

Effect of Aloe Vera Gel Extract on C-Reactive Protein Variations Induced by Methotrexate in Albino Rats

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ABSTRACT

Background: The present research attempts to investigate the efficiency of the gel form of aloe Vera against side effects that are induced by the effecting of the drug Methotrexate in adult Albino rats with highly sensitive C-reactive protein and provide protection and treatment from this deficiency, if drugs are taken alone or in combination in cases where require it, Group 1: control, Group2: Methotrexate 1mg/kg Group3: give gel of Aloe Vera 250 mg/ kg, Group4: give gel of Aloe Vera 500 mg/ kg., Group5: MTX 1 mg /kg with 250 mg/kg, Group6: MTX 1 mg/ kg with 500 mg/kg. Samples of blood were taken from every animal after the finish of the three-week study. The results indicated a significant reduction in highly sensitive C-reactive protein In adult Albino rats, animal groups orally received 250 mg /kg of Aloe Vera gel and 500 mg / kg of Aloe Vera gel . High-sensitive C-reactive protein was increased in the group of rats treated with Methotrexate 1 mg /kg, and sensitive C-reactive protein was lower compared with the MTX, groups received MTX 1 mg/ kg together with 250 mg /kg and in groups that received MTX 1 mg /kg together with 500 mg /kg. **Conclusion:** Based on the study's findings, we deduce that aloe vera gel extract, at varying dosages, can eliminate and lessen adverse effects in rats receiving methotrexate when looking at C_ Reactive Protein.

Keywords: Methotrexate (MTX), C-Reactive Protein (CRP), Aloe Vera Gel Extract.

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INTRODUCTION

C-reactive protein (CRP) is Hepatocytes generate an acute phase protein. serum CRP levels are modest (the average CRP value 0.8 mg/l). However, following the commencement of an acute-phase activity (e.g., wound infection, inflammation, trauma, delivery, serious burns, tension, and malignancy), the liver itself has a high ability to enhance CRP formation by upregulating its expression in hepatocytes. (1,2). In human blood serum, c-reactive protein (CRP) is used as a marker for infections and inflammation. Despite its widespread and widely recognized use in

blood testing and clinical diagnosis, the CRP remains important in contemporary healthcare. CRP is a protein from the acute stage whose concentration in blood serum can increase by up to by a thousandfold in response to an acute acute stimulation. Hepatocytes secrete CRP, which can be seen in hepatic and peripheral blood at typical quantities of as much as five mg per liter. (3). Methotrexate (MTX) along with various drugs are used to maintain normal joints healthy and to sluggish down or even prevent the advancement of inflammatory processes or to relieve pain. Methotrexate

(MTX) is an antirheumatic prescription drugs used for moderate to severe RA; however, hepatotoxicity is the main constraint to its use (4). Various studies (5,6) have been conducted using the outcome of both MTX and natural products in combined therapy, , to achieve a more efficient medicine with fewer adverse effects. Methotrexate is commonly used to treat the conditions of inflammation such as rheumatoid arthritis and psoriasis, but MTX has side effects including the toxic effects on the nervous system and hepatotoxicity in addition to bone marrow dysfunctions causing hematologic disorders (7). Aloe species shown a variety of therapeutic actions, such as anti-inflammatory properties, antiarthritic, wound treatment, liver-protective properties, cytotoxic, diabetes prevention, immunostimulant, and antibacterial. (8,9).

MATERIALS AND METHODS

Preparation of Aloe Vera gel extract

To prepare gel of Aloe Vera, shut the spine at both ends of the leaf, then cut the two sides of the leafs with a harp instrument to separate the interior gel. "The gel was then blended with the blander, the materials were drained through filtering paper no. one, and the filtrate gel was dried in the oven at 40 degrees Celsius for 48 hours. The extracted extract was further powdered and then diluted in purified water". (10)

Experimental Animals Design

Blood collection

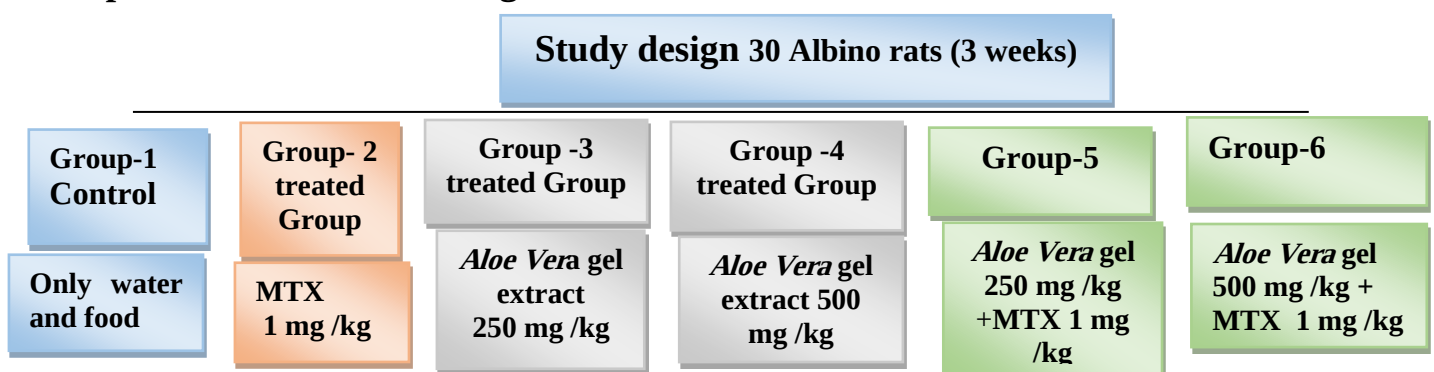
At the final stage of the experiment. "Each animal received anesthesia with 0.1 ml xylene and 0.5 ml ketamine. The rat was dissected into the heart, and blood was drawn with a five ml syringe". The blood was then placed in a serum separating container for 30 minutes at room temp, followed by centrifugation at (2000 R.P.M for a period of 10 minutes) to produce the serum. The serum followed by storage in a deep-freezing at (-20 C) for chemically assessment, and the blood was placed in a tube filled with EDTA. Samples were immediately sent for testing.(11).

Animals Experiment

The experiment involved separating the 30 lab animals into six groups (at a weight between 250 to 300 grams), each with five rats. The animals were kept in conventional settings (25 ± 2 °C, $55 \pm 5\%$ moisture, and a 12-hour light/dark period). The adaptation happened in the laboratory environment for three weeks earlier.

High sensitive C reactive protein

Two immunoassays were used to quantify plasma CRP level in the research samples. "The first procedure, an in-house competitive ELISA, utilized polyclonal anti-CRP antibodies (Calbiochem-Novabiochem) and a calibrator traceable to WHO Reference Material 85-506. This technique has been reported before". (12)



RESULTS AND DISCUSSION

In the current experiment, resulting increased in level of C-Reactive protein was elevated in group of rats treated with Methotrexate in concentration 1mg/kg to 1.08 ± 0.16 mg/dl, compared with the control group 0.53 ± 0.05 mg/dl, whereas groups of *Aloe Vera* gel 250 mg/kg and 500 mg/kg were 0.43 ± 0.12 mg/dl, 0.51 ± 0.06

mg/dl respectively. Values for High sensitive C Reactive protein were lower compared with the MTX, where the data showed Hs-CRP level was 0.80 ± 0.10 mg/dl in groups received MTX 1 mg/kg together with 250 mg/kg and 0.70 ± 0.07 mg/dl in groups received MTX 1.

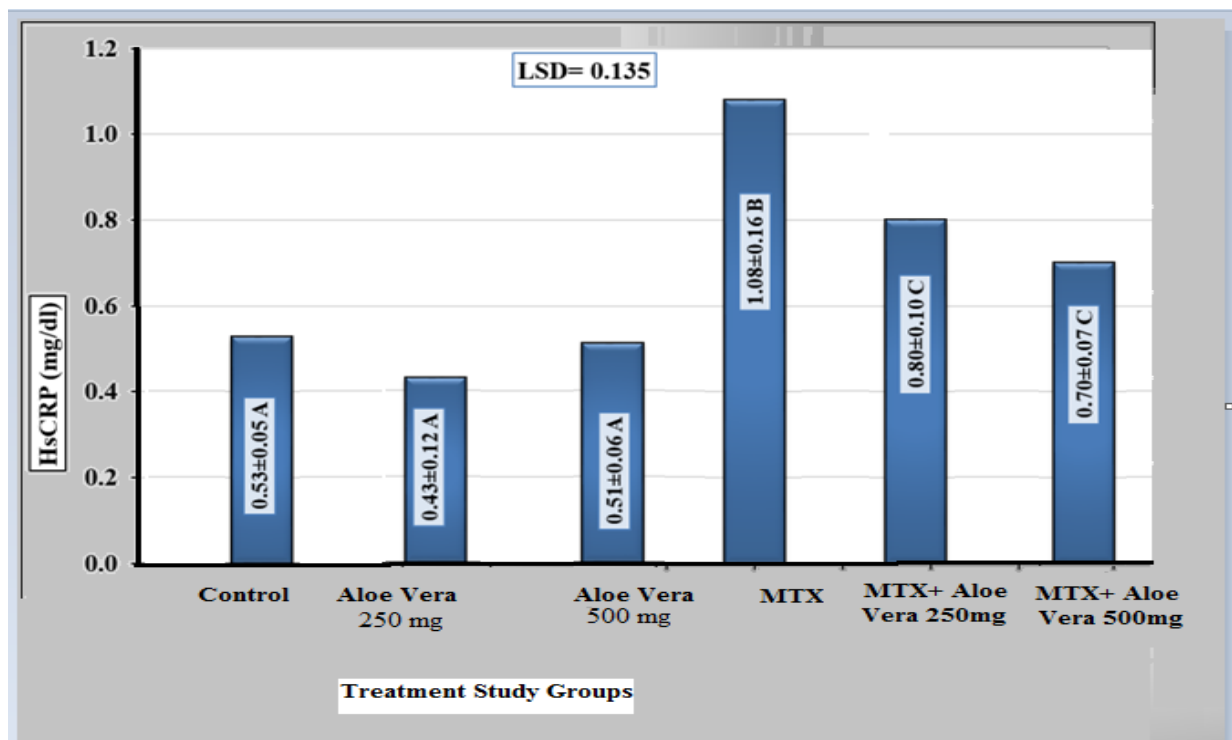


Figure which shows the effects of MTX and different concentrations of *Aloe Vera* gel on High sensitive C Reactive Protein level groups of male rats.

The treatment with Methotrexate for sure induced liver inflammation leads to hepatotoxicity occurred and detected with the obvious increase in all of parameters and decrease others like folic acid and B₁₂, the long period of treatment with MTX deemed a source to more complexity of liver disease include fibrosis and cirrhosis (13).

Values of High sensitive C reactive protein were recorded by (14) there was raising that occurred due to the liver disorder inflammation, where their result

showed clear association between High sensitive C reactive protein and liver disorder inflammation caused by cancer, their finding are agree with this finding.

C reactive protein is a indicator, forms in the cells of liver as a reaction to the inflammation in liver tissue, thus its used to diagnosis the inflammatory cases, meanwhile many functions involve identify the disorder, stimulate immune system , expulsion the target, activate the cells of proinflammatory cytokines and cells in the tissue as monocytes, and several research

mentioned the related among High sensitive C reactive protein and liver inflammation (15). Inflammation process take place together with the increase of High-sensitive C reactive protein which has been proved is related to increase of levels of hepatic enzymes of liver function tests like ALT and Alk-P, where the result showed abnormal value corresponding with abnormal value of High sensitive C reactive protein (16), their result are match to this study.

In response to the curing process, Kupffer cells appeared in liver tissues, which have an essential role in protecting the liver from inflammation related to fat accumulated through the production of cytokines that lead to elevated CRP levels (17) this findings are fall in with both physiological and histological results. High-sensitive C reactive protein (Hs-CRP) is an extra sensitive indicator of inflammations can indicate low levels of C reactive protein, hepatic diseases have correlating to the elevation of Hs-CRP, the data were recorded by (18) and where the elevation in Hs-CRP completely agree with present study as a result to the liver damage. Novel studies confirmed that the Hs-CRP is useful biomarker to detect the inflammation and damage in the tissue of liver (19).

Research on hyperglycemia submitted by (20), the altitude level of High-sensitive C reactive protein (Hs-CRP) was minimized due to impacts of *Aloe Vera* gel extract. The medical plants are characterized with huge numbers of curing benefits, like anti inflammations, this feature has been used to inhibition the High sensitive C reactive protein elevation (21).

CONCLUSION

Based on the study's findings, we deduce that aloe vera gel extract, at varying dosages, can eliminate and lessen adverse effects in rats receiving methotrexate when looking at C-Reactive Protein.

REFERENCES

1. **Osmand, A. P.**, Friedenson, B., Gewurz, H., Painter, R. H., Hofmann, T., & Shelton, E. (1977). Characterization of C-reactive protein and the complement subcomponent C1t as homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proceedings of the National Academy of Sciences*, 74(2), 739-743.
2. **Tall, A. R.** (2004). C-reactive protein reassessed. *New England Journal of Medicine*, 350(14), 1450-1452.
3. **Black, S.**, Kushner, I., & Samols, D. (2004). C-reactive protein. *Journal of Biological Chemistry*, 279(47), 48487-48490.
4. **Park, G.**, Lee, S. H., Han, J. Y., & Oh, D. S. (2018). Altered TNF- α response by Aconibal® and methotrexate in a lipopolysaccharide-induced setting of inflammatory conditions: Potential on a synergistic combination. *Journal of Ethnopharmacology*, 213, 191-197.
5. **López Mantecón, A. M.**, Garrido, G., Delgado-Hernández, R., & Garrido-Suárez, B. B. (2014). Combination of *Mangifera indica* L. extract supplementation plus methotrexate in rheumatoid arthritis patients: A pilot study.

- Phytotherapy Research, 28(8), 1163-1172.
6. **Li, F.**, Li, H., Luo, S., Ran, Y., Xie, X., Wang, Y., ... & Li, X. (2018). Evaluation of the effect of andrographolide and methotrexate combined therapy in complete Freund's adjuvant induced arthritis with reduced hepatotoxicity. *Biomedicine & Pharmacotherapy*, 106, 637-645.
7. **Celik, F.**, Gocmez, C., Bozkurt, M., Kaplan, I., Kamasak, K., Akil, E., ... & Uzar, E. (2013). Neuroprotective effects of carvacrol and pomegranate against methotrexate-induced toxicity in rats. *Eur Rev Med Pharmacol Sci*, 17(22), 2988-93.
8. **Barp, E. A.**, Soares, G. L. G., Gosmann, G., Machado, A. M., Vecchi, C., & Moreira, G. R. P. (2006). Phenotypic plasticity in *Passiflora suberosa* L.(Passifloraceae): induction and reversion of two morphs by variation in light intensity. *Brazilian journal of biology*, 66, 853-862.
9. **Nasir, A. S.** (2018). Biochemical and histological evaluation of diclofenac sodium induced acute hepatotoxicity in rats. *Journal of pharmaceutical sciences and research*, 10(4), 733-735.
10. **Macy, E. M.**, Hayes, T. E., & Tracy, R. P. (1997). Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clinical chemistry*, 43(1), 52-58.
11. **Hayashi, P. H.**, Lucena, M. I., Fontana, R. J., Bjornsson, E. S., Aithal, G. P., Barnhart, H., ... & Hoofnagle, J. H. (2020). A revised electronic version of RUCAM for the diagnosis of drug induced liver injury. In *The Liver Meeting Digital Experience™*. AASLD.
12. **Chen, W.**, Wang, J. B., Abnet, C. C., Dawsey, S. M., Fan, J. H., Yin, L. Y., ... & Freedman, N. D. (2015). Association between C-reactive protein, incident liver cancer, and chronic liver disease mortality in the Linxian Nutrition Intervention Trials: a nested case-control study. *Cancer Epidemiology, Biomarkers & Prevention*, 24(2), 386-392.
13. **Aleksandrova, K.**, Boeing, H., Nöthlings, U., Jenab, M., Fedirko, V., Kaaks, R., ... & Pischon, T. (2014). Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. *Hepatology*, 60(3), 858-871.
14. **Kerner, A.**, Avizohar, O., Sella, R., Bartha, P., Zinder, O., Markiewicz, W., ... & Aronson, D. (2005). Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology*, 25(1), 193-197.
15. **Diehl, A. M.** (2002). IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 282(1), G1-G5.
16. **Ma, L. N.**, Liu, X. Y., Lu, Z. H., Wu, L. G., Tang, Y. Y., Luo, X., ... & Xie, Y. (2017). Assessment of high-sensitivity C-reactive protein tests for the diagnosis of

- hepatocellular carcinoma in patients with hepatitis B-associated liver cirrhosis. *Oncology letters*, 13(5), 3457-3464.
17. **Zhao, X.**, Luo, J., Li, B., Liu, S., & Li, D. (2015). The association between preoperative serum C-reactive protein and hepatocellular carcinoma recurrence in patients with chronic hepatitis B virus (HBV) infection—a retrospective study. *PLoS One*, 10(1), e0116909.
 18. **Cárdenas-Ibarra, L.**, Villarreal-Pérez, J. Z., Lira-Castillo, J. C., & Nava-Alemán, A. (2017). Randomized double blind crossover trial of Aloe vera, *Cnidioscolus chayamansa* and placebo for reducing hyperglycemia in women with early metabolic syndrome. *Clinical nutrition experimental*, 14, 1-12.
 19. **Kirichenko, T. V.**, Sukhorukov, V. N., Markin, A. M., Nikiforov, N. G., Liu, P. Y., Sobenin, I. A., ... & Aliev, G. (2020). Medicinal plants as a potential and successful treatment option in the context of atherosclerosis. *Frontiers in pharmacology*, 11, 403.