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Effect of Gluten Containing Diet on Pristane Induced Lupus Prone Mice

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ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with characteristic organ involvement and autoantibodies production. The pathogenicity and etiology of the disease has yet to be elucidated. It is presently accepted that environmental factors trigger the disease in genetically sensitive individuals. Gluten, a protein fraction commonly found in wheat grains, associated with food related disorders and a number of autoimmune diseases. We hypothesized that gluten containing diet would further exacerbate an already undergoing arbitrary immune reaction in SLE patients. Pristane was injected in female BALB/c mice to induce the disease. After five months, mice in various groups were treated with prednisone and fed with gluten containing and standard diet for four weeks and applied procedure to detect minor changes in paw swelling, ANA autoantibodies, CCL11, C3c, glucose level and renal damage. We detected increased symptoms of arthritis and gastrointestinal tract involvement in gluten containing diet group compared with standard diet disease control group. ANA autoantibodies, C3c and renal damage between gluten and standard diet group was non-significant. The remission of SLE manifestations was observed in prednisone treated group except renal damage. From the study it was concluded that gluten intake could worsen the clinical manifestations in SLE patients, therefore, administration of gluten free diet might be a better strategy for SLE patients. However, further confirmatory studies are required in this regard.

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Authors' Contribution
MM, ZA and NH designed the experiments; MM and NH performed the experiments; MM and ZA did the histology, MM and NH analysed the data, MM and NH wrote the paper.

Key words
SLE, Pristane induced lupus prone mice, Gluten containing diet, Autoimmunity.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a distinctive autoimmune disease with diverse clinical manifestations involving many organs (Santhanam *et al*., 2016). The commonly affected organs include joints and muscles, skin, nervous system and kidneys. An important aspect of SLE is the presence of numerous self-antigen specific autoantibodies, principally of nuclear origin. The tissue injury and phenotypic abnormalities develop as a result of these pathogenic autoantibodies (Dema and Charles, 2016). The better diagnosis and treatment of the disease have improved the life expectancy and survival of patients, however, they still encounter recurrent disease flares and subsequent organ involvement (Choi *et al*., 2016). The etiology of SLE is still unknown. However, it is believed that the disease develops due to genetic susceptibility, epigenetics and environmental factors (Xiao and Zuo, 2016).

Various animal models of SLE have been established that play a vital role in exploring the mechanism of disease. Pristane-induced lupus mouse model is one of the SLE models, which develops a repertoire of autoantibodies and immune complex mediated glomerulonephritis (Wang *et al*., 2014). Pristane is a naturally occurring hydrocarbon derived from phytol metabolism. Intraperitoneal induction of pristane in BALB/c mice develops chronic inflammation. A Type 1 inflammatory response is evident after 2 weeks of pristane injection and 4-6 months later, high level of autoantibodies production leads to spectrum of disease manifestations such as arthritis, pulmonary vasculitis and glomerulonephritis. Hence, this experimental model represents many key immunological characteristics of human SLE (Carlucci *et al*., 2016).

Several environmental factors have been implicated in the initiation, intensification or promotion of SLE flares such as cigarette smoke, air pollutants, alcohol and UV exposure. Nutrition and diet may also play a role in the development and advancement of SLE, however, knowledge in this regard is relatively scarce (Elkan *et al*., 2012; Choi *et al*., 2016). Gluten is a protein portion found principally in wheat, oats, barley, rye and their derivatives. It is used in final products of bakery and pasta goods in order to enhance their quality. Beside enhancing food quality and providing nutritional benefits, these gluten proteins trigger food related disorders in humans (Martinez-Esteso *et al*., 2016).

Celiac disease is an immune reaction to gluten, which affects the small intestine in genetically susceptible individuals (de Lourdes Moreno *et al*., 2016). In addition

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to celiac disease, gluten also induce Type 1 diabetes development. The studies on animal models had revealed its major role in pathogenesis of T1D (Antvorskov *et al*., 2014). A number of research investigations evidenced an association between CD and SLE. The concept of shared autoimmunity is an assumption used to associate SLE to other rheumatic diseases. The association between SLE and CD could be a part of this perception (Ludvigsson *et al*., 2012). Both SLE and CD are autoimmune disorders with discrete genetic and environmental factors are implicated in their pathogenesis. There are case studies reporting SLE in patients diagnosed with CD and vice versa (Hrycek and Siekiera, 2008). However, previously there was no study that directly links risk of gluten exposure to SLE. Therefore, current study was conducted in view of the hypothesis that gluten containing diet would further exacerbate an already undergoing arbitrary immune reaction in SLE patients.

MATERIALS AND METHODS

Animals

A total of 30 female BALB/c mice at an age of 4-8 weeks (purchased from Veterinary Research Institute (VRI) Lahore). All the mice were kept in cotton-bedded cages in an animal facility of the department of MMG. The mice were allowed to get accustomed in cages (5 mice per cage) for 1 week prior to experimentation. Standard laboratory conditions were maintained in the animal facility (24±2ºC temperature, 55-65% humidity and 12 h light/dark cycle). The experimental procedures were conducted quickly in an anxiety and fear free atmosphere involving minimum psychological and physiological disturbance. All procedures were approved by institutional Biosafety and Ethical Committee.

Induction of PIL

Initially mice were divided into two major groups. Out of 30 mice, 25 were injected intraperitoneally with a single dose of 0.5 ml pristane (purchased from Tokyo Chemical Industries (TCI) Japan). The remaining 5 mice were healthy control. Pristane was eventually found to initiate autoantibodies production and clinical symptoms of SLE.

Diet

After 5 months, pristane injected mice were further divided into 4 groups (5 mice in each group). One was control group known as disease control, fed with palatable commercial diet. Other groups were treated with prednisone and gluten upto 4 weeks. Prednisone treated group (P) was orally administrated with 1 mg/kg prednisone. Prednisone + gluten containing diet group (P+G) were given prednisone and gluten containing diet. Gluten group (G) was provided with gluten containing diet. Gluten containing diet comprised of 10% of gluten (purchased from Vital Chemicals Lahore, Pakistan) to the total diet.

Paw volume measurement

Joint involvement is an important feature of SLE. Paw swelling of mice was measured using a digital plethysmometer. Paw swelling was expressed as percentage change in paw edema compared with a healthy control group.

Detection of autoantibodies

ANA antibodies were detected through Mouse Anti-nuclear antibody (ANA) ELISA kit (Catalogue # GA-E0266MS) purchased from GenAsia. This kit was used to assay Mouse Anti-nuclear Antibody (ANA) on the basis of the Biotin double antibody sandwich technology.

CCL11 quantification

The quantitative measurement of Eotaxin protein from mouse serum was carried out through Simple Step ELISA™ kit (Catalogue # Ab201277) purchased from Abcam.

C3c quantification

Serum complement protein C3 was quantified by using "RID 3-plate" (Catalogue No. RID4490) manufactured by FAR srl Verona, Italy. This combi-plate quantify serum proteins by radical immunodiffusion.

Renal damage evaluation

The blood urea nitrogen test was performed to determine kidney function. The test procedure performed was based on the enzymatic method as described by Tietz (1987). Glomerulonephritis severity was assessed histologically through kidney sectioning and the HN staining as described by Peng (2004). Mesangial thickening, hypercellularity, crescentic and sceloritic changes were evaluated to detect the intensity of kidney damage.

Statistical analysis

Data analysis was performed by using software Graph Pad Prism (6.07). To summarize data and determine distribution properties for subsequent analysis, descriptive statistics was used. To find out the significance level, one way ANOVA, student T test and Tukey HSD test and for the correlation Pearson r test were performed.

RESULTS

The experiment was started with 30 female mice five of them died during the experiment. At the end of the experiment, there were five mice in each experimental group.

Paw edema

Paw edema of all groups of mice was measured initially after two months of pristane injection. Initial paw edema values showed signs of arthritis in 35% of pristane injected mice. After 5 months of pristane injection, four mice groups were exposed to experimental diet upto four weeks. Final paw edema was measured before slaughtering the mice. The diseased control group (DC) paw edema values were higher than the healthy control group (HC) ($p<0.0001$) showed that pristane had induced paw swelling. Comparison of paw swelling values between diseased control group and prednisone treated group ($P <0.0012$) revealed that prednisone had suppressed the paw swelling. The higher values of paw edema in gluten containing diet group than prednisone +gluten and prednisone treated ($P < 0.008$) showed that the addition of gluten in standard diet increased the paw swelling in pristane injected mice (Fig. 1).

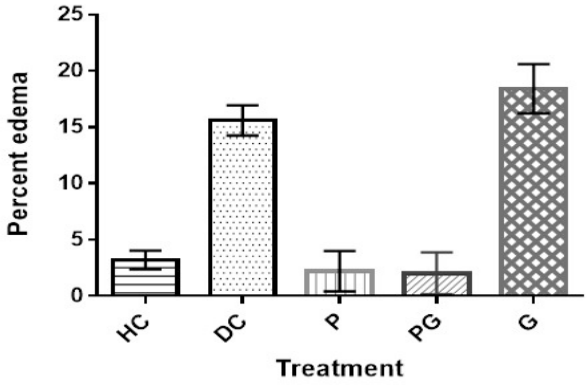

Fig. 1. Paw edema (%) after treatment in pristane induced lupus mice.

ANA autoantibodies

The level of ANA autoantibodies in mouse serum was quantified to detect autoantibodies production. One way ANOVA analysis of ANA quantification ($p<0.024$) strongly suggested the significance. Almost all mouse groups were ANA positive, however, the ANA level in the healthy control group was lower than disease control ($P < 0.02$) (Fig. 2). The ANA autoantibodies concentration was comparatively higher in diseased control than prednisone treated group ($P<0.0007$). The ANA autoantibodies concentration in gluten containing group compared with disease control group was non-significant ($P > 0.05$). However, it was significantly different when compared with prednisone treated and healthy control group (Fig. 2).

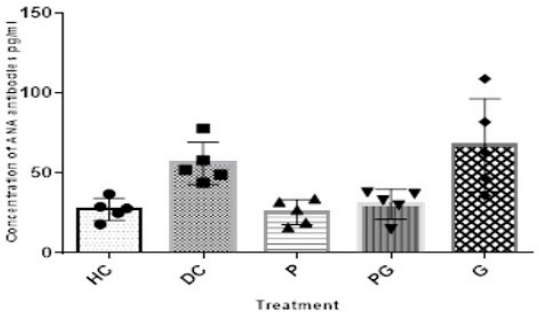

Fig. 2. ANA autoantibodies level in pristane induced lupus mice after treatment.

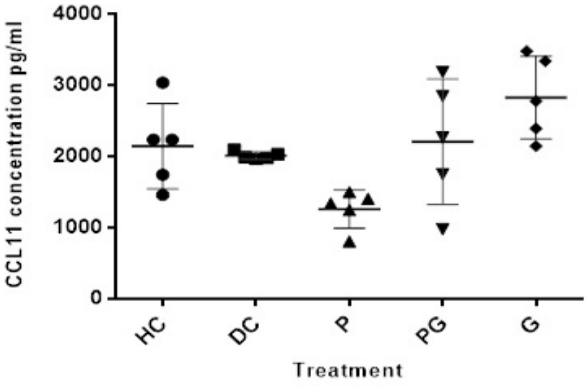

Fig. 3. CCL11 concentration in pristane injected mice after treatment.

CCL11 (Eotaxin)

The varied concentration of chemokine CCL11 (eotaxin) was noticed among different groups of mice. The p-value of one way ANOVA was lower than 0.05 suggested that the treatment group was significantly different. The CCL11 concentration in the serum of healthy control mouse group was higher than disease control group showing weak association of eotaxin with SLE ($P < 0.001$). The CCL11 concentration was decreased in the prednisone treated group compared with a disease control group ($P < 0.001$). The difference of CCL11 level between prednisone and prednisone + gluten group was non-significant ($P>0.05$). The increased level of CCL11 was detected in gluten containing diet group compared with prednisone treated ($P<0.001$) and disease control group ($P < 0.043$) suggested the role of gluten in enhancing eotaxin concentration (Fig. 3).

Complement component 3 (C3c)

In a limited number of mice concentration of C3c was also detected through radial immune-diffusion. The C3c concentration was found to be lower in disease control (n=1) compared with healthy control (n=1) (Fig. 4). The concentration was also lower in gluten containing diet group (n=3) compared with disease control (n=1) showed that level was become low due to its more consumption. There was a small difference of C3c level in gluten containing diet group and prednisone treated. The correlation between ANA autoantibodies and C3 level was found to be positive (r^2 =0.8022) and significant (p<0.0398) (Fig. 5).

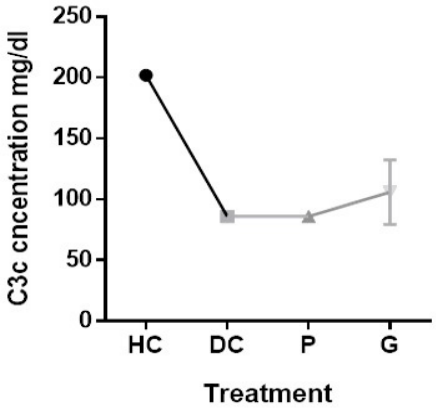

Fig. 4. C3c level in pristane induced mice after treatment.

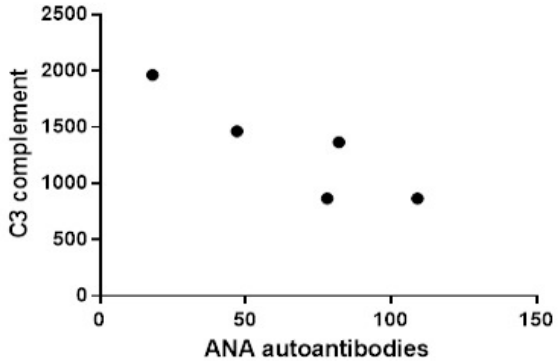

Fig. 5. Correlation between ANA and C3 level in pristane induced lupus mice.

Renal damage

The laboratory BUN concentration reference value for normal female BALB/c mice was 7-31 mg/dl. In prednisone treated group, 66% mice had BUN level above than the reference range. In disease control 33% and in gluten containing diet group, 50% mice were detected with higher BUN level than laboratory reference range.

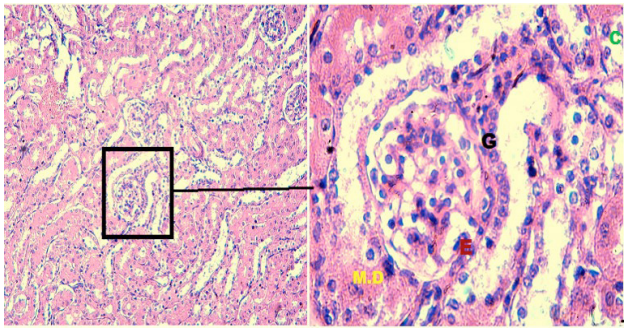

Fig. 6. Histological structure of kidney of positive control mouse. Thich black arrows in (x100) showing epithelial crescent squashing the glomerular tufts from all sides, yellow arrow showing necrocitising glomerulus, black arrow in (x400) showing segmental glomerular sclerosis.

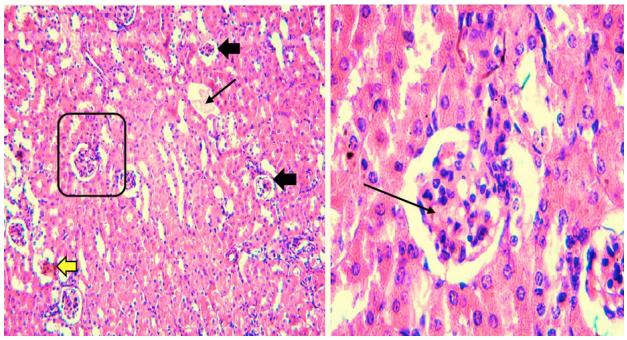

Fig. 7. Histological structure of kidney of prednisone treated mouse. Black arrows in (x100) showing affected glomeruli. Not a single glomerulus was normal showing severe lupus nephritis. Thick black arrow in (x400) showing hyaline arteriosclerosis and thin arrow showing glomerular necrosis, brown arrow showing immune deposits of membranous nephropathy, yellow arrow showing tubules with neutrophils.

Microscopic examination of kidneys of healthy controls showed the normal architecture of the kidneys. Many of the glomeruli were seen with a damaged portion or segment known as segmental glomerular sclerosis. The damaged portion was infiltrated with inflammatory cells and immune deposits. Necrotizing glomeruli and epithelial crescent was seen in disease control group (Fig. 5). Prednisone treated group was also affected. One mouse in the prednisone treated group showed severe glomerulonephritis with hyaline arteriosclerosis, membranous nephropathy and glomerular necrosis (Fig. 6). The prednisone + gluten group had least glomerulonephritis (Fig. 7). The gluten containing diet group showed rapidly progressive glomerulonephritis and glomerulosclerosis (Fig. 8).

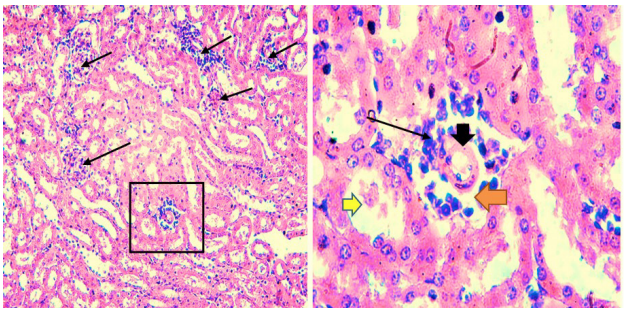

Fig. 8. Histological structure of kidney of mouse fed on prednisone + gluten containing diet. Arrow in (x100) showing glomerulonephritis. Most of other glomerulus in (x100) are normal. Thick black arrow showing partial hyaline sclerosis and thin arrow showing tubules with neutrophils.

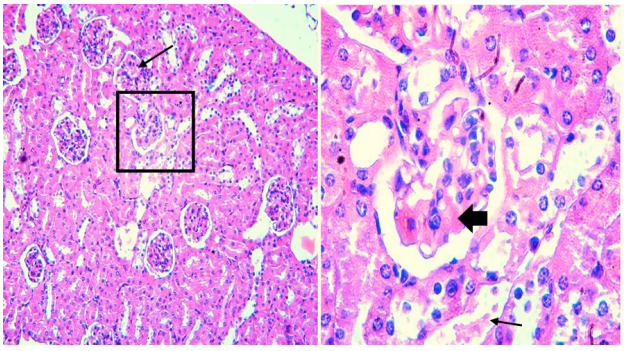

Fig. 9. Histological structure of kidney of mouse fed on gluten containing diet. Black arrows in (x100) and yellow arrow in (x400) showing rapidly progressive glomerulonephritis, red arrow showing tubules with neutrophils and black arrow (x400) showing segmental glomerulosclersis.

DISCUSSION

Systemic lupus erythematosus (SLE) is a common autoimmune disease of unknown etiology. It is marked by extreme inflammation and multi-organ damage that can prove to be fatal (Lee *et al*., 2016). The knowledge about the influence of diet on SLE is still scarce (Elkan *et al*., 2012). Gluten is an important commercial entity of starch industry. It's used as a nutrient supplement, dough strengthener, thickening and stabilizing agent. However, it causes allergies and a number of autoimmune diseases, especially celiac disease (Jones *et al*., 2016).

A distinctive feature of SLE is the vast variation in the number and type of organs involved. Joint involvement is commonly presenting feature in SLE (Ball *et al*., 2014). Pristane treated lupus model is the only model with arthritis among drug induced models of SLE (Liess *et al*., 2013). Carlucci *et al*. (2016) observed pristane induced joint inflammation as less aggressive than rheumatoid arthritis murine models. Swelling and redness of hind paw was detected after 6 months of pristane injection, a larger time period in comparison to other studies. Iqbal *et al*. (2013) investigated 590 patients with gluten intolerance. Out of which 131(37%) were diagnosed with arthritis. Elkan *et al*. (2008) found that the gluten free diet caused the remission of arthritis symptoms in rheumatoid arthritis patients. According to El-Chammas and Danner, (2011) gluten free diet regulates dyslipidemia in arthritic patients. It decreases total cholesterol, LDL and the LDL: HDL ratio. On the other hand, it increases the level of natural antibodies against Phosphorylcholine (PC). As LDL is atherogenic and anti-phosphorylcholine concentration negatively associated with atherosclerosis development, therefore gluten free vegan diet is possibly antiartherogenic.

In the current study, significant paw swelling was detected in pristane injected groups compared with a healthy control group after six months of pristane induction. Prednisone treated group showed significant reduction in paw edema when compared with disease control group. Paw edema measurement was found to be significantly higher in gluten containing diet group compared with disease control group lead to the finding that increased gluten intake in diet worsen the arthritis feature of SLE in pristane induced lupus prone mice. The comparison of the gluten containing diet group with prednisone treated group confirmed the inflammatory role of gluten instead of immunosuppressive.

The ANA-autoantibody level was found to be significantly higher in the disease control group compared with a healthy control group, whereas it was significantly lower in the prednisone treated group. Satoh *et al*. (2008) reported that pristane induced lupus specific autoantibodies, including anti- ANA, anti-dsDNA and anti-Sm in BALB/c mice. It was concluded from the study that pristane can induce lupus specific antibodies in any mouse strains regardless of its genetic background. ANA antibody level comparison between disease control group and gluten containing diet group was non-significant. This showed a weak association between gluten intake and production of antinuclear antibodies.

CCL11 is a chemokine that plays an important role in pathogenesis of digestive inflammatory disease. Its role was investigated by introducing it into human epithelial cells. An instant wheal and flare reaction occurred associated with the mast cell degranulation (Méndez-Sánchez *et al*., 2007). In the present study, the level of CCL11 between healthy and disease control groups was found to be non-significant, hence there was no direct association found

between CCL11 and SLE disease. However, its level in gluten diet group was significantly higher compared with disease and prednisone treated groups. As it was evidenced that CCL11 is involved in digestive tract pathogenesis therefore, its increase in the gluten containing diet group might be due to the inflammation of the intestine caused by gluten intake. Macroscopic examination of the intestine also revealed dilation and swelling of the intestine in gluten containing diet group. Previously, it was reported that gliadin crosses the epithelium through paracellular transport. The paracellular transport relies on intestinal permeability. Enhanced intestinal permeability was found to be associated with autoimmune diseases like type 1 diabetes, multiple sclerosis, rheumatoid arthritis and inflammatory diseases such as inflammatory bowel disease, depression and chronic fatigue syndrome (De Punder and Pruimboom, 2013). Therefore, CCL11 rise in pristane induced gluten diet group might be due to the impairment of gastrointestinal tract caused by the gluten intake.

Hussain *et al*. (2008) quantified the level of C3 and C4 in lupus nephritis patients. It was reported that depletion of C4 was more than C3. Wang *et al*. (2013) detected low concentration of C3 in severe SLE patients, during his 4 year of study. Experimental studies found that a complement deficiency impairs the clearance of immune complexes from tissue injury sites that leads to inflammation and autoantigen release. This autoantigen release results in enhanced autoimmune response (Robson and Walport, 2001). In the present study, C3c concentration was detected in a small number of mice. It was significantly higher in the healthy control group compared with remaining experimental groups. The deficiency of C3c level in disease control and gluten treated group can be attributed to its high consumption in tissue inflammation. The correlation between ANA autoantibodies and C3 level was positive and significant (r^2 =0.8022; $p<0.0398$), showed that with the increase in the ANA antibody level C3 level decreases.

Histopathology of kidneys revealed glomerulonephritis in majority of mice in all experimental groups except healthy control group. The comparison of kidney damage among various experimental group was found to be non-significant. Necroticising glomeruli were observed in the disease control group. Prednisone treated group was also found to be severely affected and not recovered. The gluten containing diet group and prednisone + gluten diet group were detected with glomerulosclerosis and membranous nephropathy. From previous investigations, it was evidenced that IgA nephropathy (IgAN) is one of the commonest glomerulonephritis. In the late 1980s, it was proposed that food antigens can be the reason for IgAN emergence. A large number of studies suggested gluten as the potential antigen responsible for IgAN onset. Others, such as casein, rice proteins and soy bean proteins had also been identified in the mesangium. Coppo *et al.* have reported that IgAN can be induced in experimental mice through gliadin (Smerud *et al*., 2009). Thamer *et al*. (2009) assessed the prednisone effect on organ damage among SLE patients. The results of the experiment revealed that low dose prednisone was unable to reverse the organ damage. Zonana-Nacach *et al*. (2000) evaluated the association between corticosteroid use and organ damage in SLE patients. The organ damage risk associated with cumulative prednisone dose and high prednisone dose was estimated. Cumulative and high prednisone dose were significantly linked to permanent organ damage in SLE patients. In the current study, we observed negative effects of gluten containing diet on SLE manifestations in pristane induced lupus mice, especially for arthritis and gastrointestinal tract. Therefore, gluten free diet might be a better strategy for SLE patients to avoid and cure SLE complications. However, further confirmatory studies are needed in this regard.

CONCLUSION

From the present study, it was concluded that gluten intake could worsen the clinical manifestations in SLE patients. Therefore, administration of gluten free diet might be a better strategy for SLE patients in order to prevent the disease from getting flare-up.

Statement of conflict of interest

Authors have declared no conflict of interest.

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