RESEARCH ARTICLE

Modulation of Inflammation and Immune Response by Herbal Extract in *Escherichia coli* Infected Broiler Chickens

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Abstract

This experiment was conducted to evaluate the immunomodulatory and anti-inflammatory effects of a fermented polyherbal formulation in *Escherichia coli* infected broiler chickens over a period of 28 days (from day 0 to 28th day of age). A total of 120 day-old broiler chicks (Vencobb 400) were divided into four equal groups each of 30 birds, *viz.,* Environmental control (G1), infection control (G2), non-fermented extract treated (G3) and fermented extract treated (G4). *E. coli* infection was experimentally induced in all groups, except the environment control group. Significant reductions in CRP and TNF-α concentrations were observed in the polyherbal treated groups compared to the infection group. Antibody response to Newcastle disease virus (NDV) was assessed using the haemagglutination inhibition (HI) test. Administration of the polyherbal formulation resulted in higher HI titres in both treated groups, reflecting an improved humoral immune response when compared with the infection group. In conclusion, fermented polyherbal extract effectively suppressed systemic inflammation, as evidenced by lower CRP and TNF-α levels, and strengthened humoral immune responses through increased NDV antibody titres in *E. coli* infected broiler chickens.

Key words: Anti-Inflammatory, Boiler chickens, *Escherichia coli*, Polyherbal formulation.

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Introduction

Poultry farming has always been a key part of the global livestock economy, and its growth continues to accelerate steadily over time. Poultry represents a main source of animal protein and is among the most intensively reared livestock species worldwide. *Escherichia coli* is a common bacterial pathogen in the poultry industry, responsible for significant economic losses due to decreased productivity, increased mortality, and the cost of treatment and prevention. *Escherichia coli* causes various health problems in poultry, including colibacillosis, omphalitis, septicemia, yolk sac infection, issues with respiratory tract, swollen head syndrome, polyserositis, coligranuloma, enteritis, and cellulitis (Nolan *et al*., 2020). Antibiotics are effective in treating colibacillosis, but using them improperly can develop resistance against *E. coli*. Because of this, antibiotic growth promoters (AGPs) are no longer used and farmers are now exploring different strategies to manage *E. coli* infections. Various substitutes for AGPs including exogenous enzymes, phytogenic compounds, medicinal herbs, probiotics, prebiotics, and synbiotics have demonstrated efficacy in improving gut health and overall performance of broiler chickens, thereby enhancing productivity (Adli *et al*., 2023; Sholikin *et al*., 2023).

Herbal products have emerged as commonly adopted AGP alternatives in broiler production. Active compounds in herbal products have been shown to improve the morphology and functions of the digestive tract, physiological conditions, antioxidant status and immune competences of broiler chickens (Sugiharto and Ayasan, 2023). For better growth, food conversion ratio (FCR), and other purposes, many scientists used single or multiple herbs formulations in poultry birds (Bhushan *et al*., 2008; Mahajan *et al*., 2013). But literatures showed issue of palatability in poultry birds (Meimandipour *et al*., 2017), which can be resolved by using polyherbal formulations and fermented herbs. Hence, the present study was planned to explore immunomodulatory and anti-inflammatory effects of herbal extract in *E. coli* infected broiler chickens.

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Materials and Methods

This study was conducted following approval of Institutional Animal Ethics Committee (No. 128-VCN-VPT-2023). The general managemental procedures were followed in accordance with the method described by Kamani *et al*. (2024). A total of one hundred twenty (120), day-old commercial broiler chicks of Vencobb-400 strain were purchased from a registered commercial hatchery and used as experimental birds. The Birds were kept at Poultry Unit, Department of Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat (India) throughout the experimental period of 42 days. The deep litter system of management was used to raise the birds.

Experimental Design

The day-old broiler chicks were divided into four equal groups each comprising 30 birds*, viz.,* Environmental healthy control (G1), Infection control (G2), *E. coli* infected plus LPS and non-fermented polyherbal extract treated (G3), and *E. coli* infected plus *E. coli* LPS (lipo-polysaccharide) and fermented polyherbal extract treated (G4). Experimental *E. coli* infection (1.5×10^8 CFU/mL) was induced in group 2, 3 and 4 through intraperitoneal route on 7^{th} day of experiment. On the same day LPS solution (200 µg/mL) prepared in normal saline was administered through intraperitoneal route (50 µg/bird) in group 2, 3 and 4. Further in groups 2 and 3, non-fermented and fermented liquid polyherbal formulations (100 mL/litre of drinking water) were administered daily from day 0 to day 28^{th}. Liver swab samples were collected and inoculated on eosin methylene blue (EMB) agar to confirm green metallic colonies of *Escherichia coli*.

Preparation of Liquid Fermented Polyherbal Formulation

The liquid fermented polyherbal formulation was prepared by inoculating *Lactic acid bacillus* (120 million spores, Lactolus DS, Intas Pharmaceuticals Ltd.) along with 25 % sugar and 10 % corn starch with polyherbal formulations. Botanical name, common name, parts and proportion of herbal ingredient used for preparation of polyherbal formulation are narrated in Table 1. The fermentation was conducted at 35°C to 37°C for 1-3 days. The fermented polyherbal extract was prepared and filtered through filter paper. Following filtration, the extract was kept at 4 °C to preserve its integrity until use. This fermented extract was used as such without any further concentration or dilution. This experiment was carried out over a period of 42 days.

Evaluation of Inflammatory Response (CRP and TNF-α)

Blood samples were collected randomly from six birds of each group through wing vein into clot activator tubes on 9^{th}, 12^{th}, 18^{th} and 42^{nd} day of experiment. Harvested serum was used to analyze CRP (C-reactive protein) and TNF-α (tumor necrosis factor-alpha) concentration by using chicken specific ELISA assay kit (G-Biosciences, USA). All steps were performed according to the manual provided in the kit. The standard curve for CRP and TNF-α was established by plotting corrected absorbance values (Y-axis) against standard concentrations (X-axis). The equation of this linear curve ($y = mx + c$) was then used to calculate the concentration of CRP and TNF-α in each experimental sample from their respective corrected absorbance readings.

Evaluation of Immune Status against NDV

The HI test was performed to determine the HI antibody titer of the sera from the chickens vaccinated with New Castle Disease Virus (NDV) vaccine. HI test for NDV vaccine was performed according to the method described by Bansal (1996). Initially, a haemagglutination (HA) test was carried out, in which the appearance of mat, lattice, shield, granular, or agglutination pattern was interpreted as a positive reaction, while button formation was taken as negative. The highest dilution showing mat formation was considered the HA titer. Subsequently, 4 HA units (calculated as HA titer/4) of NDV antigen were prepared, and a haemagglutination inhibition (HI) assay was performed, wherein button formation was taken as indicative of haemagglutination inhibition.

Table 1: Ingredients used for preparation of polyherbal formulation

Sr. No.	Botanical name of Plant	Common name	Part to be used	Proportion
1	*Glycyrrhiza glabra*	Jethi Madhu	Dried roots & Rhyzomes	4 parts
2	*Piper longum*	Pipper	Dried roots	2 parts
3	*Piper nigrum*	Black pepper	Dried fruit	2 parts
4	*Emblica officinalis*	Amla	Dried Fruit	2 parts
5	*Adhatodavasica*	Ardusi	Dried leaves	1 part
6	*Ocimum sanctum*	Tulsi	Dried leaves	1 part
7	*Withania somnifera*	Ashwagandha	Dried roots	1 part
8	*Zingiber officinale*	Sunth	Dried roots	1 part
9	*Cinnamomum zeylanicum*	Dalchini	Dried bark	1 part
10	*Coriander sativum*	Coriander	Dried fruit	1 part
11	*Curcuma longa*	Haldar	Dried rhyzomes	1 part
12	*Andrographis paniculata*	Kariyatu	Dried leaves	¼ part
13	Rock salt	–	–	¼ part

Statistical analysis

All collected data from different parameters were expressed as Mean ± SE. One-way ANOVA was used to analyze data at 1 and 5% significance level using Duncan's New Multiple Range Test through SPSS-20 software.

Results and Discussion

Inflammatory Response (CRP and TNF-α)

The dynamics of inflammation related parameters, C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α), are depicted in Table 2. Levels are shown for infected birds treated with fermented (G4) or non-fermented (G3) polyherbal extracts, compared to infected (G2) and control (G1) groups across the 9th, 12th and 18th days of the experiment.

On day 9, group 1 exhibited significantly lower CRP concentrations compared to groups 2, 3, and 4. By day 18, group 4 recorded the lowest CRP levels, which was significantly reduced in comparison to Group 2. The progressive decline in CRP levels observed in groups 3 and 4 over the experimental period highlights the anti-inflammatory potential of both the water-based and fermented polyherbal formulations against *E. coli* challenge. Regarding TNF-α, significantly reduced level was observed in G1 on day 9 when compared with G2, G3, and G4. By day 12, both G1 and G4 maintained significantly lower TNF-α concentrations than G2. By day 18, TNF-α level dropped across all groups, with no significant differences detected among them.

Fermented herbal formulation (G4) showed a strong trend of improvement over time in both CRP and TNF-α levels in comparison to G2 group birds and closer to G1 group. Our findings in this study are consistent with previous report (Qu *et al.,* 2025), which evaluated modulatory effects of polyherbal mixture (PHM) (*Portulaca oleracea L., Radix sophorae flavescentis, Thalictrum glandulosissimum, Terra flava usta*, and *Pogostemoncablin*) on the immuno-antioxidant capacity and intestinal health of chicks infected with *Escherichia coli* O78. They also concluded that *E. coli* group had the highest TNF-α level (52.90 ± 1.619 pg/mL) than the control (45.75 ± 1.619 pg/mL; p< 0.05). Their findings confirmed that *E. coli* infection triggers a pro-inflammatory response, consistent with TNF-α's role in systemic inflammation. Both PHM2 (2 g/kg diet) and PHM4 (4 g/kg diet) significantly suppressed TNF-α levels (PHM2; 38.65 ± 1.619 pg/mL and PHM4; 37.12 ± 1.619 pg/mL

Haemagglutination and Haemagglutination Inhibition (HA and HI) Test

Antibody response to Newcastle disease virus (NDV) was assessed using the haemagglutination inhibition (HI) test at 18th and 42nd day of experimentation. The average HI titers for each treatment group, indicating levels of NDV specific antibodies, are detailed in Table 3.

On day 18, group 2 (G2; *E. coli* infected) exhibited the lowest antibody titers, indicative of *E. coli*-induced immunosuppression. In contrast, group 4 (fermented herbal extract) recorded the highest titers, significantly surpassing G2 ($p < 0.05$), while groups 1 and 3 showed no statistical difference from each other. By day 42, G4 demonstrated a markedly elevated mean titer, which was significantly higher than all other experimental groups. Among the remaining groups, G3 maintained the highest titers, followed by G1, with G2 showing the lowest response. The outcomes presented here are in agreement with findings of other investigators (Bhardwaj *et al*., 2012; Dharmaraj *et al.,* 2017; Kamani *et al*., 2024). In the polyherbal supplemented (*Allium sativum*, *Cinnamomum zeylanicum*, *Coriander sativum*, *Cuminum cyminum*, *Mentha piperita*, *Syzygium aromaticum* and *Withania somnifera*) and *E. coli* infected broilers group, Kamani *et al*. (2024) found significantly highest titre against NDV and IBDV as 362.67 ± 133.91 and 469.33 ± 122.18 in comparison to infection control group (85.33 ± 13.50 and 160.00 ± 32.00, respectively).

Ashwagandha, or *Withania somnifera*, is a well-known immunostimulant and boost the levels of immunoglobulins, interferons, T-helper cells, CD3+, CD4+/CD8+ ratios, and a number of cytokines, this potent herb aids in boosting the immune system's function (Tharakan *et al*., 2021).

Table 2: Inflammation related parameters in various treatment groups on 9th, 12th and 18th day

Parameters	Days	Treatment Groups			
		G1	G2	G3	G4
CRP (ng/mL)	9th	7.27 ± 0.50^{a}	14.05 ± 1.24^{b}	12.08 ± 1.17^{b}	12.34 ± 1.48^{b}
	12th	6.90 ± 1.23^{a}	10.40 ± 0.82^{b}	8.91 ± 0.87^{ab}	6.59 ± 0.59^{a}
	18th	7.52 ± 1.12^{ab}	9.23 ± 1.50^{b}	6.72 ± 0.60^{ab}	5.34 ± 0.84^{a}
TNF-α (pg/mL)	9th	48.60 ± 6.30^{a}	195.57 ± 40.98^{b}	185.98 ± 44.18^{b}	161.89 ± 51.38^{ab}
	12th	33.56 ± 6.50^{a}	97.78 ± 27.01^{b}	61.86 ± 8.11^{ab}	40.70 ± 12.24^{a}
	18th	36.03 ± 1.61^{a}	47.18 ± 10.30^{a}	38.63 ± 6.15^{a}	29.95 ± 6.28^{a}

Means bearing different superscripts between treatment groups differ significantly (p<0.05)

Table 4: Mean antibody titre against NDV in various treatment groups

Titre (NDV)	Treatment Groups			
	G1	G2	G3	G4
18th day	56.00 ± 16.40^{ab}	18.67 ± 4.46^{a}	66.67 ± 20.41^{ab}	74.67 ± 17.85^{b}
42th day	149.33 ± 35.70^{a}	74.67 ± 17.85^{a}	181.33 ± 34.73^{a}	320.00 ± 64.00^{b}

Means bearing different superscripts between treatment groups differ significantly (p<0.05)

Dharmaraj *et al.* (2017) investigated the effects of herbal immunomodulator powder (mandukaparni, yasthimadhu, guduchi, vriddadaru, amalaki, nimba) and liquid form himsara, kasani, vasaka, guduchi, daraksha, jhavuka, shatavari *etc*., without and with administration of vaccines on immune organ weights and immunological indices in Giriraja birds. Results indicated that herbal immunomodulator powder supplement showed significant impact than standard immunostimulant levamisole with regards to antibody titre against NDV compared to levamisole treated group (11.40 ± 6.00 Vs. 6.60 ± 1.27). Bhardwaj *et al*. (2012) estimated effect of *Tinospora cordifolia* aqueous extract on immunity in broiler birds. In levamisole treated group, they found significantly higher mean HI antibody titre against ND (log_{10}) as 3.16 ± 0.13 followed by *Tinospora cordifolia* aqueous extract supplemented group as 2.76 ± 0.05 and lower titre in control group as 2.21 ± 0.10 at 42nd day.

Histopathological Examination

In the present study histopathological alterations in the liver was assessed following inoculation of *E. coli* on day 7 of the experiment. Histologically, G1 birds showed normal hepatic architecture with well preserved hepatocytes and sinusoids. In contrast, the *E. coli* infected group (G2) revealed with prominent gross changes, including pale and swollen liver. Histopathological findings in G2 revealed severe hepatic degeneration characterized by hepatocellular vacuolation, sinusoidal congestion, and inflammatory cells infiltration. Polyherbal treated broiler groups typically showed normal hepatocytes with minimal congestion of central veins and sinusoids based on histopathological analysis. Our results aligned closely with the previous research (Kamani *et al*., 2024, Bhushan *et al*., 2012). The findings also revealed degenerative changes in hepatocytes as well as congestion and dilation of central veins in *E. coli* infected broilers.

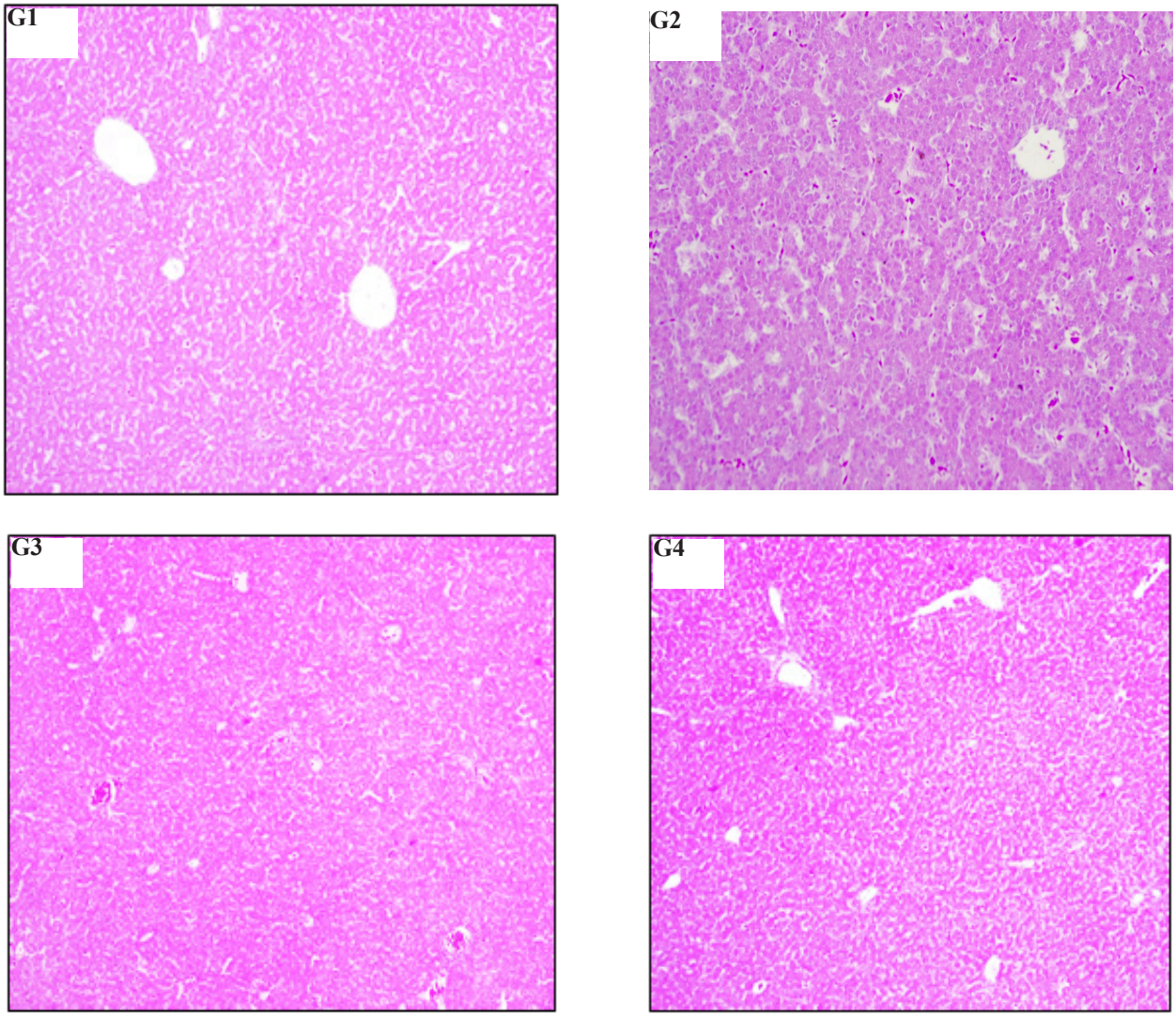

Fig. 1: Liver histoarchitecture (G1-Apparently normal central vein and hepatocytes; G2- Infiltration of inflammatory cells in liver parenchyma; G3- Normal architecture of hepatocytes with minimal signs of inflammation; G4- Uniform hepatocytes with no visible infiltration of inflammatory cells), H&E Staining 4x.

Conclusion

The findings of present study suggests that supplementation of fermented polyherbal extract at 100 mL/litre in drinking water up to 28 days can be used as a natural and safe alternate in broiler chickens for *E. coli* management. The elevated levels of CRP and TNF-α in the infection group were significantly reduced in the polyherbal treated groups, indicating anti-inflammatory activity of herbal extract. Both fermented and non-fermented polyherbal treated groups exhibited higher HI titres compared to the infection group, with minimal histoarchitectural alterations in liver, suggesting enhanced humoral immune response induced by the polyherbal formulation.

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References

Adli, D.N., Sadarman, S., Irawan, A., Jayanegara, A., Wardiny, T.M., Prihambodo, T.R., Nayohan, S., Permata, D., Sholikin, M.M., & Yekti, A.P.A. (2023). Effects of oligosaccharides on performance, egg quality, nutrient digestibility, antioxidant status, and immunity of laying hens: A meta-analysis. *Italian Journal of Animal Science*, *22*(1), 594-604.

Bansal, R.P. (1996). Techniques in viral diagnosis. In: *Technology in Diagnostic Virology*. published by ICAR, New Delhi, India, pp. 76-82.

Bhardwaj, U., Tiwary, B.K., Prasad, A., & Ganguly, S. (2012). Effect of *Tinospora cordifolia* extract on immune response and serum biochemical profile in broilers. *Indian Journal of Animal Sciences*, *82*(4), 379-381.

Bhushan, B., Choudhury, S., Garg, S. K., & Ram, R. (2012). Pathomorphological studies on the effect of polyherbal feed supplement on certain vital organs of healthy and *E. coli* challenged broiler chicks. *Indian Journal of Poultry Science*, *47*(3), 326-329.

Bhushan, B., Garg, S.K., Kumar, J., & Shukla, P.K. (2008). Effect of poly-herbal feed supplement on production performance and nutrient utilization in broiler chicks. *Indian Journal of Poultry Science*, *43*(1), 67-70.

Dharmaraj, G.Y., Naik, J., Indresh, H.C., & Indresh, H.C. (2017). Effect of herbal immunomodulator on immune organ and immunological parameters in Giriraja birds. *International Journal of Current Microbiology and Applied Sciences*, *6*(8), 3740-3746.

Kamani, R.H., Varia, R.D., Patel, J.H., Modi, F.D., Patel, D.R., Vihol, P.D., Padheriya Y.D., & Patel, A. (2024). Immunomodulatory and protective effects of polyherbal formulation against *Escherichia coli* infection in broiler birds. *Annals of Phytomedicine*, *13*(1), 770-779.

Mahajan, N., Rawal, S., Verma, M., Poddar, M., & Alok, S. (2013). A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctum*. *Biomedicine & Preventive Nutrition*, *3*(2), 185-192.

Meimandipour, A., Nouri Emamzadeh, A., & Soleimani, A. (2017). Effects of nano encapsulated aloe vera, dill and nettle root extract as feed antibiotic substitutes in broiler chickens. *Archives Animal Breeding*, *60*(1), 1-7.

Nolan, L.K, Vaillancourt, J.P., Barbieri, N., & Logue, CM. (2020). Colibacillosis. In: *Diseases of Poultry*. 14th Edn., Ed: Swayne, D.E., Boulianne, M., Logue, C.M., McDougald, L.R., Nair, V.L., Suarez, D. L., (s.). Hoboken: John Wiley and Sons, Inc., pp 770-830.

Qu, Q., Liu, M., Hu, Y., Huang, G., Xuan, Z., Lun, J., Chen, X., Lv, W., & Guo, S. (2025). Modulatory effects of polyherbal mixture on the immuno-antioxidant capacity and intestinal health of chicks infected with *Escherichia coli* O78. *Poultry Science*, *104(6)*, 1-13.

Sholikin, M.M., Irawan, A., Sofyan, A., Jayanegara, A., Rumhayati, B., Hidayat, C., Adli, D.N., Julendra, H., Herdian, H., Manzila, I., &Hudaya, M.F. (2023). A meta-analysis of the effects of clay mineral supplementation on alkaline phosphatase, broiler health, and performance. *Poultry Science*, *102*(3), 102456.

Sugiharto, S., & Ayasan, T. (2023). Encapsulation as a way to improve the phytogenic effects of herbal additives in broilers–an overview. *Annals of Animal Science*, *23*(1), 53-68.

Tharakan, A., Shukla, H., Benny, I.R., Tharakan, M., George, L., & Koshy, S. (2021). Immunomodulatory effect of *Withania somnifera* (Ashwagandha) extract - A randomized, double blind, placebo controlled trial with an open label extension on healthy participants. *Journal of Clinical Medicine*, *10*(16), 3644.