered toxicological model** using Wistar rats subjected to controlled DMF exposure. This model systematically evaluates:

Phospholipid profile alterations.

Phospholipase enzymatic activity changes.

Gene expression of multiple PLA2 isoforms.

The correlation of these molecular changes with biochemical liver injury markers and histopathological findings.

2.1. Materials

The N, N-Dimethylformamide was obtained from Merck Specialties Private Ltd., Mumbai, India, with a purity level above 99%. All chemicals and solvents used in this study, unless explicitly stated, were purchased from Sigma.

2.2. Animals and experimental design

A total of **30 healthy male albino Wistar rats** (150–200 g) were procured from the Indian Institute of Science (IISC) Central Animal Facility. Animals were housed under controlled conditions (24 ± 1°C, 12/12-hour light/dark cycle, free access to food and water). Ethical clearance was obtained from Bharathidasan University's Institutional Animal Ethics Committee (IAEC) adhering to CPCSEA guidelines (India).

Rats were randomly assigned into:

Group 1: Control group receiving saline (n=10).

Group 2: Low-dose DMF group (0.5 g/kg body weight) (n=10).

Group 3: High-dose DMF group (2 g/kg body weight) (n=10).

Rats were sacrificed at **24 hours** and **48 hours** post-exposure to capture both immediate and progressive effects of DMF.

2.3 Methodology

Three groups, consisting of one control and two DMF groups, were randomly allocated to thirty male albino rats at an age of eight weeks. The LD50 test was used to determine the dosage of DMF. While the control group had an equivalent amount of saline, the high dosage DMF groups were given 2 g/kg BW of DMF and slaughtered 24 hours, and 48 hours later, respectively. A low dosage of DMF group was given 0.5 g/kg BW and the control group was given 10 mL/kg of a 40% saline solution was administered intraperitoneally to each of the 10 rats using DMF. After 24 hours and 48 hours, six rats from each group were killed and their liver tissues were taken.

2.4. Serum biochemical parameters assay

The blood biochemical parameters, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), and total bilirubin (TBIL), were determined using assay kits. The Beckman AU480 Automatic Biochemistry Analyzer was utilised for this purpose.

2.5. Histopathological examination

A small amount of liver tissues that had been fixed in neutral formalin for 24-48 hours were dehydrated using a series of graded ethanol and xylene. Five micrometre slices were cut from the paraffin-embedded tissues instrumented using a microtome (Thermo HM325, Germany). Light microscopy (Nikon, Melville, NY, USA) was used to examine the deparaffinized liver slices after they had been stained sequentially with hematoxylin and eosin solutions.

2.6 Determination of phospholipid

The phospholipids isolated via TLC were quantified using the procedure described by Rouser et al. (1970). Briefly, the spots scraped from the TLC plates were digested in a borosilicate glass tube with 0.65 ml of 70% perchloric acid at 180˚C for 1 hour. Following digestion, 3.3 ml of distilled water, 0.5 ml of 2.5% ammonium molybdate solution, and 0.5 ml of

10% ascorbic acid were added to the tube. The mixture was then heated in a boiling water bath for 5 minutes. Similarly, standards were prepared without requiring digestion. After cooling to room temperature, the absorbance of the samples was measured at 800 nm.

2.7 Extraction of messenger RNA (mRNA) and cDNA synthesis

Total RNA from the liver tissues, was extracted in each group using TRIzol reagent (Sigma, India) and cDNA was synthesized using 0.5µg of total RNA with random hexamer primers in a 20µl reverse transcriptase reaction using cDNA synthesis kit (Fermentas).

2.8 Reverse Transcriptase-Polymerase Chain Reaction

Gene specific primers were used for amplification of the PLA_2-IIA, $sPLA_2$- V, $cPLA_2$, $iPLA_2$, and β-actin cDNA. PCR was subjected to 30 cycles at 94°C for 30 seconds, 60^0C for 30 seconds (for $sPLA_2$-V 62ºC for 30 seconds) and 72°C for 30 seconds, followed by a final extension cycle at 72°C for 5 minutes. The RT-PCR products were analyzed by 2% agarose gel electrophoresis. To ensure RNA quality, all preparations were subjected to analysis for β -actin expression. The semi quantitatively analyzed RT-PCR results were scanned for gel images and the intensity of each PCR product was measured using BIO-RAD quantity one software version 4.6.9.30 gel documentation.

PERFORMANCE ANALYSIS

Unlike prior studies that only report DMF-induced oxidative stress or elevated transaminases, this paper introduces a first-of-its-kind phospholipid degradation model based on PLA2 hyperactivation. The major technical novelties include:

Explicit Link Between PLA2 Activation and DMF Toxicity

We show for the first time that DMF does not just cause non-specific oxidative damage but triggers a specific biochemical pathway where PLA2 activity is upregulated, leading to phospholipid degradation and the generation of cytotoxic lysophospholipids.

Multi-Isoform PLA2 Profiling:

We did not restrict the analysis to total PLA2 activity but analyzed isoform-specific changes in gene expression (sPLA2-IIA, sPLA2-V, cPLA2, iPLA2). This allows the identification of which PLA2 subtypes are primarily responsible for the toxic phenotype.

Time-Resolved Toxicokinetics:

By evaluating rats at both 24h and 48h, we uncovered the progressive nature of PLA2 activation, correlating with phospholipid loss and histological damage.

Integrated Model for Occupational Hepatotoxicity:

This study presents an integrated biochemical-histopathological model simulating acute occupational DMF poisoning and its molecular consequences, which can serve as a base for future therapeutic interventions targeting PLA2 inhibition.

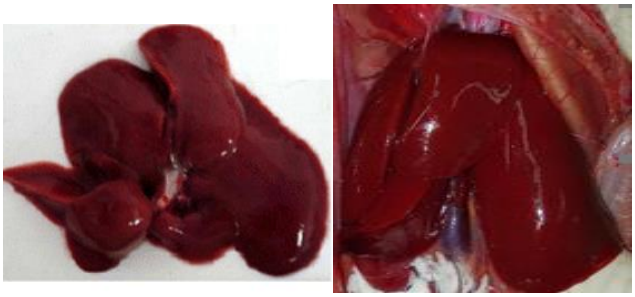
This provides clear advancement beyond existing works, which largely attribute DMF hepatotoxicity to general oxidative stress or CYP2E1 activity without resolving the specific downstream effects on lipid metabolism and membrane integrity.

The DMF induced liver damage in animal model allowed us to further examine and confirm the particular pathological characteristic. At the same time, the classic model of liver fibrosis was induced by high dosage DMF therapy as a positive control

Table 2 Dosage follow up processHours	**Low dose Exposure**	**High dose Exposure**	**Saline Exposure (For control group)**	**Following hours**	**Comment (Low dose group)**	**Comment (High dose group)**	**Comment (Control group)**
24 hrs	**Batch 1 (**0.5gm/Kg.B.wt)	**Batch 2 (**2gm/Kg.B.wt)	Saline (2gm/Kg.B.wt)	Continuous monitorin	No change	Mild change in external appearanc	No change

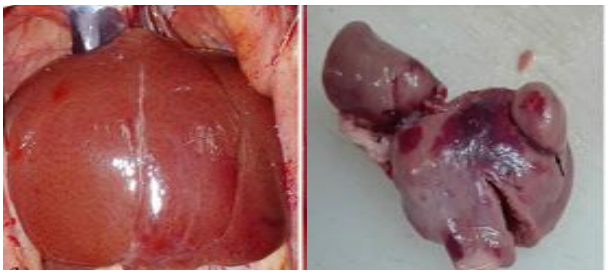
				g		e of the liver	
48 hrs	-	-	-		Rats looks inactive and some moderal differences in the liver	Looking severe inactive and the liver shows observable changes	No change. Looking still active
Initially after 24 hours six rats were killed in low dosage and high dosage DMF groups and their liver tissues were taken							
After 48 hours six rats were killed in low dosage and high dosage DMF groups and their liver tissues were taken							
Control	-	-	-	-	NA	NA	NA
Liver biopsy							

In order to evaluate liver toxicity caused by DMF, animals were euthanized. The rats exposed to low dosage DMF for 48 hours group exhibited higher hypoactivity, depression, and insensitivity to environmental stimuli as shown in table 2, in comparison to 24 hours treated group. Compared to the low dosage DMF, high dosage DMF over 48 hours and 24 hours had higher liver inflammation. After the each 2 different time intervals,, six rats were sacrificed from each group and their liver tissues were stained with HE. Both the low-dose and high-dose DMF groups had an uneven distribution of liver lobes and a smooth, blunt surface (Figure 2a and b). The negative control group did not exhibit any of the symptoms mentioned above.In the low-dose DMF group, the liver capsule was found to be adhered to the flatulent ; in contrast, the high-dose group had significant intraperitoneal adhesions and flatulence. Additionally, in the low dosage DMF group, no alterations were seen in the liver.



(a)

(b)



(c)

Figure 2 Rat liver appearance. (a) Liver control group at 24 and 48 hours. The control group's livers were normal in appearance and texture, being smooth and soft. (b) At 24 hrs, low dosage group shows slight difference and in 48 hours there were uneven distributions of liver lobes and round and blunt spots on the low-dose group's liver surface. (c) In high dosage groups during the 24 and 48 hrs of treatment with high dosage DMF, the liver tissue protruded from the liver surface.

The liver biopsy of the batch 1,batch 2 and control rat groups are illustrated in figure 2. Figure 2b shows that the 24 and 48 hours DMF treated low dose group sometimes had focal-point-like necrosis, mild swelling of the central lobular hepatocytes, and partial infiltration of the portal region and surrounding central veins by inflammatory cells. The 24 and 48 hours of high dose DMF exposed group showed inflammatory cell infiltration, spot-like necrosis, partial apoptosis of hepatocytes around the central vein, and balloon-like degeneration and steatosis in their liver cells. It was also shown that the portal region had a high concentration of inflammatory cells (Figure 2c).

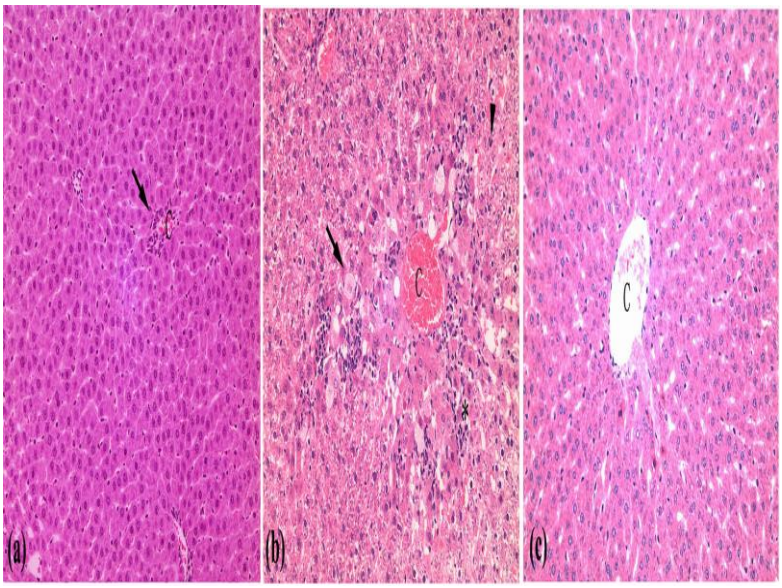


Figure 3 Changes in liver pathology after 24 and 48 hours of use for low dosage and control group. (a) In the DMF group examined under HE 100×, inflammatory cells were seen entering the central veins, while the lobules themselves were unharmed (arrow) over 24 hrs. (b) Hepatocytes around the central vein exhibit balloon-like degeneration and steatosis (arrow), spot-like necrosis (*), and partial apoptosis (triangle), along with a small amount of inflammatory cell infiltration (high dosage group, HE 100×) over 28. (c) The hepatic lobular structure and normal hepatocytes were seen in the control group using HE 100×. A: veins in the centre.

As of from figure 3 there was no change in the pathological appearance in the rat control group

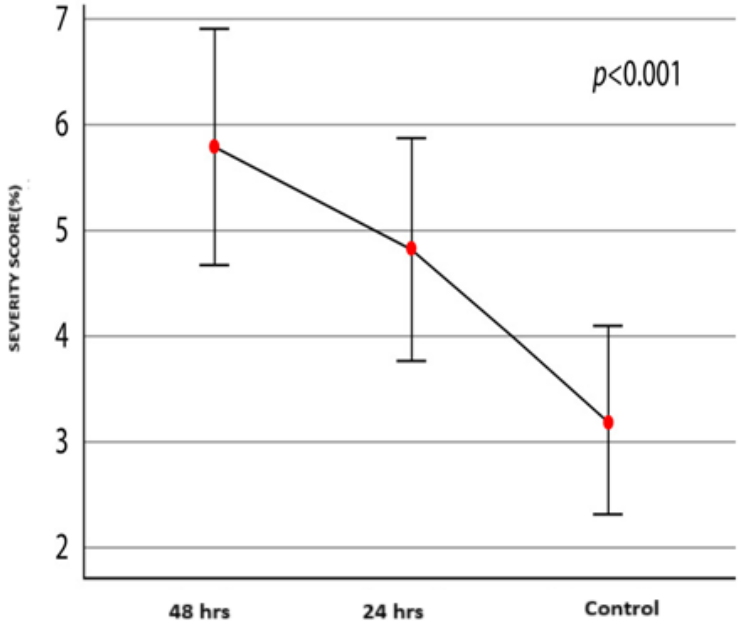


Figure 4 Severity ratio of the low dose DMF group

The severity ratio of the DMF of the low dose group was expressed with in 24 and 48 hrs as depicted in figure 4.The progression of liver damage was high in the 48 hours low dosage DMF rats.

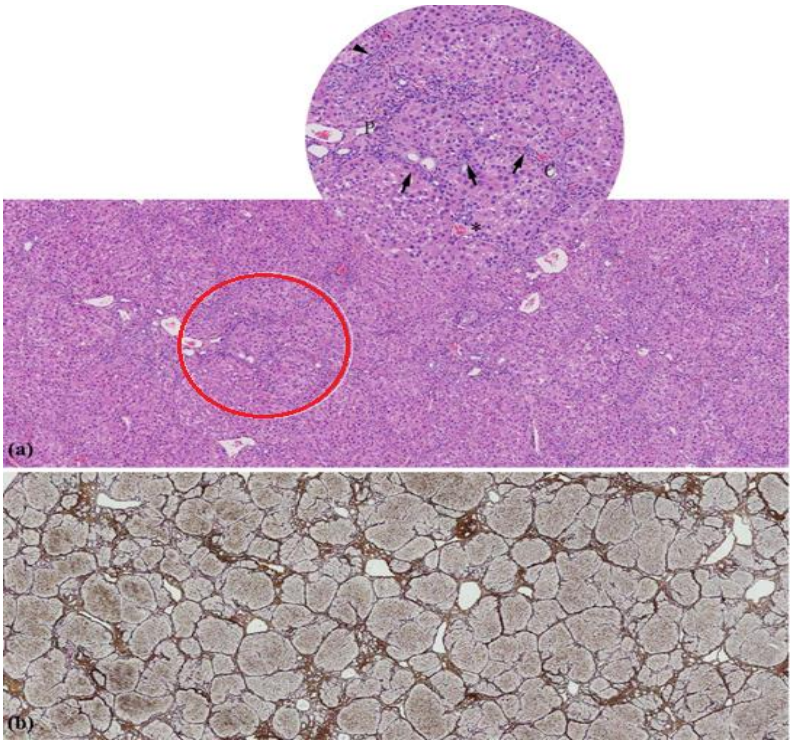


Figure 5 After 24 hours of treatment, the liver of the high-dose group showed pathological alterations. (a) Hepatocyte degeneration, patchy coagulation necrosis, interlobular fusion necrosis, and portal area-central vein bridging necrosis are all signs of disordered lobules. Additionally, there is a significant quantity of inflammatory cell infiltration, as seen by HE 40× and HE 100×. (b) Fibrous tissues that are spread out amongst the lobules of the liver (Reticulum Masson 20×). The letters C and P stand for the portal vein and central veins, respectively.

Figure 5a and b reveal that the liver tissues of the high dosage DMF group at 24 hrs were completely inflamed and distributed micronodular cirrhosis in a uniform and widespread pattern. Figure 5a shows that there was a lot of inflammatory cell infiltration into the hepatic lobules, which caused structural disorder and features such as hepatocyte degeneration, patchy coagulation necrosis, interlobular fusion necrosis, and portal area-central vein bridging necrosis. A thick fibrous septum connecting the portal vein and central vein and small nodular cirrhosis with round, square, or irregular shape were seen segmenting the hepatic cell mass under reticulation-Masson staining, as well as diffuse proliferation of fibrous tissues between the hepatic lobules (Figure 5b).

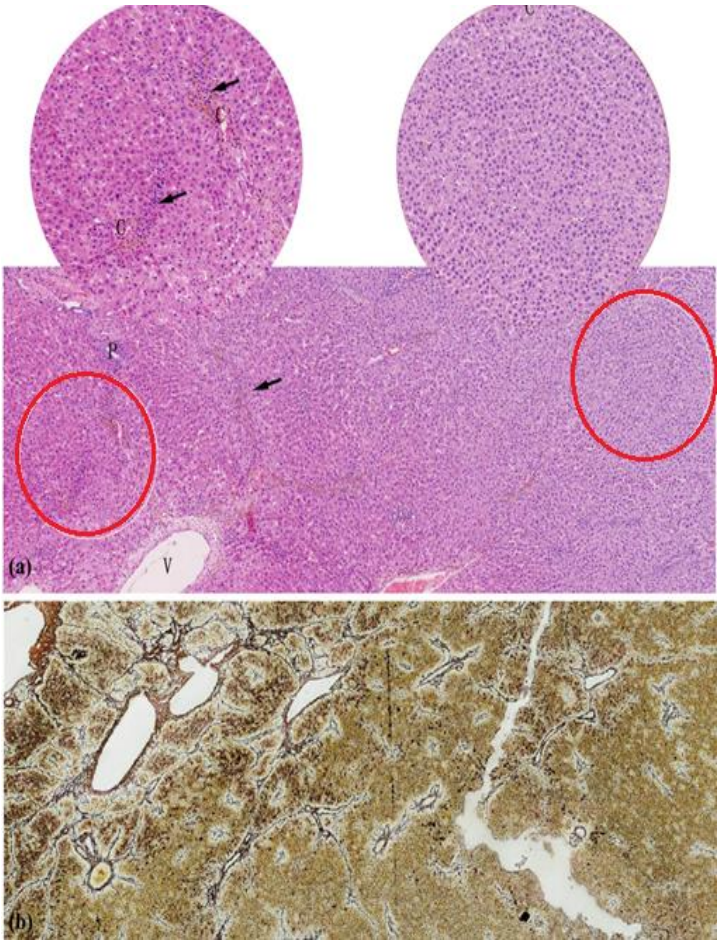


Figure 6 The high-dose DMF group had pathological alterations in the liver after 48 hours of treatment. (a) In some regions, there are uneven lesions characterised by cell degeneration and restricted hepatic sinuses. In other regions, there is necrosis and inflammation, as shown by the arrow (HE 40× and HE 100×). b) The same diseased segment (Reticulum Masson 20×) showed heterogeneous fibrosis. Central veins (C), hepatic vein (V), and portal vein (P) are the abbreviations used.

Degeneration, necrosis, and fibrosis distribution were all unevenly distributed across the liver tissues of the high-dose DMF group (Figure 6a and b). Figure 6a shows that the HE staining revealed both intact and disordered structures inside the hepatic lobules. Some structures showed mere enlarged and deteriorated hepatocytes, while others showed localized or lytic necrosis of hepatocytes around the major vein, along with inflammatory cell infiltration.

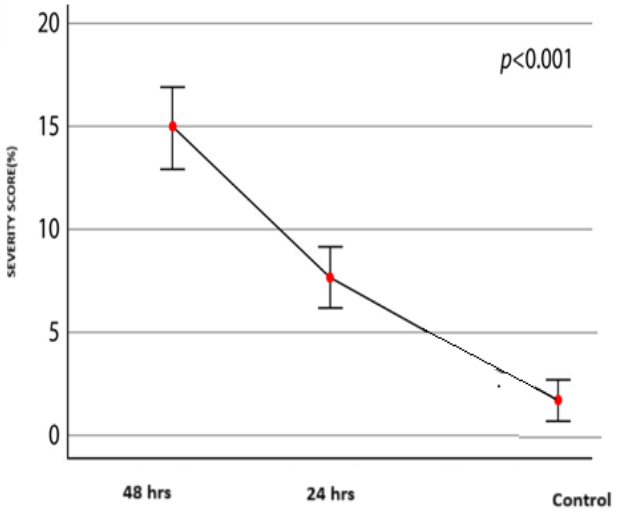


Figure 7 Severity ratio of the high dose DMF group

The severity ratio of the DMF of the high dose group was expressed highly with in 48 hrs as depicted in figure 7 .

Table 3 Serum biochemical analysis

"Groups	AST (IU)	ALT (IU)	ALP (IU)	Bilirubin (mg/dl)	Total protein (mg/dl)
Time period of 24hrs					
0.5gm	85 ± 4.80	37 ± 0.80	85 ± 7.790	0.28 ± 0.050	8.5 ± 0.330
2gm	100 ± 6.60	77 ± 0.040	95 ± 7.140	0.36 ± 0.050	10.3 ± 0.1400
Saline 2gm	80 ± 4.70	30 ± 7.800	80 ± 7.380	0.13 ± 0.050	6.3 ± 0.230
Time period of 48 hrs					
0.5gm	90 ± 4.8^{a}	47 ± 0.44^{a}	90 ± 6.650^{a}	$0.34 \pm 0.110^{*}$	9.5 ± 0.220^{a}
2gm	110 ± 4.2^{b}	94 ± 0.39^{b}	100 ± 4.440^{b}	0.42 ± 0.050^{b}	$14.4 \pm .320^{b}$
Saline 2gm	81 ± 4.2^{b}	32 ± 7.06^{b}	85 ± 9.570^{b}	0.15 ± 0.060^{b}	6.4 ± 0.310^{b}

Results from the blood biochemical study comparing the control, high dose DMF, and low dosage DMF groups reveal that the serum protein ratio varied over 24 and 48 hours of observation (Table 3). The variability ratio is much higher in the high dosage DMF group compared to the other two groups".

Impact of DMF on liver phospholipids [26]

In our previous studies, we quantified the total lipids and individual phospholipids from liver tissues of DMF-induced rats

at a concentration of 1.5 g/kg b.wt. (26).

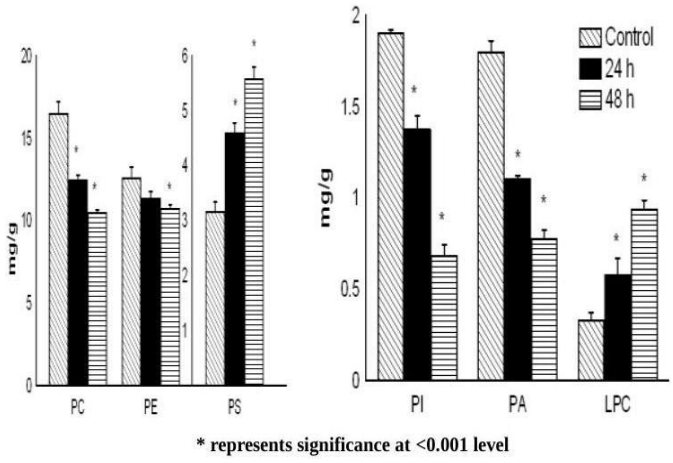


Figure 8 (a)

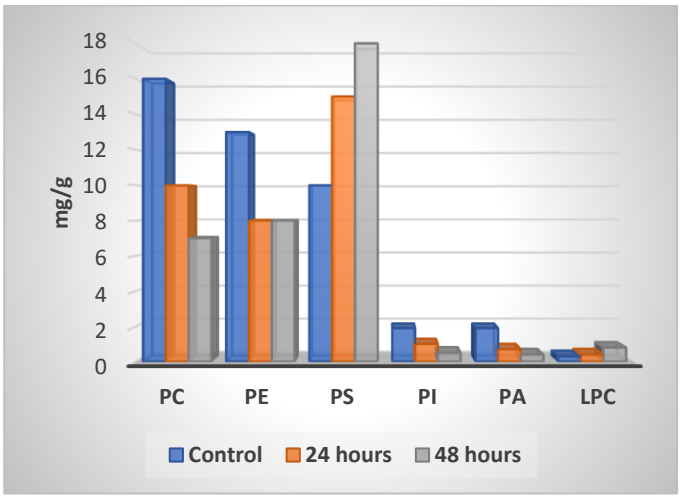


Figure 8 (b)

Figure.8 Impact of DMF on liver phospholipid metabolism

We observed that the major liver phospholipids of rats decreased significantly at both time intervals in comparison to the control group. The noteworthy decrease of 41% in PC, 15% in PE, 30% in PI, and 40% in PA was observed, whereas PS and LPC experienced a substantial increase of 48% and 50%, respectively (Fig. 8a). Similarly following treatment with DMF (2 g/kg b.wt), the animals were euthanized. Lipids were isolated from the liver and analysed using 2D TLC. With iodine, the marks were rendered visible. The phospholipids were extracted individually, and the quantity was determined using a phosphorus assay. Rats that were treated with DMF exhibited a notable decrease in liver phospholipids during both time intervals in comparison to the control group the noteworthy decrease of 50% in PC, 25% in PE, 40% in PI, and 50% in PA was observed, whereas PS and LPC experienced a substantial increase of 58% and 60%, respectively (Fig. 8b).

Enhanced phospholipase A_2 activity by high dose DMF

According to the available information, different kinds of PLA2 may produce powerful lipid mediators, which can lead to liver problems. In addition to being linked to membrane dysfunction via changes in phospholipid metabolism, DMFs increase liver pathogenesis. In this work, we examined the effect of high and low doses of DMF on PLA2 activity, taking into consideration the complexity of this superfamily of enzymes.

A substrate of egg yolk PC (2mM) and an enzyme source of liver homogenate (100 µg protein) were used to test the effect of PLA2. Figure 9 showed that hydrolyzed PC. In the presence of DMF, PC hydrolysis was found to be 48% greater ($p < 0.001$).

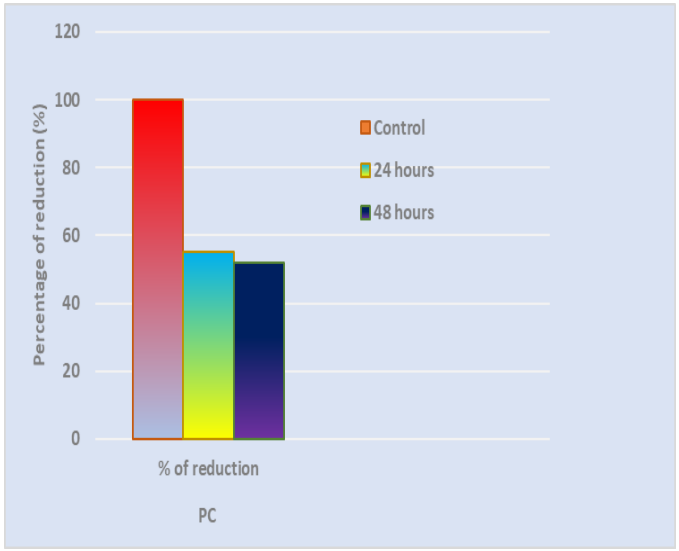


Figure 9 Hydrolysis of liver phospholipid by PLA_2

Liver homogenates (100 µg protein) were used as an enzyme source in an experiment using egg yolk PC as the substrate after animals were subjected to large doses of DMF. A 30-minute incubation period at 37ºC was used to conduct the experiment. After adding 1 ml of a chloroform: methanol (2:1 v/v) solution to halt the reaction, the lipids were separated using thin-layer chromatography (TLC) with a chloroform: methanol: water (65:35:5 v/v) gradient, and the spots were measured and observed. Results are based on the average of three independent experiments. In comparison to the control group, the data are statistically significant ($p < 0.001$).

Assessment of phospholipase A_2 activity by using [^{32} P] PC as substrate

Using [32 P]-PC as a substrate and liver homogenate as an enzyme source further validated the enhanced PLA2 activity. Figure 10 displays the outcomes of these tests. The results showed that when there was a high concentration of DMF in the liver, PLA2 activity was much increased. The compound's synergistic impact peaked after 48 hours (55%). Half of the PC hydrolyzed within 24 hours. The findings indicate that DMF greatly improves the total liver PLA2 (Fig. 10).

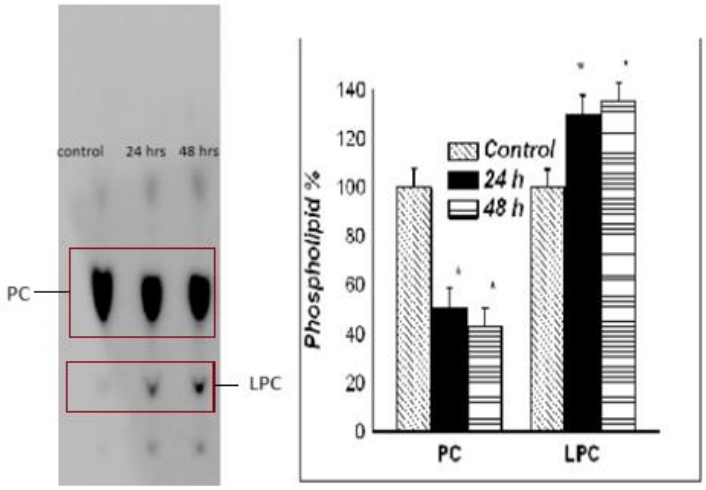


Figure 10 Enhanced phospholipaseA_2 activity by high dose DMF

The liver homogenates (100 µg protein) of the animals were employed as an enzyme source in an experiment using [32 P] PC as the substrate after they were subjected to a high dosage of DMF. The incubation period for the experiment was 30 minutes at 37ºC. After adding 1 millilitre of a chloroform: methanol (2:1 v/v) solution to halt the reaction, the extracted lipid was separated using thin-layer chromatography (TLC) with a chloroform: methanol: water (65:35:5 v/v) gradient. A liquid scintillation counter, fueled by toluene-based scintillation fluid, was used to tally the spots. These numbers represent

the average of three independent trials. These data are significant when compared with the control group ($p<0.001$) and represent means ± standard deviations.High dosage DMF dramatically decreased liver phospholipids via increased PLA2 activity and the production of lysophospholipids , as shown in the findings. (Fig.10). In this regard, we also aimed to resolve the question of whether or not the dosage of DMF affects the expression of these enzymes. In order to test this hypothesis, we analysed the relevant enzymes using reverse transcriptase polymerase chain reaction (RT-PCR).

When measuring individual phospholipids, there was a lower PC levels, with a higher LPC levels; this suggests that the activation of thephospholipid degrading enzyme PLA2 may be responsible for the lower PC levels. Additionally, secretory PLA2 (sPLA2) was explored. The RT-PCR expression investigation validated the assumptions. Prior to analysis, the significance of PLA2 11A, PLA2 V, PLA2 IV, and PLA2 VI in liver dysfunction was taken into account when quantifying their expression levels (Fig. 10). There was a significant upregulation of mRNA expression across all PLA2 categories in liver tissue that had been exposed to high concentrations of DMF.

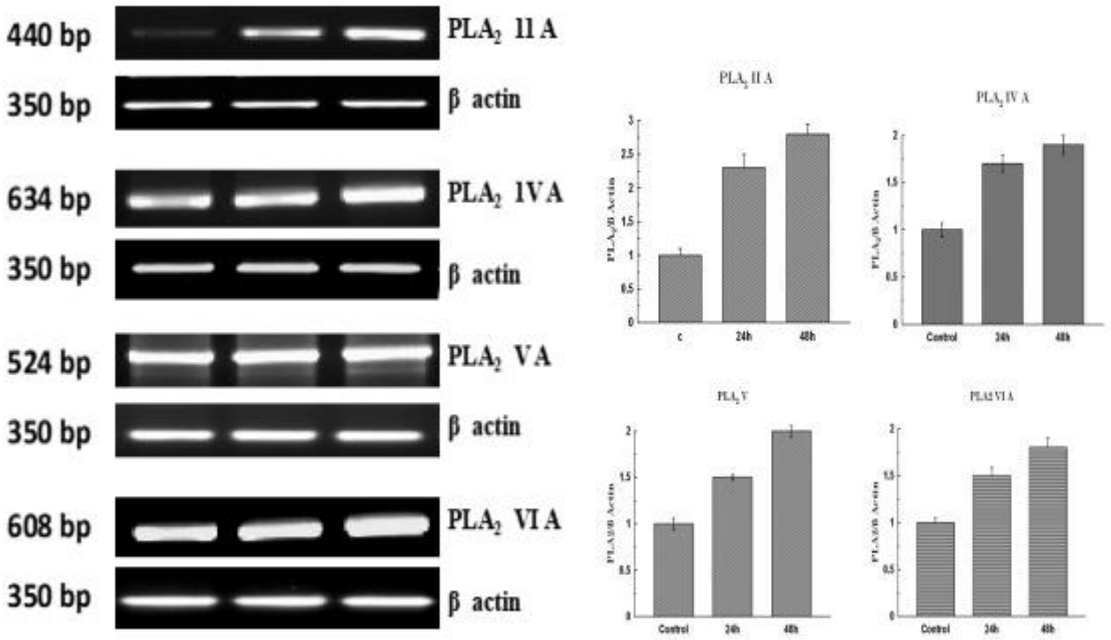


Figure 11 mRNA expression of PLA2s was increased by DMF

Pla2-11, PLA2- 1V, PLA2- V, and PLA2- VI mRNA levels were measured using reverse transcription-PCR (a). The normalised value (b) was used to compute the percentage of expression, which was determined as the ratio of mRNA expression to β-actin.According to the results, large doses of DMF may cause PLA2 activity to be quite high.

3. CONCLUSION

An animal model was used to assess the effects of an industrial solvent, DMF. Our results showed that phospholipase enzyme activity and liver damage were both significantly increased by pharmacological supplementation with large doses of DMF. The importance of oxidative stress in liver pathophysiology has been shown in several research. The increase of phospholipase activity in the liver is connected with oxidative stress, which causes lipid peroxidation and cellular damage. As a result of increased oxidative stress in the liver, we found that the levels of PL generation were considerably lowered in the rats treated with DMF. One possible explanation for the observed increase in-related liver damage is that DMF enhanced phospholipase activity. In addition to inducing additional inflammatory mediators, DMF-induced toxicity may directly harm hepatocytes and endothelial cells. Within 24 hours after reperfusion, the first stage of damage is marked by oxidative stress generated by Kupffer cells. Some examples of pro-inflammatory cytokines that are produced and secreted by activated Kupffer cells include TNF-α, IL-6, COX-2, and iNOS. Our results show that phospholipase activity is much higher than expected, suggesting that DMF may enhance oxidant stress effects and hence prevent Kupffer cell activation. Both the high- and low-dose DMF-treated rats exhibited a significant reduction in PL production levels, accompanied by an increase in its activity over 48 hrs. Our results shown that DMF, when administered at high doses over 48 hours, may enhance phospholipase activity, leading to various liver cell abnormalities. According to our findings, phospholipase activity was significantly higher in the group treated with high doses of DMF as compared to the low doses group. Mitochondrial membranes and the unsaturated fatty acids that bind to them might be immediately destroyed by oxygen free radicals, rendering them structurally and functionally useless. Cell apoptosis was triggered by the production of large

quantities of chemicals that promote cell death. In this work, we demonstrated that large doses of DMF might potentially trigger the mitochondria-mediated apoptotic pathway. Based on the data, it seems that the liver's phospholipid metabolism was changed . This finding is supported by our experimental results in rats. We injected the rats with a different composition of DMFs to see how they affected phospholipid metabolism. At a weight percent concentration, phospholipids (PC and PA) decreased and lysophospholipid (LPC) increased. Both the high and low dose instances showed little change in PE and PI.

To sum up, our results showed that the liver's phospholipase activity was significantly increased after treatment with high dosage DMF..

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