

# Immunomodulatory Activity of *Gentiana Macrophylla* Pall on C57bl/6 Mice

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**Abstract:** Background: High level of ATP and NADPH and carbohydration (CHO) to formation of reduced metabolite such as gentiopicroside, secoiridoid glycosides and triterpenes, and loganicacide, gentians, is the bioactive components of the (*Gentianamacrophylla*) gentian species of plants. Several days after antigen exposure, antigen-specific antibodies, predominantly of the IgM isotype, are generated and released into the general circulation by B-cells and plasma cells. The method: Sheep blood was put into a sterile heparinized tube. The solution was rinsed three times with saline before centrifugation at 2000 rpm for 10 min. The supernatant was abandoned and the Sheep red blood cells (SRBC) were prepared. 0.2 ml 10% SRBC immunized was lateral tail vein injection into each mice (N.K.Jerne and A.A.Nordin., 1963). Result: A significant decrease ( $P < 0.05$ ) in the hemagglutination (antibody titre) was observed in the *Gentianamacrophylla* (GM) treated group (37,8%) when compared with the control group (56%) Table 1. Conclusion: From the protonation compounds generate polyphenol compound with rich hydroxyl and alkaloid. These compounds prevent to lipid peroxidation or antioxidation and membrane protection and immunomodulation and protection of immune system.

**Keywords:** immunomodulation, *Gentianamacrophylla pall*, hemagglutination, antibody

## 1. Consideration of Experimental Animals

The United States National Academies of Science estimates that as many as 22 million vertebrate animals are used every year in the United States alone for research and testing. About 85 percent of these animals are rats and mice. The scientific community is well aware of the invaluable role these rodents have played in the development of modern medicine and the lengthening of the average human lifespan from just 40 years at the turn of the 20<sup>th</sup> century to over 70 years today. The monument to the experimental animals humanity has used to study genetics, molecular biology, pathophysiology, immunology, toxicology, surgery and mechanism of disease, pharmacology, as well as for the development of new drugs. Experimental animals of monument such as Pavlov's Dog (1902), is located in the garden of the institute of Experimental Medicine at the Apothecary island in Saint - Peterburg, Russia, Bronze composition of dog and a cute mongrel puppy (2010) Ufa, Russia. The baboon monument is located on the campus of the Scientific Research Institute of experimental pathology and Therapy, in Sukhumi, a city on the coast of the Black Sea. The monument of a sad-looking dog can be seen at the entrance to the Grodno Medical University, in Belarus. Laika (Space dog) was a stray dog found on the streets (2008) of Moscow (The Soviet space dog who became one of the first animals in space, and the first animal to orbit the Earth). Monument to lab mouse (2012) in Novosibirsk, Russia. A statue located in front of the institute of Cytology and Genetics of the Russia Academy of Sciences in Akademgrodok (The statue depicts a mouse in a lab coat and glasses, knitting the DNA double helix). Monument of experimental rats (2019), is located in the square of the New Medicine Medical university in Ulanbator, Mongolia (According to the director of University, Ambaga Miyegombo, rat (Wistar) monument (figure 1) is symbolizes gratitude for the animal that humanity has used to study all

medical science, research development of new drugs and scientific degree, anti-pseudoscience or prevent to anti-science).



**Figure 1:** Locates in the square of the new medicine Medical University in Ulanbator, Mongolia

## 2. Background

Medicinal plant is water molecules are great, in cool environment, sunlight is low, In that case reduced reaction such as HADH, NADPH, and high level of protonation compounds (multiple hydroxyl), and high flow of proton to ATP generation by ATP synthetase enzyme. ATP and NADPH, FADH<sub>2</sub> to synthesis of reduced formation

polyphenols and alkaloids in plant cell environments (Ambaga M et al., 2010). High level of ATP and NADPH and carbohydrate (CHO) to formation of reduced metabolite such as gentiopicoside, secoiridoid glycosides and triterpenes, and loganicacide, gentians, is the bioactive components of the (*Gentianamacropylla*) gentian species of plants (Yondonperenlei R et al.,2018, Na Jia et al., 2012). The species are strictly restricted to the high alpine lands of the *Tibetan Plateau* (between 2400-3500 meters) and high *Khuvsgul* and *Khentii*lands (low sun light, high level of water, cold environmental, increased of reduced reaction and the potential of antioxidant and anti-inflammatory, immunomodulation, protection of immuno cell induced cell damage and the regulation of apoptotic process, protection and inhibition of reactive oxygen species release from phagocytic cells. Chemical composition by Photosynthetic system (*Calvin cycle*) in the *Gentianamacrophylla*) of the *Mongolia* (Na Jia et al., 2012, Yondonperenlei R et al., 2019, Farong Yu et al., 2004, Teng Gong et al., 2018, Chih-Yang Huang et al., 2015). Used in Tibetan and Chinese, Mongolian traditional medicine for treatment of diverse disease, such as toxic disease and fever, rheumatoid arthritis, diabetes, apoplexy, and liver disease, stomachache, gallbladder pain and enteritis (Na Jia et al., 2012, Yondonperenlei R et al., 2019, Farong Yu et al., 2004, Teng Gong et al., 2018, Chih-Yang Huang et al., 2015).

The membrane-based mechanism for making ATP were formed very early in life history and its essential features retained in the long evolutionary journey from the time of the early prokaryotes to modern cells during last 4.5 billion years by converting to membrane-redox potential three state (alpha state with high oxidation potential, beta state with high reduction potential, gamma state with low redox potential) line system (Ambaga M, Tumen-Ulzii A., 2018). The evolutionary development of this set of v-genes in phylogeny is traced back to the requirements for cell to cell recognition in all metazoa. Antibody diversity reflects differences in the amino acid sequences of the polypeptide chains of antibody molecules of different specificity (N.K. Jerne et al., 1971). The genetics of immune responsiveness have revealed the importance of the histocompatibility pattern of an animal in restricting its range capabilities in antibody formation (N.K. Jerne et al., 1971). The total number of lymphocytes represent a little more than 1% of the body weight of an animal thus, it would not be wrong to say that our immune system is an organ consisting of about  $10^{12}$  lymphocytes (Niels.K. Jerne et al., 1984).

The production of antigen-specific antibodies represents a major defense mechanism of humoral immune responses. Following antigen exposure, the generation of antigen-specific antibody response involves the cooperation and interaction of several immune cell types (i.e. antigen-presenting cells, T-helper and B-cells,) and cell products (Gregory S. Ladics et al.,2007). Several days after antigen exposure, antigen-specific antibodies, predominantly of the IgM isotype, are generated and released into the general circulation by B-cells and plasma cells (Ellen J. McAllister et al.,2017). As the assessment of the primary antibody response to a T-dependent antigen (eg.,SRBC) has been reported to provide one of the best predictors of

immunotoxicity in mice (Gregory S. Ladics et al., 2007, Zhoujie Ding et al.,2013).

As the assessment of the primary antibody response to a T-dependent antigen (e.g.,SRBC) has been reported to provide one of the best predictors of immunotoxicity in mice (*Lustar et al.*, 1992). The SRBC-specific ELISA is relatively new, with the first publication of the method appearing in 1993 (*Temple et al.*, 1993). N.K. Jerne and Nordin developed the hemolytic plaque assay in 1963 to measure the number of IgM antibody-forming cells specific to SRBC (Gregory S. Ladics et al.,2007). IL-17 is a T cell-derived proinflammatory cytokine originally named cytotoxic T lymphocyte-associated serine esterase-8 (Susumu Nakae et al.,2002). IL-17 is produced by TCR $\alpha/\beta^+$  CD4 $^-$  CD8 $^-$  thymocytes, as well as activated CD4 $^+$  and CD4 $^+$ CD45 RO $^+$  memory T cells (Yao et al.,1995). IL-17 has pleiotropic activities including induction of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, G-CSF, and MCP-1 on various types of cells (Susumu Nakae et al.,2002). To assess the kinetics of the production of SRBC-specific Abs over the course of the immune response, we measured IgG and IgM levels in serum obtained from mice at days 0, 7 and 14 after the immunization, challenged the mice with a second SRBC immunization on day 14, and measured IgG and IgM levels 7 and 13 day later (days 21 and 27 of the immunization, respectively). As expected, titers of SRBC-specific Abs increased after the immunization, and the increase in IgG was more prominent than the increase in IgM. The immune response to SRBCs peaks 7-10 day after immunization; thus, serum collected at day 7 was expected to contain high level of SRBC-specific antibodies (Ellen J. McAllister et al., 2017).

**Purpose:** We aimed at studying immunomodulatory effects of *Gentianamacrophylla pall* in animal model induced by SRBC immunization.

### 3. Material and methods

**Plant Material** The plant *Gentianamacrophylla pall*. Was collected during July 2017 from the forest region *Galt sum, Khuvsgulaimakh, Mongolian*. The taxonomical identification of the plant was done by Botanical survey of Ganbold E. (ScD) *Mongolian* university subsidiary of Ulanbator institute, *Ulanbator*. The study was based on the New medicine medical university bio-model, pharmacology laboratory and ELISA-biochemistry laboratory.

**Animals:** Male *C57BL/6* (miceweight 18-22g (n=40)) were obtained from the *Mongolian* national university of Medical sciences animal house. Experimental procedures were conducted in accordance with the regulations of Animal Ethical committee.

**The method of SRBC induced immunization:** Sheep blood was put into a sterile heparinized tube. The solution was rinsed three times with saline before centrifugation at 2000 rpm for 10 min. The supernatant was abandoned and the Sheep red blood cells (SRBC) were prepared. 0.2 ml 10% SRBC immunized was lateral tail vein injection into each mice (*N.K. Jerne and A.A. Nordin.*, 1963). Control group the vehicle, comparing group Salimon (immunostimulation product of Mongolia, by *Salsolaricifolia*) 1ml/kg (minimal

dose) and Dexamethazone 1mg/kg at a dose orally, experimental group *Gentianamacrophylla* extract at a dose of 100mg/kg orally daily for 8 days respectively. Last day (8 days later), blood was drawn from the heart, after isolation of serum and blood cells by centrifugation. The assess of IL-17 (pg/ml), Anti-SRBC IgM (ng/ml) in plasma by ELISA kit (Temple et al., 1993 and) (Shanghai MLBIO Biotechnology Co.Ltd) and hemagglutination (Sarantsetseg B and Ambaga M et al., 1999., Promoda Kumar Sahoo et al., 1996).

#### 4. The Result

**Table 2:** The compared result of *Gentianamacrophylla*, Salimon, Dexamethazone and Control group in immunization by T-cell dependent antigen (SRBC)

No.	Group	Dose	Hemagglutination (%)	IL-17 (pg/ml)	Anti-SRBC IgM (ng/ml)	Thymus index (gr)	Spleen index (gr)
1	Controll		56,1±5.1	19,32±2,3	13,36±1,23	0,00222±0,00012	0,00309±0,00003
2	<i>Gentianamacrophylla</i>	100mg/kg	37,8±3.3**	10,73±2,2*	8,02±1,03*	0,00011±0,00001**	0,00343±0,00023
3	Salimon	1ml/kg	62,5±2.2*	30,00±2,67*	15,95±1,35*	0,00137±0,00022	0,00295±0,00026
4	Dexamethazone	1 mg/kg	38,5±3.4**	9,60±0,8**	8,19±1,24*	0,00014±0,00002**	0,00138±0,00021**

\*p<0.001, \*\* p<0.05 comparing with the control

#### 5. Conclusion

Evolution has different chemical compositions because of plant environment (Photosystem), the sun light and the wet soil. Protonation compounds are dominated in the following plant environments such humid ground (high level water), less sun light, upland and cool temperature. From the protonation compounds generate polyphenol compound with rich hydroxyl and alkaloid. These compounds prevent to lipid peroxidation or antioxidation and membrane protection. The results of the study show that *Gentianamacrophylla* is inhibition of immune cell dependent cell damage and detoxification of reactive oxygen species release from phagocytic cells, and treatment of autoimmune disease and hypersensitivity reaction. Maybe a *Gentianamacrophylla* is immunomodulation and protection of immune system.

#### 6. Acknowledgment

The authors thank to New medicine medical university of experimental laboratory and ELISA-Bio chemistry, pharmacology laboratory. Galindav B (*Mongolian* national university of Medical sciences animal house).

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A significant decrease (P<0.05) in the hemagglutination (antibody titre) was observed in the *Gentianamacrophylla* (GM) treated group (37,8%) when compared with the control group (56%) Table 2. A significant decrease (P<0.001) in the Anti-SRBC IgM was observed in the *Gentianamacrophylla* (GM) treated group (8,02ng/ml) when compared with the control group (13,36 ng/ml). Condition of SRBC induced immunization in *C57BL/6* mice a significant decrease (P<0.01) in the IL-17 was observed in the *Gentianamacrophylla* (GM) treated group (10,73 pg/ml) when compared with the control group (19,32pg/ml).

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