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Article

Preliminary evaluation of five herbal Ayurved medicines commercially available in Bangladesh for the proliferation of murine spleen cells and production of IgM & IgG antibodies *in vitro*

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Abstract

Although Ayurved medicines have 3000 years long history of traditional use for the treatment of diseases, scientific evidences in support of the therapeutic effect and safety of most of the preparations yet to be explored and justified. The present study aimed to evaluation of the immunostimulating activity of five preparations of Ayurved medicines namely Kanakasav, Dasamularista, Draksharista, Saribadi and Bramhi Rasayan manufactured and marketed in Bangladesh. Freshly prepared Balb/c mice splenocytes were treated with 1, 5 and 10% (v/v) of different Ayurved preparations and the cells were sub-cultured at 37ºC, humidified atmosphere containing 5% CO_2 for 120 hours. The production of IgM and IgG antibodies, and proliferation of cells were determined by enzyme-linked immunosorbent assay (ELISA), and 3-(4,5-dimethylthiazol-2-y)-2,5-diphenylterazolium bromide (MTT) methods, respectively. Among the five preparations tested, Kanakasava and Saribadi stimulated the proliferation of splenocytes and enhanced production of both IgM and IgG antibodies. Interestingly, KNK at the dose of 1% exerted immunostimulating activities but the higher doses 5% and 10% did not show any stimulating activities, rather those concentrations of KNK were found to be toxic to the spleen cells. Similarly, 1% concentration of SDI stimulated the splenocytes proliferation and IgM and IgG antibodies productions. Higher doses resulted with the decreased production of IgM and IgG antibodies by the 5% and 10% concentrations of SDI because of the similar reason as illustrated for KNK. This result is also an indicator of narrow safety profile of both of these Ayurved medicines, KNK and SDI. The other three Ayurved preprations - Dasamularista, Draksharista and Bramhi Rasayan failed to induce immunostimulation *in vitro*. Although Bramhi Rasayan (BRS) showed stimulating activity at the dose of 10% (v/v), it could not induce the production of antibody. This is the first report on the evaluation of immunostimulating activities of Kanakasav, Dasamularista, Draksharista, Saribadi and Bramhi Rasayan which resulted the preliminary immunostimulating activities of Kanakasav and Saribadi. Further investigations are required with the comprehensive experimental studies to explore its immunostimulating potential and safety of the two preparations.

Keywords: Ayurvedic medicine, Kanakasav, Dasamularista, Draksharista, Saribadi, Bramhi Rasayan, Immunostimulant, Splenocytes, IgM, IgG, Differentiation, Proliferation, BALB/c mice.

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Introduction

Ayurved medicines originated in India have about 3000 years long history of traditional use for the treatment of diseases. In spite of its long history of use and practice among the people of India and some Asian developing countries including Bangladesh, reports of scientific evidences for the efficacy and safety of those Ayurved medicines are very negligible. The popularity of herbal medicine is increasing due to the tremendous side-effects of synthetic or modern medicines (Sarker, 2014a). Besides, negative feedbacks of several commercially available drugs lead to the investigations of new therapeutic approaches from natural sources and alternative medicines (Imam et al, 2013; Sarker, 2014b). Recently, it has been given great emphasis on the scientific evaluation of traditional complementary and alternative medicines for the justification of their traditional use as well as for the assessment of new therapeutic value that have not yet been explored scientifically. WHO has recently focused its attention to the traditional, complementary and alternative medicines to include them in the national healthcare system, and suggests researches on these medicines in order to ensure their safety, efficacy and quality (Sarker et al, 2012). The national health policy of Bangladesh has similar objective to encourage the systemic improvement of the practice of indigenous medicines and emphasized scientific evaluation of those indigenous and herbal medicines. Recently lot of investigations are carried out for the evaluation of scientific basis of different forms of herbal medicines, functional foods, nutraceuticals, complementary and alternative medicines intended for the prevention and treatment of diabetes (Munira et al, 2020; Khan et al, 2019; Chen et al, 2019; Nesa et al 2018; Ullah et al, 2017; Shah et al, 2016b; Sarker et al, 2015; Kifayatullah et al, 2017b), cancer (Sheikh et al, 2017a; Sheikh et al, 2017b; Jesuraj et al, 2017; Shajib et al, 2018), immunity (Goto et al, 2010; Sarker et al, 2014; Sarker et al, 2012; Sarker et al, 2011), hyperlipidemia and obesity (Sarker et al, 2015; Rouhi et al, 2017; Kazemipoor et al, 2014; Kifayatullah et al, 2017a, Kifayatullah et al, 2017c), inflammation (Kamarudin et al, 2017; Imam et al, 2013), infections (Yasmin et al, 2009), oxidation (Karim et al, 2015), neurological disorders (Das et al 2017), toxicological (Kifayatullah et al, 2015; Shah et al, 2016a), and other studies for the isolation, identification and different properties (Hasan et al, 2015; Sikder et al, 2015; Nahar et al, 2015; Rashid et al, 2012) of herbal medicines and phyto-compounds. In order to improve the quality as well as to ensure the therapeutic potential and safety of herbal medicines, there is no other alternative for scientific studies on herbal medicines including the Ayurved medicines which is widely used among the people of Bangladesh as well (Pelkonen et al., 2014).

The defensive system of human body, composed of innate and adaptive immunological arms, protects it from possible infections from microorganisms and prevents the formation of cancerous cells and/or eliminates them as soon as they appear as an antigen (Goto et al, 2010; Sarker and Gohda, 2013). In certain conditions, such as, diabetes, obesity, burns, sepsis, stress, insomnia, AIDS, lack of exercise, poor nutrition and aging, the immunity is greatly suppressed (Godbout and Glaser, 2006; Sarker and Gohda, 2013; Keusch, 2003; Sheridan et al, 2012). Immunity is also suppressed in case of receiving radiotherapy, chemotherapy, and antibiotics (Sarker et al, 2012; Zitvogel et al 2008). The impaired immune system is greatly susceptible to several diseases including infections, cancers, allergy, asthma, ulcerative colitis and asthma (Ahmad et al 2015; Turvey et al 2010). Immunopotentiators play a vital role to upgrade the immunity to protect the body from possible infection and cancers under those immunocompromised conditions.

Ayurvedic medicines are prepared from the multiple combination of a number of medicinal plants which are believed to act as an immunomodulating agent. Therefore, herbal medicines recently have gained attention for the evaluation of immunopotentiality and the treatment of immune disorders (Sarker et al 2016). This study was designed for the preliminarily evaluation of the immunopotentiality of five Ayurvedic preparations named “Kanakasav (KNK)”, “Dasamularista (DSM)”, “Draksharista (DRK)”, “Saribadi (SDI)” and “Bramhi Rasayan (BRS)” by determining the effect of the above five Ayurved preparations on the promotion of humoral immunity by measuring the production of Immunoglobulin M (IgM) and proliferation of cells in cultured murine splenocytes *in vitro*.

Materials and Methods

Preparation of samples

Kanakasav (KNK), Dasamularista (DSM), Draksharista (DRS), Saribadi (SDI) and Bramhi Rasayan (BRS) used in this study were prepared according to Bangladesh National Formulary of Ayurvedic Medicine (Annonymous, 2011). The in-process and quality control for the preparation was strictly controlled and monitored by an experienced Ayurved specialist.

Chemicals and reagents

Roswell Park memorial institute (RPMI)-1640 and Eagle’s minimum essential medium (MEM) were purchased from ICN Biomedicals (Irvine, CA, USA) and Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Lipopolysaccharide (LPS) from Escherichia coli 055: B5, bovine serum albumin (BSA) (Fraction V), Tween 20, and fetal calf serum (FCS) were purchased from Sigma-Aldrich (Japan). Purified mouse IgM, goat anti-mouse IgM antibody (Ab), and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgM Ab were obtained from Zymed Laboratories Inc (San Francisco, CA, USA), Organon Teknika Corporation (Durham, NC, USA), and Kirkegaad & Perry Laboratories (Gaithersburg, MD, USA), respectively.

Mice

BALB/c female mice were purchased from Charles River Japan (Yokohama, Japan). They were maintained under specific pathogen-free conditions in the animal facility of Okayama University and used between 8 and 12 weeks of age. All experimental procedures concerned with mice were performed according to the guidelines established by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and to the Guidelines for Animal

Experiments at Okayama University and were approved by the Animal Research Control Committee of Okayama University, Japan.

Preparation of spleen cells from Balb/c mice

Spleen cells from BALB/c female mice, depleted of erythrocytes, were prepared by lysis of erythrocytes with ammonium chloride as previously mentioned by Sarker et al (2012). Mice were killed and spleens were collected aseptically. The spleens were mashed with spatula through the strainer to pass the cells into MEM (pH adjusted to around 7.0 with 1N NaOH) in a Petri dish (Iwaki, Japan). Cells were suspended by Pasteur pipette and screened by passing through a mesh into the centrifuge tube. The cell suspensions were then centrifuged for 5 min at 4°C and 2000 rpm. The supernatants were removed and ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA; pH 7.2) was added to the cells into the centrifuge tube for lysing the erythrocytes for 5 min at room temp. MEM was added to the centrifuge tube, suspended and centrifuged for 5 min at 4°C and 2000 rpm. The supernatants were removed and the cell pellets were washed twice with MEM. The cells were re-suspended in MEM and passed through a mesh into another centrifuge tube to collect the spleen cells. The viability of the prepared splenocytes was determined by the Trypan-blue exclusion technique and cells having viability higher than 70% were used for the experiments.

Cell culture

Cell culture was done as described by Sarker et (2012c). Freshly prepared spleen cells were suspended in basal culture medium (RPMI 1640 medium, supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml of penicillin G and 100 µg/ml of streptomycin). The cells (2.5×10^5 cells/100 µl/well) were plated in 96-well U-bottom plates (Nunc, Roskilde, Denmark) and incubated for 30 min at 37°C in a fully humidified atmosphere containing 5% CO_2. Fifty µl of 2-mercaptoethanol (2-ME) (0.2 mM), diluted with the basal culture medium, was added into each well and plates were incubated for 5 days with or without the addition of PRS and LPS, diluted with the basal medium, at 37°C in the CO_2 incubator. The cultured supernatants were then collected and frozen at −30°C for IgM-ELISA and the cells pellets were used for MTT assay.

Measurement of splenocytes proliferation by MTT assay

The growth of cultured cells was determined by MTT assay as previously described (Sarker and Zhong, 2014; Sarker and Gohda, 2013; Hansen et al, 1989). Briefly, at the end of incubation of splenocytes for 120 h, 160 µl supernatants were removed. Sixty µl of fresh medium and 25 µl of MTT solution were added in each well and the plate was incubated for 2 h. After addition of 100 µl stop solution in each well, the plate was incubated overnight in dark at 37°C and the absorbance was measured at 570 nm by using a plate reader (Bio-Rad Laboratories, USA).

ELISA for the determination of IgM and IgG antibody productions in cultured supernatants

The IgM and IgG antibody production levels in the serum were measured by a sandwich ELISA as described earlier (Sarker et al, 2011; Sarket et al, 2012b; Sarket et al, 2012c; Sarket et al, 2014; Sarket et al, 2016; Sarker, 2012). Briefly, each well of 96-well microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with 50 µl/well of goat anti-mouse IgM (10 µg/ml) diluted with phosphate buffer saline (PBS), and incubated the plates overnight in the dark at 4°C. The plates were washed three times by PBS containing 0.05% Tween 20 (wash-buffer) (200 µl/well). The wells were blocked with 200 µl of 1% bovine serum albumin (BSA) in PBS for 2 h at room temp. After washing the plates, 100 µl/well of cultural supernatants (diluted with 1% BSA-PBS-Tween 20) or standard mouse-IgM were added into each well, and the plates were incubated for 2 h at room temp. The plates were again washed three times by wash buffer (200 µl/well). Fifty µl per well of horseradish peroxidase-conjugated goat anti-mouse IgM (0.2 µg/ml) was added into each well and the plates were incubated for 1 h at room temp. After washing the plates 100 µl/well of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and 0.17% H_2O_2 were added. The plates were then incubated for 10 min at room temp and the optical densities at 405 nm were measured using an automatic plate reader (Bio-Rad Laboratories, USA).

Results

Determination of λ max of MRT

The maximum wavelength of measuring MRT absorbance (λmax) was 289 nm in phosphate buffer (PH 6.8) as illustrated in Figure (1). This absorbance peak was in agreement with previous study conducted by Prabhat et al (Prabhat *et al.*, 2021a).

Effect of KNK, DSM, DRK, SDI and BRS on the Proliferation of Murine Splenocytes

BALB/c female mice spleen cells were sub-cultured with or without varying doses of KNK, DSM, DRK, SDI and BRS for 5 days and the proliferation of cells were measured by MTT assay as mentioned in the Methodology section. As shown in the Fig. 1, KNK at the concentration of 1% (v/v), DSM 10%, SDI 1% and BRS 10% concentrations increased the proliferation of the number of murine spleen cells in culture compared to the control. The highest enhancement of proliferation (4 times higher than that of control) was measured by BRS at the concentration of 10% (v/v). Other doses of all the preparations were could not stimulate the proliferation of cultured spleen cells.

Effect of KNK, DSM, DRK, SDI and BRS on the Production of Polyclonal IgM Antibody

BALB/c female mice spleen cells were sub-cultured with or without the five Ayurved preparations (KNK, DSM, DRK, SDI and BRS) for 5 days and the amounts of IgM antibody produced in the cultured supernatants were determined by IgM antibody specific ELISA as mentioned in the methodology section. Lipopolysaccharide (LPS) was used as a positive control. Our data (Fig. 2) showed that KNK at the dose of 1% (v/v) augmented IgM production by 3.75 fold higher than that of control, respectively. Similarly, SDI at the dose of 1% (v/v) promoted the production of polyclonal IgM

Fig. 1

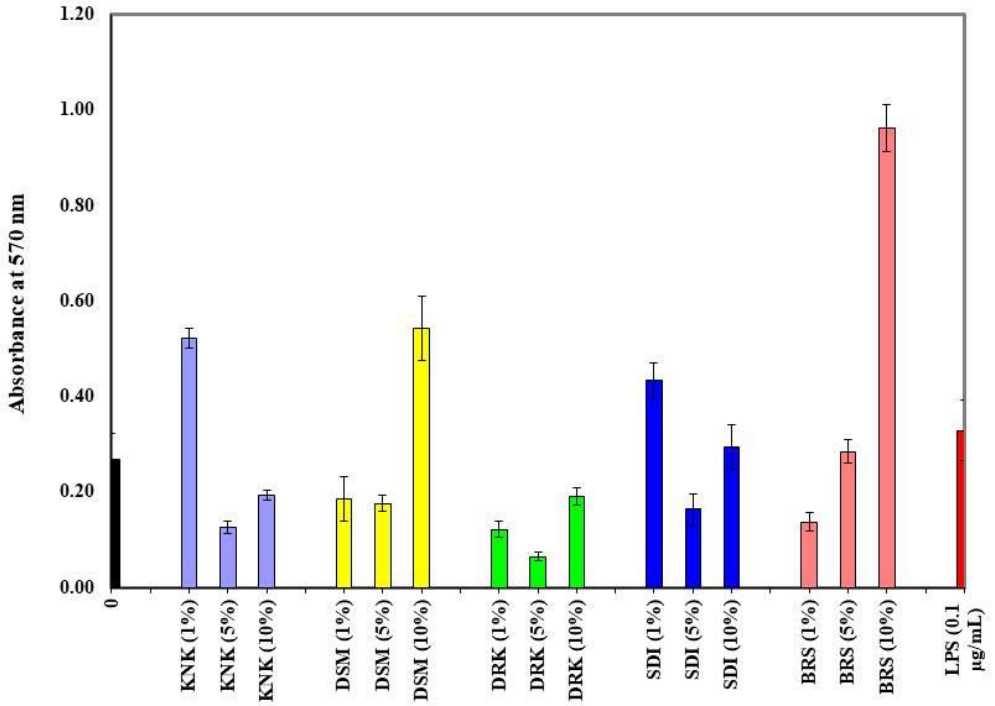

Fig. 1: Effect of different Ayurved medicines on the proliferation of murine splenocytes in culture. BALB/c mice whole spleen cells (2.5×10^5 cells/well) were incubated with the indicated concentrations of KNK, DSM, DRK, SDI and BRS at 37º C in the 5% CO_2 incubator for 5 days. The proliferations of cells were determined by MTT assay. The data are means ± S.D. of triplicate cultures.

Fig. 2

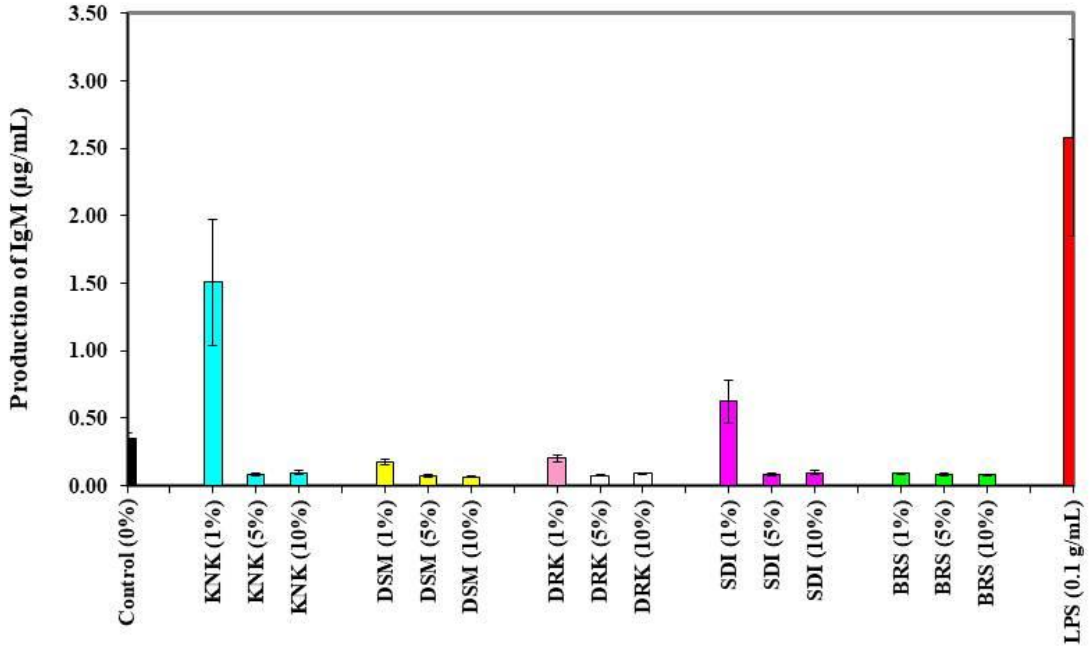

Fig. 2: Effect of KNK, DSM, DRK, SDI and BRS on the production of polyclonal IgM antibody in cultural supernatants of murine splenocytes. BALB/c mice whole spleen cells (2.5×10^5 cells/well) were incubated with the indicated concentrations of PRS at 37º C in the 5% CO_2 incubator for 5 days. The IgM levels in the cultured supernatants were determined by an IgM-ELISA. The data are means ± S.D. of triplicate cultures.

Fig. 3

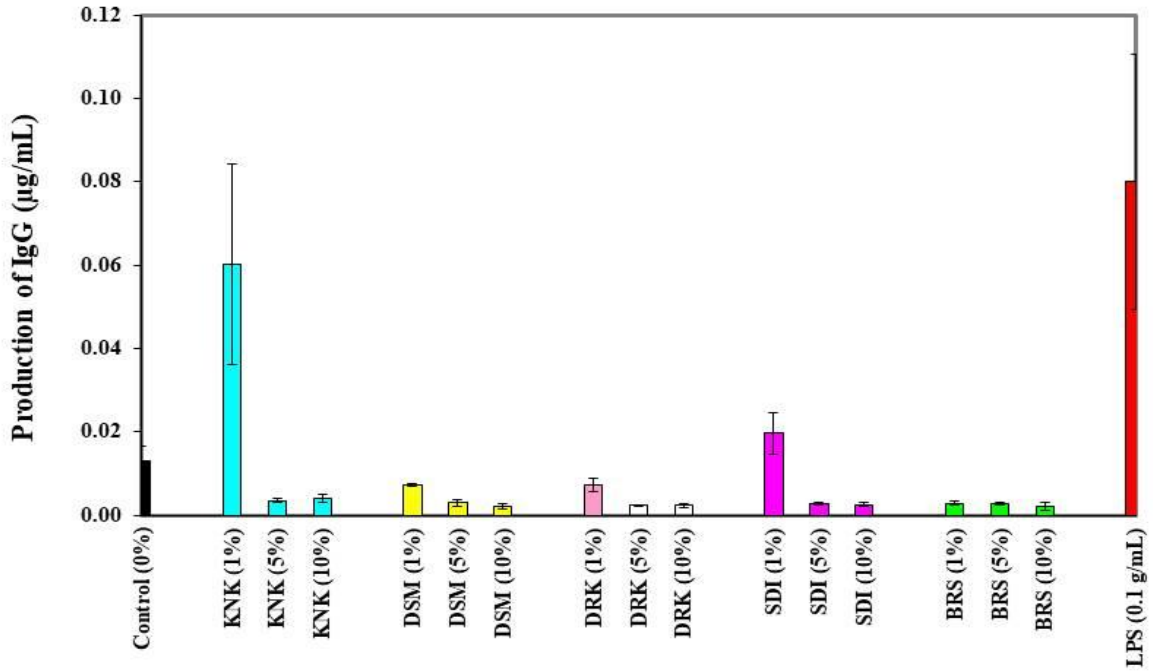

Fig. 3: Effect of KNK, DSM, DRK, SDI and BRS on the production of polyclonal IgG antibody in cultural supernatants of murine spleen cells. BALB/c mice whole spleen cells (2.5 ×10^5 cells/well) were incubated with the indicated concentrations of PRS at 37º C in the 5% CO_2 incubator for 5 days. The IgG levels in the cultured supernatants were determined by an IgM ELISA. The data are means ± S.D. of triplicate cultures.

antibody production by 2 folds compared to control group. The higher doses of both the KNK and SDI could not induce the production of IgM (Fig. 2). LPS (0.1 µg/ml) was used as a positive control which also increased IgM production 1.5 times higher than that of control. The 5% and 10% concentration of KNK and SDI degraded the production of IgM level, hence those concentrations were considered to be toxic to the cultured cells. No concentration of DSM, DRK and BRS could induce the production of IgM in the culture of spleen cells (Fig. 2).

Effect of KNK, DSM, DRK, SDI and BRS on the Production of Polyclonal IgG Antibody

BALB/c female mice spleen cells were sub-cultured with or without the five Ayurved preparations (KNK, DSM, DRK, SDI and BRS) for 5 days and the amounts of IgG antibody produced in the cultured supernatants were determined by IgG antibody specific ELISA as mentioned in the methodology section. Lipopolysaccharide (LPS) was used as a positive control. The data has been shown in the Fig. 3. KNK at the dose of 1% (v/v) enhanced polyclonal IgG production by 5 times higher than that of control, although the amount of the IgG in both of the cases of KNK and control is very negligible. SDI at the dose of 1% (v/v) exerted the similar quantity of IgG production as produced by control. The higher doses of both the KNK and SDI could not induce the production of IgG, rather degraded the production of IgG level, hence those concentrations were considered to be toxic to the cultured cells. The quantity of IgG production by three different doses (1%, 5% and 10%) of DSM, DRK and BRS could not induce the production of IgG in the culture of spleen cells, rather all the doses of those preparations were found to be slightly toxic to the cells as the production of IgG by those Ayurved medicnes were found to be less than that of control (Fig. 3).

Among the five preparations of Ayurved medicines we have tested, two preparations – Kanakasava (KNK) and Saribadi (SDI) showed the stimulating activities for the preparation of spleen cells and the enhancement of both IgM and IgG antibodies. Interestingly, KNK at the dose of 1% exerted immunostimulating activities but the higher doses 5% and 10% did not show any stimulating activities, rather affected negatively for the stimulation of the proliferation of spleen cells and the production of antibodies. The proliferation of splenic cells and the production of both the IgM and IgG antibodies are lower than that of control (Fig. 1, 2 and 3) which indicates that the 5% and 10% doses of KNK are toxic to the spleen cells; that's why the number of cells are reduced because the cells were killed, and hence the amount of antibody productions were also decreased. Similarly, 1% concentration of SDI stimulated the splenocytes proliferation and IgM and IgG antibodies productions. Higher doses resulted with the decreased production of IgM and IgG antibodies by the 5% and 10% concentrations of SDI because of the similar reason as illustrated for KNK. This result is also an indicator of narrow safety profile of both of these Ayurved medicines, KNK and SDI. The other three Ayurved medicines (Dasamularista,

Draksharista and Bramhi Rasayan) experimented in this study failed to induce immunostimulation in the culture of splenic cells.

Although Bramhi Rasayan (BRS) showed stimulating activity at the dose of 10% (v/v), it could not induce the production of antibody. Previously, we reported the immunostimulating activity of another Ayurvedic preparation: Chandanasav which was found to be contaminated with bacterial endotoxin, LPS (Sarker et al, 2012a). Ayurved medicines are natural medicines that are prepared from natural plants mainly; hence, their production cost is very cheap. Yet, negligence of modern research on Ayurved medicines, lack of prominent scientific advancement in its preparation techniques, and, above all, microbial contamination of these preparations during the manufacturing process are still considered as great problems, especially in Bangladesh.

Conclusion

On the basis of present investigation, it can be concluded that Kanakasava and Saribadi may have immunostimulating activities which is evident from the preliminary screening of these two preparations. Dasamularista, Draksharista and Bramhi Rasayan did not have any immunostimulating activity. However, further studies are required for the confirmation of its immunostimulating activities whether the immunostimulating activities are due to the presence of bioactive phytochemicals present in the preparation or because of the contamination of bacterial endotoxin.

Data Availability Statement

All the data generated by the study has been included in the article. However, the raw data and analytical report of the research are available from the corresponding author on reasonable request.

Authors Contributions

Conceptualization, research design, conducting experiment, data analysis and interpretation, manuscript writing, review and editing everything was performed by MMRS as the exclusive one author of this manuscript.

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References

Ahmad W, Jantan I, Kumolosasi E, Bukhari SN. Immunostimulatory effects of the standardized extract of Tinospora crispa on innate immune responses in Wistar Kyoto rats. Drug Design, Development and Therapy 2015:2961-2973.

Anonymous. *Bangladesh National Formulary of Ayurvedic Medicine*, National Unani and Ayurvedic Formulary Committee Bangladesh, Board of Unani and Ayurvedic System of Medicine, Bangabandhu Avenue, Dhaka, Bangladesh, 2011.

Chen Y, Liu D, Wang D, Lai S, Zhong R, Liu Y, Yang C, Liu B, Sarker MR, Zhao C. Hypoglycemic activity and gut microbiota regulation of a novel polysaccharide from Grifola frondosa in type 2 diabetic mice. Food and Chemical Toxicology 2019; 126:295-302.

Das SK, Sengupta P, Mustapha MS, Sarker MM. An experimental evaluation of adaptogenic potential of standardized epipremnum aureum leaf extract. Journal of Pharmacy and Bioallied Sciences 2017;9(2):88-93.

Godbout JP, Glaser R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. Journal of Neuroimmune Pharmacology 2006; 1:421-427.

Goto T, Sarker MM, Zhong M, Tanaka S, Gohda E. Enhancement of immunoglobulin M production in B cells by the extract of red bell pepper. Journal of Health Science 2010;56(3):304-309.

Hasan MA, Hasan MR, Uddin MJ, Begum G, Abdullah N, Sarker MMR, Chowdhury MMH and Choudhuri MSK. Preclinical blood chemistry safety profile studies of "Rohitakarista" on the kidney function after chronic administration to male sprague-dawley rats. International Journal of Pharmacy 2015; 5(2): 367-373.

Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. Journal of immunological methods 1989;119(2):203-210.

Imam H, Mahbub NU, Khan MF, Hana HK, Sarker MM. Alpha amylase enzyme inhibitory and anti-inflammatory effect of Lawsonia inermis. Pakistan journal of biological sciences: PJBS. 2013 Dec 1;16(23):1796-800.

Jesuraj SA, Sarker MM, Ming LC, Praya SM, Ravikumar M, Wui WT. Enhancement of the production of L-glutaminase, an anticancer enzyme, from Aeromonas veronii by adaptive and induced mutation techniques. PloS one. 2017 Aug 16;12(8):e0181745.

Kamarudin MN, Sarker MM, Kadir HA, Ming LC. Ethnopharmacological uses, phytochemistry, biological activities, and therapeutic applications of Clinacanthus

nutans (Burm. f.) Lindau: A comprehensive review. Journal of Ethnopharmacology 2017; 206:245-66.

Karim MF, Imam H, Sarker MM, Uddin N, Hasan N, Paul N, Haque T. Free radical scavenging, antidiarrheal and anthelmintic activity of Pistia stratiotesL. extracts and its phytochemical analysis. Pakistan Journal of Pharmaceutical Sciences 2015;28(3):915-20.

Kazemipoor M, Cordell GA, Sarker MM, Radzi CW, Hajifaraji M, En Kiat P. Alternative treatments for weight loss: Safety/risks and effectiveness of anti-obesity medicinal plants. International Journal of Food Properties. 2015;18(9):1942-63.

Khan F, Sarker MM, Ming LC, Mohamed IN, Zhao C, Sheikh BY, Tsong HF, Rashid MA. Comprehensive review on phytochemicals, pharmacological and clinical potentials of Gymnema sylvestre. Frontiers in Pharmacology. 2019 Oct 29;10:1223.

Keusch GT. The history of nutrition: malnutrition, infection and immunity. The Journal of nutrition. 2003;133(1):336S-40S.

Kifayatullah M, Mustafa MS, Sengupta P, Sarker MM, Das A, Das SK. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice. Journal of Acute Disease. 2015;4(4):309-15.

Kifayatullah M, Mustapha MS, Sarker MM, Amin M, Ali F. Possible role of a compound identified from Pericampylus glaucus plant in controlling blood glucose in experimental animals. Egyptian Pharmaceutical Journal. 2017a;16(1):37-42.

Kifayatullah M, Mustapha MS, Sarker MM, Amin M. Effect of compounds identified in the active fraction of Pericampylus glaucus on blood glucose and lipid profiles in streptozotocin-induced diabetic rats. Egyptian Pharmaceutical Journal 2017;16(1):8-15.

Kifayatullah Muhammad KM, Mustapha Mohd Shahimi MM, Aravinth Vijay Jesuraj AV, Sengupta Pinaki SP, Khan Muhammad Shahid KM, Kaleemullah K. Effect of ethanolic extract of Pericampylus glaucus (Lam) Merr on biochemical parameters in Sprague-Dawley rats with high fats diets. Journal of Coastal Life Medicine 2017c; 5(5): 219-223.

Munira S, Nesa L, Islam MS, Begum Y, Rashid MA, Sarker MR, Ahmed T. Antidiabetic activity of Neolamarckia cadamba (Roxb.) Bosser flower extract in alloxan-induced diabetic rats. Clinical Phytoscience. 2020; 6:33.

Nahar K, Hasan MR, Chowdhury SK, Chakma P, Begum G, Abdullah N, Sarker MMR and Choudhuri MSK. *Preclinical blood chemistry safety profile studies of "Basanta Kusumakar Ras" after chronic administration to male Sprague-Dawley rats*. International Journal of Pharmacy 2015; 5 (1): 146-152.

Nesa ML, Karim SS, Api K, Sarker MM, Islam MM, Kabir A, Sarker MK, Nahar K, Asadujjaman M, Munir MS. Screening of Baccaurea ramiflora (Lour.) extracts for cytotoxic, analgesic, anti-inflammatory, neuropharmacological and antidiarrheal activities. BMC complementary and alternative medicine. 2018; 18:35.

Pelkonen O, Xu Q, Fan TP. Why is research on herbal medicinal products important and how can we improve its quality?. Journal of Traditional and Complementary Medicine. 2014;4(1):1-7.

Rashid MH, Gafur MA, Sarker MM, Karim N. A Spinasteryl Glycoside from Ipomoea turpethum L. Herb (Stem) Growing in Bangladesh. Journal of Bangladesh Academy of Sciences. 2012;36(1):13-17.

Taheri Rouhi SZ, Sarker MM, Rahmat A, Alkahtani SA, Othman F. The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of Langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague–Dawley rats. BMC Complementary and Alternative Medicine 2017; 17(1):156.

Sarker MMR. Adulteration of herbal medicines and dietary supplements with undeclared synthetic drugs: dangerous for human health. International Journal of Pharmacy and Pharmaceutical Sciences2014a; 6(4): 1-2.

Sarker MR. Therapeutic efficacy and safety of traditional Ayurvedic medicines: demands for extensive research and publications. Journal of Homeopathy Ayurvedic Medicine 2014;3:e113.

Sarker MMR, Choudhuri MS, Zhong M. Effect of Chandanasav, an Ayurvedic formulation, on mice whole splenocytes for the production of polyclonal IgM and proliferation of cells: a preliminary study. International Journal of Pharmaceutical Sciences and Research 2012; 3:1294-1299.

Sarker MMR, Gohda E. Promotion of anti-keyhole limpet hemocyanin IgM and IgG antibody productions in vitro by red bell pepper extract. Journal of Functional Foods 2013; 5(4):1918-1926.

Sarker MMR, Nahar S, Shahriar M, Seraj S, Choudhuri MS. Preliminary study of the immunostimulating activity of an ayurvedic preparation, Kanakasava, on the splenic cells of BALB/c mice in vitro. Pharmaceutical Biology 2012; 50(11):1467-1472.

Sarker MMR, Ming LC, Sarker MZ, Choudhuri MS. Immunopotentiality of Ayurvedic polyherbal formulations "Saribadi" and "Anantamul Salsa" with augmentation of IgM production and lymphocytes proliferation: a preliminary study. Asian Pacific Journal of Tropical Biomedicine 2016; 6(7):568-573.

Sarker MMR, Nimmi I, Kawsar MH. Preliminary screening of six popular fruits of Bangladesh for in vitro IgM production and proliferation of splenocytes. Bangladesh Pharmaceutical Journal 2012;15(1):31-37.

Sarker MMR, Imam HA, Bhuiyan MS, Choudhuri KM. In vitro assessment of prasarani sandhan, a traditional polyherbal ayurvedic medicine, for immunostimulating activity in splenic cells of balb/c mice. International

Journal of Pharmacy and Pharmaceutical Sciences 2014;6(9):531-534.

Sarker MMR. Induction of Humoral Immunity Through the Enhancement of IgM Production in Murine Splenic Cells by Ethanolic Extract of Seed of Piper nigrum L. Journal of Scientific Research 2012;4(3):751-756.

Sarker MMR, Mazumder ME, Rashid MH. In vitro enhancement of polyclonal IgM production by ethanolic extract of Nigella sativa L. seeds in whole spleen cells of female BALB/c mice. Bangladesh Pharmaceutical Journal 2011;14(1):73-77.

Sarker MMR. Antihyperglycemic, insulin-sensitivity and anti-hyperlipidemic potential of Ganoderma lucidum, a dietary mushroom, on alloxan-and glucocorticoid-induced diabetic Long-Evans rats. Functional Foods in Health and Disease 2015; 5(12):450-466.

Shah MA, Sarker MMR, Gousuddin M. Antidiabetic potential of Brassica Oleracea Var. Italica in type 2 diabetic Sprague Dawley (SD) rats. International Journal of Pharmacognosy and Phytochemistry Research 2016;8(3):462-469.

Shah MA, Sarker MMR, Gousuddin M. Toxicity study of Brassica oleracea var. italica extracts in Sprague Dawley (SD) rats. International Journal of Pharmacognosy and Phytochemistry Research 2016; 8:735-741.

Shajib MS, Rashid RB, Ming LC, Islam S, Sarker MMR, Nahar L, Sarker SD, Datta BK, Rashid MA. Polymethoxyflavones from Nicotiana plumbaginifolia (Solanaceae) exert antinociceptive and neuropharmacological effects in mice. Frontiers in Pharmacology 2018; 9:85.

Sheikh BY, Sarker MMR, Kamarudin MN, Ismail A. Prophetic medicine as potential functional food elements in the intervention of cancer: A review. Biomedicine & Pharmacotherapy. 2017;95:614-648.

Sheikh BY, Sarker MMR, Kamarudin MN, Mohan G. Antiproliferative and apoptosis inducing effects of citral via p53 and ROS-induced mitochondrial-mediated apoptosis in human colorectal HCT116 and HT29 cell lines. Biomedicine & Pharmacotherapy 2017; 96:834-846.

Sheridan PA, Paich HA, Handy J, Karlsson EA, Hudgens MG, Sammon AB, Holland LA, Weir S, Noah TL, Beck MA. Obesity is associated with impaired immune response to influenza vaccination in humans. International Journal of Obesity 2012;36(8):1072-1077.

Sikder MM, Hasan MR, Lopa SS, Abedin N, Uddin MJ, Sarker MMR. Toxicological studies of an Ayurvedic medicine Karpur Ras used in diarrhoea. International Journal of Pharmacy 2015;5(2):472-477.

Turvey SE, Broide DH. Innate immunity. Journal of Allergy and Clinical Immunology 2010;125(2): S24-S32.

Ullah K, Sarker MMR, Khan MS, Mustapha MS and Ullah MK. Anti-diabetic activity of compound "2-[(trimethylsilyl) oxy]-methyl ester (cas) methyl-o-trimethyl-silylsalicylate" isolated from Pericampylus glaucus (Lam) Merr in STZ-induced diabetic rats. Journal of Basic and Clinical Pharmacy 2017; 8:68-73.

Yasmin H, Kaiser MA, Sarker MMR, Rahman MS, Rashid MA. Preliminary anti-bacterial activity of some indigenous plants of Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 2009;8(1):61-65.

Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nature Reviews Immunology 2008;8(1):59-73.