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EVALUATION OF THE TREATMENT EFFICACY OF TIGECYCLINE AND REISHI SHIITAKE MAITAKE MUSHROOM EXTRACT IN MICE WITH THE VISCERAL LEISHMANIASIS MODEL

VİSERAL LAYŞMANYAZ MODELİ OLUŞTURULAN FARELERDE TİGESİKLİN VE REİSHİ SHİİTAKE MAİTAKE MANTAR EKSTRESİNİN TEDAVİ ETKİNLİĞİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Visceral leishmaniasis (VL) is an infection that can be fatal if left untreated. Treatment of VL is becoming increasingly difficult due to the development of resistance to some drugs used. We aimed to investigate the efficacy of tigecycline (Tig) and Reishi-Shiitake-Maitake (RSM) mushroom extract alone and in combination in BALB/c mice infected with the *Leishmania donovani* strain (ATCC 30030).

Materials and Methods: To compare the treatment efficacy, the mice that were treated with amphotericin B (AMB) were used as the control group. BALB/c mice (n=40) were intravenously inoculated in the lateral vein of the tail with 10^7 stationary-phase *L. donovani* promastigotes in 100 µL of PBS. BALB/c mice were divided into 5 groups of 8. Tig group received 3.7 mg/kg tigecycline intraperitoneally for 5 days, RSM group received 10 mg/kg RSM extract by oral gavage for 5 days while Tig+RSM group received the same doses of both drugs via the same routes. Also, the AMB group received 15mg/kg amphotericin B by oral gavage for 5 days. The spleen and liver of all mice that were sedated with ketamine were collected on the 12th day. Parasite load was determined by Leishman Donovan Unit (LDU) and quantitative RT-PCR.

Results: When all groups were statistically evaluated according to LDU and RT-PCR findings, the lowest value was obtained in the AMB group compared to the value in the control group, while the second lowest value was obtained in the Tig+RSM group. The data obtained in the Tig+ RSM group were significantly lower

ÖZET

Amaç: Viseral layşmanyaz (VL) tropikal ve subtropikal bölgelerde yayılım gösteren, tedavi edilmediğinde ölümcül olabilen bir enfeksiyondur. VL tedavisi kullanılan bazı ilaçlara direnç gelişimi nedeni ile giderek güçleşmektedir. Çalışmamızda *Leishmania donovani* (ATCC 30030) suşu ile viseral layşmanyaz modeli oluşturulan BALB/c farelerde, tigesiklin (Tig), Reishi-Shiitake-Maitake (RSM) mantar ekstresi ve her iki ilacın birlikte tedavi etkinliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Tedavi etkinliğinin karşılaştırılmasında amfoterisin B (AMB) ile tedavi edilen fare grubu kullanılmıştır. BALB/c farelerine (n=40), 100 µL PBS içinde 10^7 strasyoner fazlı *L.donovani* promastigotları kuyruğun lateral damarına intravenöz yolla verildi. BALB/c fareler sekizli 5 gruba ayrıldı. Tig grubuna 5 gün intraperitonal yoldan 3.7 mg/kg tigesiklin, RSM grubuna 5 gün oral gavaj ile 10 mg/kg RSM ekstresi, Tig+RSM grubuna aynı yol, gün ve dozlarda tigesiklin ve RSM ekstresi, AMB grubuna 5 gün OG ile 15 mg/kg amfoterisin B verilmiş, kontrol grubuna hiçbir işlem yapılmamıştır. On ikinci günde ketamin ile sedatize edilen farelerin dalak ve karaciğeri alınmıştır. Parazit yükü Leishman Donovan Unit (LDU) ve kantitatif RT-PCR ile belirlenmiştir.

Bulgular: Tüm gruplar, LDU ve RT-RCR bulgularına göre istatistiksel olarak değerlendirildiğinde, kontrol grubuna göre en düşük değer AMB grubunda elde edilirken ($p<0,001$), ikinci en düşük değer Tig+RSM grubunda elde edilmiştir ($p<0,001$). Tig+RSM grubunda elde edilen değerler AMB grubu hariç ol-

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than the data in other groups, except for the AMB group.

Conclusion: Our study suggested that the use of RSM extract together with tigecycline may be an alternative in the treatment of VL. Further studies using different doses and routes of administration are needed to evaluate the efficacy of this combination.

Keywords: Visceral leishmaniasis, tigecycline, reishi shiitake maitake, treatment

mak üzere diğer gruplardan anlamlı ölçüde düşük bulunmuştur.

Sonuç: Çalışma bulgularımız tigesiklin ile birlikte RSM ekstresinin kullanılmasının VL tedavisinde alternatif olabileceğini göstermekle birlikte, farklı doz ve veriliş yollarının denendiği yeni çalışmalarla desteklenmesi gerektiğini düşündürmüştür.

Anahtar Kelimeler: Viseral layşmanyaz, tigesiklin, reishi shiitake maitake, tedavi

INTRODUCTION

Visceral leishmaniasis (VL) is a vector-associated infection caused by the species included in the *L. donovani* complex. It is estimated that 20,000 to 40,000 people have died due to this infection, which spreads in subtropical and tropical regions. It is noted that an estimated 50.000 to 90.000 new cases of VL occur worldwide each year, and only 25% to 45% of the cases are reported to the World Health Organization. VL is a parasitic infection with a fatal potential of over 95% if left untreated. VL is observed sporadically in eastern Anatolia, the Aegean, central Anatolian and the Mediterranean regions in Turkiye. seventy-one cases in 1997, 24 cases in 2000 and 2003, 20 cases in 2006, 6 cases in 2008, 22 cases in 2014 and 37 cases in 2016 were reported as VL in Turkiye (1, 2).

Pentavalent antimony compounds are still the first treatment option in VL. Despite the main advantages being the low cost and 90-95% efficacy, it has adverse effects that are temporary but life threatening, such as intramuscular administration, longer treatment time, development of resistance, cardiac arrhythmia, pancreatitis, and pneumonia. Liposomal amphotericin B is the first treatment option in developed countries with rapid and up to 100% recovery rates. However, it is expensive and toxic, and patients usually need close monitoring and hospitalization during treatment. Miltefosine is the only oral drug and is used against VL and Cutaneous Leismaniasis. However, resistance to miltefosine has been observed. Compounds with synergistic and/or additive activity can reduce the duration of treatment and the need for doses, toxic side effects and cost can be reduced, and most importantly may prevent the development of drug resistance (1-4).

Tigecycline is the first representative of the glycylcycline class, and has a broad spectrum of antibacterial activity similar to tetracyclines. It inhibits protein synthesis by binding to the 30S ribosomal subunit on the m-RNA ribosome complex of the amino acid t-RNA. It has been determined that tigecycline is well distributed in bone, bone marrow, spleen and thyroid in experimental animal models. Interestingly, although the mechanism has not been fully understood, tigecycline has shown significant antimalarial activity against the in vitro *Plasmodium falciparum* and in-vivo *P. berghei* infection (5, 6).

Innate immune system cytokines play an important role in early protection against leishmaniasis. interleukin (IL)-12, produced by dendritic cells, triggers natural killer (NK) cell activation. IFN-γ produced by NK cells can limit the spread of the parasite until the T-cell response develops. interferon gamma (INF-γ) is very important for increasing the killing capacity of macrophages, which are the primary target of promastigotes. Tumor necrosis factor (TNF) plays a critical role in the elimination of parasites in the liver and spleen, as well as in tissue damage. TNF production controls the formation of granuloma and the reproduction of the parasite. However, the excess amount of TNF causes cell damage. The immune response in patients with VL is characterized by high levels of antibodies, the presence of Leishmania-specific T-cell proliferation, and a low level or no existence of IL-2, IFN-γ. Recovery from VL is achieved by the stimulation of the Th1 response, which is prepared by IL-12, dendritic cells and macrophages. IFN-γ produced by the T cells induces nitric oxide (NO) -mediated killing of parasites. In contrast, the progression and worsening of the clinical picture in VL are associated with the intensive production of IL-10, Transforming growth factor beta (TGF-β), and IL-4 which are Th2-type of cytokines (7, 8).

Reishi (*Ganoderma lucidum*) is a medicinal mushroom that is a lanostane type source of triterpenoid (9). The pharmacological potential of *G.lucidum* is based on an existing polysaccharide-peptide complex, β–glucans and lectine triterpenoids which have a strong immunomodulating action supporting and improving the immune system. Particularly, the polysaccharides of *G. lucidum* trigger the release of cytokines, the functions of T and B lymphocytes, dendritic cells, macrophages and NK. The in-vitro pharmacological studies showed that *G. lucidum* polysaccharides have strong effects on macrophage functions by increasing the release of IL-1α, IL-6, IL-10, and TNF. One study showed that *G. lucidum* polysaccharides increased the concentration of IL-2, TNF-α and IFN-γ, promoting the functional maturation of dendritic cells by strengthening the cytotoxic activity of T cells and NK cells (10-12). Shiitake (*Lentinus edodes*) is an edible mushroom. The oral intake of its polysaccharides was shown to have modulated the functions of immunity, and increased the fagocytosis and TNF-α levels in macro-

phage cell cultures (13). Maitake (*Grifolia frondosa*) immunomodulation is the most well-known efficacy of *G. frondosa* biocomponents, and has been confirmed by various studies. These immunomodulatory components have been shown to increase the efficacy of many other immune-related cells, such as macrophages, cytotoxic T-cells, and NK cells (14, 15).

The aim of the present study was to investigate the therapeutic efficacy of tigecycline (Tig), RSM mushroom extract, and combined treatment efficacy of both drugs in BALB/c mice that were created in a VL model with the *L. donovani* strain (ATCC-30030).

MATERIAL AND METHODS

The Novy–MacNeal–Nicolle (NNN) medium was used for the production of the ATCC-30300 *L. donovani* strain in the present study. The solid phase of the medium was sterilized in an autoclave for 15 minutes by adding 1.4 g agar and 0.6 g NaCl to 90 ml of distilled water. 10 mL of defibrinated rabbit blood was added into the medium that was heated to approximately 50-55°C and transferred to the medium tubes to obtain 4 mL in each tube and was allowed to cool by tilting. 0.2 mL of penicillin G and streptomycin sulfate were added to the solidifying medium and taken to 8°C, and 1 ml of 10% fetal calf serum (FCS) containing RPMI-1640 was added to the medium tubes before culturing to create a liquid phase (16). As recommended by the ATCC, the ATCC-30030 *L. donovani* strain was opened and planted on the checked medium.

Six-week-old BALB/c female mice were used in the study. The mice were obtained from the laboratory of the Istanbul University, Aziz Sancar Institute of Experimental Medicine (DETAE), Department of Laboratory Animal Science and were monitored throughout the experiment at 20-22°C, with 50-60% humidity, 12 hours light, 12 hours dark cycle environment, and each cage including four mice. Food and water were provided as ad-libidum. Approval was obtained from HADYEK (Animal Experiments Ethics Committee) for the study (Date:25.02.2016, No: 2016/17).

The mice were divided into five groups, each group involving eight mice (n:40). Group 1: Control group (C), Group 2: Tig, Group 3: RSM, Group 4: Tig+RSM, and Group 5: Amphotericin B (AMB) group.

All mice were infected with 10^7 stationary phase *L.donovani* promastigotes in 100 µL of phosphate buffered saline (PBS) intravenously through the lateral vein of the tail.

1. Control group (C): No procedure was performed after the *L. donovani* strain was given to the mice, and the daily health conditions were monitored in this group (figure 1).

2. Tig group: Starting from the Day 7 after giving the *L. donovani* strain, 0.5 mL intraperitoneal 3.7 mg/kg tigecycline (Tygacil) was administered at the same time for 5 days.

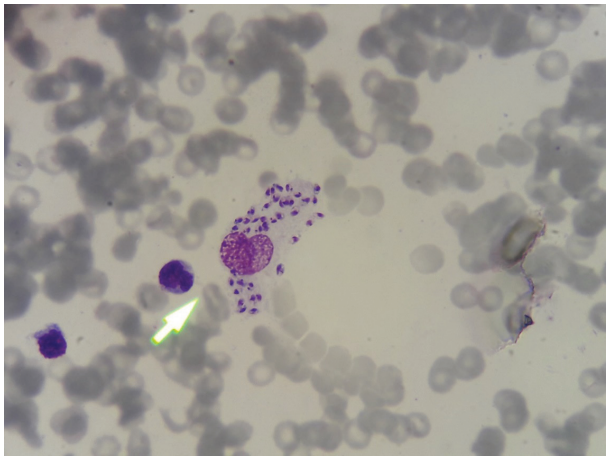

Figure 1: Amastigotes in spleen swab sample of positive control group mice (Giemsa stain x100)

3. RSM extract (Solgar) group: Starting from the Day 7 after giving the *L. donovani* strain, 0.2 mL oral gavage (OG) as to include 10 mg/kg was given at the same time for 5 days.

4. Tig+RSM group: Starting from the Day 7 after giving the *L. donovani* strain, intraperitoneal 3.7 mg/kg Tig was administered at 0.5 mL, and 10 mg/kg RSM 0.2 mL OG was administered at the same time for 5 days.

5. AMB (Ambisom-Gilead) group: Starting from the Day 7 after giving the *L. donovani* strain, 0.2 mL 15 mg/kg amphotericin B was administered with OG at the same time for five days.

At day 12, the spleen and liver were removed by opening the chest cavity after the mice in all groups were sedated with ketamine. The spleen and liver were weighed, and a swab was taken on the slide from the transverse cut surface of the right median lobe of the liver, stained with Giemsa, the number of amastigotes / 1.000 host cell nucleus was multiplied by the total weight of the liver (gr), and the Leishman Donovan Unit (LDU) and parasite load were determined by the quantitative PCR by DNA isolation from the spleen and liver samples (17). For DNA isolation, 25 mg of liver/spleen fragments were broken into pieces in a homogenizer and transferred to 1.5 ml tubes. The ZYMO RESEARCH Quik-DNA kit (USA) was used for the procedure, and the DNA isolation was performed in according to the manufacturer's recommendations. Samples were kept at -80°C. For qRT-PCR, the Techne qPCR *L. donovani* & *L. infantum* (UK) kit was used and studied in accordance with the test procedure.

Statistical evaluations were given as mean, standard deviation, median, minimum, and maximum values with

descriptive statistics of the results. The Shapiro-Wilk test was used to determine the suitability of the measurement results for the normal distribution. The differences in the measurements between the groups were performed by one-way analysis of variance (ANOVA), and multiple comparison tests were evaluated by Tukey-HSD. The relationships between the measurement levels were given by calculating the Pearson correlation coefficient. The statistical significance was accepted as $p<0.05$ and two-ways.

RESULTS

In our study, the number of amastigotes LDU and amastigote DNA copy numbers in mice in the RSM, Tig and Tig+RSM groups and in the control (C) and AMB groups were compared (table 1, figure 2). The mean LDU and copy/mL values were found to be statistically significantly higher in the control group compared with the values in all other groups ($p<0.05$). The parasite load was found significantly lower in Tig, RSM, and Tig+RSM groups when compared with the levels in the control (C) group

Table 1: Distribution of the amastigote count (LDU) and the number of DNA copies (copies/ml) in mice in the experimental groups

	Groups	N	Median	Std. deviation	Minimum	Maximum	Two way significance	
LDU	C	8	95.74	11.71	73.83	110.97	F=135.627	P<0.001
	Tig	8	71.98	10.91	53.50	84.50		
	RSM	8	54.81	4.95	48.45	61.10		
	AMB	8	3.64*	1.38	1.80	5.60		
	Tig + RSM	8	42.27§	8.18	32.64	59.80		
RT-PCR	C	8	61.50	4.93	52.00	67.00	F=208.3	P<0.001
	Tig	8	52.50	3.70	48.00	58.00		
	RSM	8	46.88	1.36	45.00	49.00		
	AMB	8	2.49*	0.99	1.20	3.80		
	Tig + RSM	8	29.55§	7.97	22.00	48.00		

*p<0.05 ; §:p<0.05; C: Control group, Tig : Tigecycline treatment group, RSM: Reishi Shiitake Maitake treatment group, AMB:Amfoterisin B treatment group, Tig+RSM:Tigecycline+Reishi+Shiitake Maitake treatment group

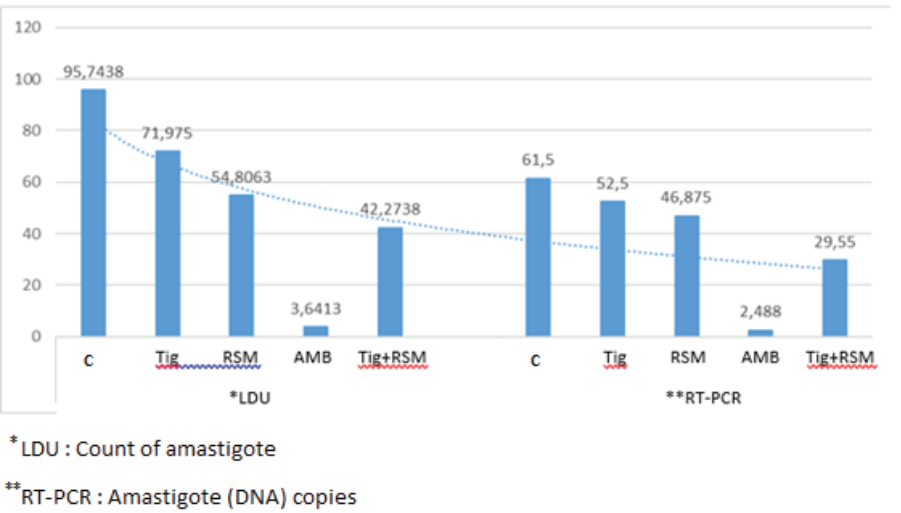

Figure 2: Graphical representation of the values obtained between the treatment groups according to the two measurement methods

C : Control,Tig : Tigecycline, RSM: Reishi-Shiitake-Maitake mushroom extract, AMB: Amphotericin B

($p<0.001$). The evaluation of the mean values showed that the lowest value was obtained in the AMB group, while the second lowest value was obtained in the Tig+RSM group. The values obtained in the Tig+RSM group were found to be significantly lower than in other groups, except for the AMB group.

DISCUSSION

Due to the fact that most anti-leishmania drugs are toxic, treatment requires hospitalization, their higher cost and the problem of increasing resistance make the treatment harder. Although in recent years improvements which reduce the treatment time and costs have been enabled with the combination treatment options, there is still a need for new drugs (4,17).

Tigecycline is the first representative of glycylcyclines. In a study, Tigecycline was shown to reach higher concentrations rapidly in human polymorphonuclear cells (5). Tigecycline was found to have antimalarial activity against the in-vitro *P. falciparum* and in-vivo *P. berghei* infections in the experimental mouse model. Researchers also found that the tigecycline antimalarial activity was strengthened by the combined association with chloroquine against the *P. falciparum* strain resistant to chloroquine. Malaria was completely eradicated using tigecycline, which was given 3.7 mg/kg IP for four days in combination with chloroquine in mice that were infected with *P. berghei* (6). Another study conducted in the same year reported that tigecycline had antimalarial activity against the culture-adapted, chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum* (18). In 2006, tetracyclines were suggested to be effective antimalarials and because they blocked the protein synthesis in prokaryotics, despite their unclear effect mechanism, it is suggested that they disrupted the mitochondria, and apicoplast including ribosomal subunits of prokaryotic origin in their genomes (19). In recent years, various antibiotics including tetracyclines and lincosamides have been shown to target apicoplast in *Plasmodiums* (20,21). The apicoplast is a plastid-like organelle from an endosymbiotic ancestor and is not found in humans. However, it is a good drug target because it is required for the survival of the parasite. Antibiotics that are effective against apicoplasts cause a typical "delayed death" effect on *Plasmodiums*, and the apicoplast function is lost in the parasite generations after exposure to the antibiotic (22,23).

The kinetoplast DNA (kDNA) involved in the single mitochondria of kinetoplastid parasites consists of mini- and maxi-circles adherent to a two-dimensional DNA network. The maxi-circles encode the mitochondrial proteins and the rRNA, while the mini-circles encode the guide RNA molecules that function in the regulation of mRNA transcripts (24). In light of this data, we may suggest that the rRNA in the kinetoplast DNA might be affected by the tigecycline used in our study.

In kinetoplastid parasites, all kinetoplasid-derived enzymes of trypanothion metabolism have been confirmed as drug targets by the genetic studies. Trypanothion reductase (TR) is one of the most well-protected gene regions in kinetoplastid parasites and is important for parasite survival. All attempts to obtain a mutant strain of trypanothione reductase on have failed in *L donovani*. Disruption of two TR alleles caused a significant decrease in the survival capacity of mutants with a low TR mRNA within the macrophage (25). Tigecycline used in the present study may disrupt the transcription by affecting the TR mRNA. In our study the number of amastigotes has significantly decreased in mice which were treated with only tigecycline compared to the numbers in the control group ($p<0.05$). One study investigating the in vitro synergistic efficacy of various antibiotics against L. donovani showed the synergistic efficacy of amodiaquine /quinine as 61.22%, pentamidine / quinine as 89.8%, pentamidine /amodiaquine 83.67% and gentamicin/amodiaquine as 100%, however, tetracycline and tigecycline were found to have no efficacy (26). No other studies which used the tigecycline against *L. donovani* were found in accessible resources. Our study is the first in vivo study which investigated the efficacy of tigecycline against *L. donovani*.

Reishi (*G.lucidum*) is a mushroom which has antifungal, antiviral effect in addition to its immunomodulatory and antiparasitic efficacy (11,12). *G. lucidum* extracts have shown antimalarial activity in mice infected with *P. berghei*. A group of mice infected with the *P. berghei* strain were given 100 and 250 mg/kg terpenoid extract of *G. lucidum*; 30 mg/kg chloroquine was given to the other group of mice with OG as standard therapy, and the results were compared with the control group of mice. In addition to antimalarial activity, *G.lucidum* has also been reported to provide improvement in liver damage associated with malaria and enabled significant decrease in AST, ALT, ALP and GGT levels (27). Similarly, RSM included 100 mg *G. lucidum* in our study. In another study, one ethyl acetate extract of *G. lucidum* was found to have antiplasmodial activity with 79% inhibition (at 4.9 µg/ml) (28). Seberi et al. suggested that the hydroalcoholic extract of *G.lucidum* at concentrations of 150 and 200 mg/ml enabled a significant decrease in the number of *L. major* living promastigotes and this effect was due to components such as tannins, flavanoids, terpenoids and polysaccharide (29). We also found in our study that it significantly reduces the number of amastigotes in vivo.

G.lucidum β-glucans have been shown to induce macrophages, neutrophils, monocytes, NK and dendritic cells. (30). The in vitro pharmacological studies with *G.lucidum* showed that *G.lucidum* polysaccharides induced macro-

phage phagocytosis by increasing the release of IL-1α, IL-6, IL-10, and TNF-α (11).

G. lucidum polysaccharides were found to have a positive effect on the Th1 response by increasing the IL-2 and IFN-γ levels. However, they have little or no effect on the production of IL-4, and IL-10 (31). Various experimental models and clinical studies showed that IFN-γ production and Th1 response in VL triggered a strong leishmanial mechanisms in phagocytes. In contrast, IL-4 and IL10 production and Th2 response inhibited the macrophage activation resulting with the intracellular replication of the parasite (32). With this data, *G. lucidum* included in RSM that was used in our study probably activates the Th1 response by increasing the IFN-γ level. However, not affecting the release of IL-4 and IL-10 was consistent with the results obtained from RSM and Tig+RSM group mice. IL-4, and IL-10 are the cytokines that contribute to pathogenesis in VL (32).

In many studies Shiitake (*L. edodes)*, were reported to have immunomodulatory, antitumoral, antiviral, and antioxidant efficacy and these activities were mainly due to its pharmacological components such as proteins, peptides, polysaccharides, terpenoids, and sterols (12).

Individuals who consumed 5 gr or 10 gr of mushrooms or who did not consume mushrooms for four weeks were investigated in a comparison in this study; in the group consuming mushrooms, the T lymphocytes and NK cell proliferation, and the IL-4, IL-10, TNF-α, and IL-1α levels were found to have increased (33). Crespo et al. compared rabbits that were fed a conventional diet. The conventional diet consisted of 5% purified β-glucan in their in vivo study. They reported that in rabbits provided with β-glucan, the indicator of immune stimulation of IL-10 expression was downregulated with the expression induced by macrophage and IL-4, IFN-γ. Shiitake included high a amount of β-1-3 and β-1-6 D-glucan (lentinant), and revealed that the β-glucans induced the intracellular signaling, and interaction with different cellular receptors may activate the different profiles of the immune response (34). IL-10 is a cytokine that contributes to pathogenicity in human VL and experimental mouse models of VL, whereas IFN-γ has a protective effect (32). This data is consistent with the significant decrease in parasite load that occurred in the RSM mice compared to the control group in our study ($p<0.001$).

Goldman et al. found that 50 mg, 200 mg, and 400 mg/kg IV and IP administration of β-glucan four times separately to BALB/c mice 4 days after they were infected with the *L.major* strain prevented the development of lesions, and 400 mg/kg administration prevented even the first phase of lesion development. In another study, the IP administration of 0.45 mg glucan seven, five, three, and one day before infection on BALB/c mice which were infected with *L. major* promastigotes showed a significant decrease in the amastigote count on the spleen and liver compared with the levels in mice in the control group ($p<0.001$) (35,36). Ghosh et al. reported that β-glucan obtained from *Alcanigenes faecalis* through 10 mg IP administration 10, 15 and 20 days after infection in BALB/c mice of the VL model decreased the parasite load as 99% in the liver and spleen (37). They also observed in β-glucan-treated mice that the IL-12, IFN-γ, TNF-α and IL-1 β levels increased 4.6, 5.7, 8.2, and 5.1 times compared to the levels in the control group, respectively. In the interpretation of their study, they considered that the activation of Th1 cytokines could directly stimulate NO production.

Similarly, although the route of administration was oral gavage, the number of amastigotes decreased in the liver and spleen in mice receiving 10 mg/kg RSM for five days compared to the levels in the control group in our study ($p<0.001$).

Sandvik et al. administered 20 mg/kg *Saccaromyces cerevisiae* type β-1-3 and β-1-6 glucan for 14 days to mice who were created to have endotoxemia with *Escherichia coli*, and reported that OG administration was more effective compared to subcutaneous administration, and they found a significant decrease in the indicators of kidney and liver damage (38). They found that IL-10 expression, which is an indicator of immunostimulation, is downregulated in combination with macrophage, IL-4, IFN-γ-induced expression of β-glucan administered with oral gavage. Similarly, we administered RSM extract with OG in our study.

IL-10 contributes to pathogenesis in VL in humans and in experimental mouse models, while IFN-γ has a protective effect (32). Our study results are consistent with this data.

Maitake (*Grifolia frondosa*) is a type of edible mushroom. This mushroom is known as a natural immunomodulator as having no side effects related to increasing immunity. It contains high β-glucan and has β-1-6 branching points in addition to β-1-3 bonds in the main chain of β-glucan. In in-vitro studies β-glucan was shown to increase the production of TNF-α, IL-1 and IL-6 by activating the macrophages (15). In experimental animal models, IP administration was found to have significantly increased the production of TNF-α and the cytotoxicity of NK cells (14). Meng et al. found that the administration of polysaccharides obtained from *G. frondosa* with OG as 30, 60, 120 mg/kg for 14 days induced the fagocytosis, and increased the IL-1β , IL-2, IL-6 and IFN-γ levels in splenocytes in the immunosuppressed mice that were induced with IP cytoxane. The RSM extract given with OG includes 100 mg of maitake in our study (39). Sultana et al. showed in their study against the promastigote and amastigote forms of *L. donovani*, *L. tropica* and *L. major* strains that in macrophage cultures the semi-purified

extract of *G.frondosa* reduced amastigote replication in macrophages and the level of IL-10 and TGF-β from inflammatory cytokines more effectively than the reference drugs of amphotericin B, paromomycin, miltefosine and sodium antimony gluconate in VL treatment. In addition, the extract was also found to induced the apoptosis in promastigotes (40).

CONCLUSION

Our study results suggest that the combined use of RSM extract together with tigecycline may be used as an alternative approach in the treatment of VL, however, there is a need for further research that investigate the different doses and administration routes in order to show the treatment efficacy.

Ethics Committee Approval: This study was approved by Istanbul University Local Ethics Committee of Experimental Animals (Date: 05.02.2016, No: 2016/17).

Peer Review: Externally peer-reviewed.

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