RESEARCH ARTICLE

Effects of ethyl acetate fraction of *Psychotria vogeliana* (*Benth*) leaf on liver function, kidney function, and cytokines level of albino rats induced with rheumatoid arthritis

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ABSTRACT

Plant-based medicines contained bioactive molecules and functions, as reported in our previous study in *Psychotria vogeliana* leaf using HPLC. This study investigated the effects of *Psychotria vogeliana* leaf ethyl acetate fractions on liver, kidney, and cytokine levels in albino rats with rheumatoid arthritis. Six groups of eight female albino rats each were constituted from a total of 48. Group A was used as the control group, and they were given simply normal saline. With 0.1 mL of complete Freund's adjuvant (CFA), rheumatoid arthritis (R.A) was induced in Group B (toxic control). Group C received a standard drug (methotrexate) after being infected with R.A. Groups D, E, and F were given ethyl acetate extract fraction of *Psychotria vogeliana* leaf at 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight, respectively, for 28 days after being induced with R.A using CFA. Biochemical parameters measured in R.A rats revealed increased levels of IL-6, IL-1β, TNF-α, urea, creatinine, BUN, AST, ALT, and ALT compared to the control group. However, albumin and total bilirubin levels decreased. The extracts showed time and dose-dependent ameliorative effects on R.A rats, with anti-arthritic potentials comparable to standard drugs. The extract effectively treated the arthritis by restoring liver, kidney, and cytokine levels against the toxic group. The extracts showed both time and dose-dependent ameliorative effects on arthritic rats, with their anti-arthritic potentials comparable to that of the standard drug. The extract effectively treated arthritis by restoring liver, kidney, and cytokine levels against the toxic group.

Keywords: Rheumatoid arthritis, complete Freund's adjuvant, cytokine, *Psychotria vogeliana*, liver, kidneys.

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1.0 INTRODUCTION

Although the human system uses inflammatory processes as a defense mechanism in reaction to potentially harmful stimuli like allergenic substances and/or damage to tissues, an unchecked inflammatory response is the main driver of a wide range of conditions, like malignancies, other metabolic disorders, rheumatoid arthritis (R.A), and many others that have a significant financial cost to both individuals and society as a whole (1). According to reports, bacterial, viral, or fungal diseases, external factors, and compromised immune responses are the main causes of harm to the biological system resulting in inflammation (2). The first objective of the body's response to inflammation is to identify and eradicate the pathogenic invaders; subsequently, it seeks to eliminate damaged tissue components, ultimately leading to the restoration of affected tissues, organs, or system (3). Rats' pro-inflammatory cell inflow has been shown to trigger swelling in a large amount of joint cartilage, one-sided breakdown, and remodeling in a rheumatoid model (4). Approximately 1.5 million Americans suffer from RA, a widespread lifelong progressive immunological illness that is primarily found in women (5).

This autoimmune illness occurs when the body's defense mechanisms against foreign substances inadvertently target human joints (6). Joint damage results from the immune system's inadvertent attack on the body's connective tissue, which causes RA. Production of cytokines and transcription factors, such as interleukins like IL-6, TNF-a, IL-1b, and IL-1, affects the course of RA. TNF-a boosts inflammation by inducing synovial fibroblasts and encouraging leukocyte migration into joints; IL-1 modifies prostaglandin and nitric oxide production, which facilitates bone resorption and cartilage degradation. IL-6 promotes vascular growth. PGE2 can trigger pain receptors and raise the temperature(7).

Medication used to control and decrease inflammatory crises includes immunosuppressants, steroids, and nonsteroid anti-inflammatory medicines; however, these drugs are costly and have deleterious effects (8). Many plant components, including leaves, stems, bark, roots, and so forth, are employed to prevent, relieve, or return anomalies to normal (9). Across the world, *Psychotria vogeliana L. (Rubiaceae)* is mostly found in tropical and subtropical areas. Numerous studies have been published on the chemical makeup and biological functions of this genus's members(10). There are very few reports regarding to its anti-inflammatory effects using various parts of *Psychotria vogeliana*(11). Some scientists used leaves to screen its anti-inflammatory effects. The plant leaf is used to cure infectious and parasitic ailments; its bark is most frequently used to treat *gonorrhea*, *diarrhea*, and bronchopulmonary conditions, either separately or in combination(12). Though there is no scientific proof, Nigerian rural residents have long used *Psychotria vogeliana* leaf, which is effective in treating inflammatory conditions like rheumatoid arthritis. Therefore, in this work, the effects of *Psychotria vogeliana* L on liver function parameters of albino rats with rheumatoid arthritis caused by Complete Freund's were assessed.

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2.0 MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used for this study are of standard analytical grade.

Biological samples

The biological materials used are albino rats and leaf-extract of *Psychotria vogelian*

Collection and authentication of plant materials

Psychotria vogeliana leaves were freshly plucked from a bush in Uburu, Ohaozara council area of Ebonyi state, Nigeria in the month of January 2022 and were authenticated using www.theplantlist.org, www.worldfloraonline.org. The leaves samples were kept with voucher number: EBSU/H/119 in the herbarium of the Applied Biology Department of Ebonyi State University, Nigeria.

Extraction of plant material

Psychotria vogeliana leaves were dried at room temperature of about 25^0C for four weeks and powdered with an electrical blending machine sterilized using ethanol. The powdered leaf material (700g) was soaked in 3200 mL of ethanol for 48 hours. Muslin cloth was used for filtration. The concentration of the filtrate was done by evaporation to dryness using a rotary evaporator **(13)**.

Fractionation using ethyl acetate

Chromatographic columns, which had an inner diameter of 2.5 cm and a height of 50 cm, were the separation technique employed. To maintain the silica gel inside the column, a layer of glass wool was placed into the base of the column. After that, a gel-like solution was created by combining 70 grams of silica gel with 150 milliliters of ethyl acetate and stirring. A packed bed column was determined to have a length-to-diameter ratio larger than 10, which might result in an elevation of up to 30 cm. The length to diameter of our column is 12 with a diameter of 2.5 cm. The ethyl acetate solution was combined with samples of reflux ethanol extracts *(Psychotria vogeliana*) that had already been made. After that, the column was filled with the extract and ethyl acetate solution combination. Above the compacted bed that had been penetrated before, the sample was placed (14).

Experimental animals purchase and handling

A total of 48 female albino rats weighing between 100 to 200g were bought from the Veterinary Medicine Department of the University of Nigeria and transported in a steel cage to the animal house at the Department of Biochemistry, Ebonyi State University, Nigeria. The rats were acclimatized for 24 hours and kept in laboratory metal cages with good ventilation and 12-hour night and day cycles. The livestock were given commercial feed (Vital Feeds®, Ebonyi, Nigeria) and unlimited access to water.

Induction of Rheumatoid Arthritis

Complete Freund's adjuvant (CFA) was bought from Sigma Aldrich in Saint Louis, Missouri, and kept out of direct sunlight at 2-4 °C. One subcutaneous injection of 0.1 ml of CFA, a suspension of heat-killed Mycobacterium tuberculosis in mineral oil, into the right hind leg footpad of male rats resulted in adjuvant arthritis. Only the injected paw displayed the inflammatory symptoms, such as redness, edema, and hyperresponsiveness to painful stimuli (15).

Experimental animals and research design

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In this investigation, 48 female Wistar albino rats weighing between 100 and 200 g were divided into eight groups as follows: Group 1 (Control) received normal saline and feed. Group 2 (Toxic) was induced with RA using CFA and left untreated. Group 3 (Standard was induced with RA using CFA and treated with a standard drug (methotrexate) Group 4 (AE200) was induced with RA using CFA and later treated with 200mg/kg of *Psychotria vogeliana* aqueous leaf extract. Group 5 (AE400) was induced with RA using CFA and later treated using 400 mg/kg of *Psychotria vogeliana* aqueous leaf extract. Group 6 (AE600 was induced with RA using CFA and later treated with 600 mg/kg of *Psychotria vogeliana* aqueous leaf extract (16).

After 28 days of oral administrations, the rats were mildly anesthetized using an inhaled anesthetic agent, isoflurane (13). Isoflurane was delivered by the drop jar method.

Blood sample collection

Blood was collected from the rats via the femoral vein into plain sample tubes. Within an hour of blood clotting, sera were separated from the whole blood at 3000 rpm by centrifuging for 15 minutes to remove the clear, non-hemolyzed supernatant sera using a Pasteur pipette into plain tube samples and kept at the temperature of -20°C until used

Assessment of liver function parameters

Assessment of alanine aminotransferase activity

The enzyme activity of ALT in blood serum was measured following the method described by (17).

Alkaline Phosphatase (ALP)

The usual procedure was followed, by the (18) guidelines. Alkaline phosphatase Randox test reagents we used according to the manufacturer's instructions.

Assessment of aspartate aminotransferase activity

The enzyme activity of AST in blood serum was measured using the (19) method. Aspartate aminotransferase Randox test reagents were used based on the manufacturer's instructions.

Determination of plasma albumin

The method described by **(20)** was used in the determination of plasma albumin.

Assessment of Renal Function Marker.

Determination of total bilirubin

The procedure outlined by **(21)** was used to measure total bilirubin.

Measurement of serum creatinine concentration

Measurement of serum creatinine concentration was done according to the method of **(22)**.

Urea concentration

Measurement of serum urea concentration was done according to the method of **(23)**.

Blood urea nitrogen (BUN) concentration

Measurement of blood urea nitrogen (BUN) concentration was done according to the method of (24).

Blank, standard, and test samples were labeled and test tubes were arranged. The working reagent (1.0 ml) was introduced to the test tube with the label "blank." 1.0 ml of working reagent and 0.02 ml of standard solution were added to the tube labeled "standard," while 1.0 ml of working reagent and

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0.02 ml of the test sample were put into the test tube labeled "sample." The components were combined, and allowed to sit at room temperature for five minutes, and then the absorbance of the standard and test samples was measured at 520 nm in comparison to the reagent blank

Determination of inflammatory parameters.

Determination of serum tumor necrosis factor- α (TNF-α)

The concentration of TNF-α in the serum was determined using a specific Elabscience ELISA kit according to the manufacturer's instructions

Determination of serum IL-6 concentration

The concentration of IL-6 in the serum was determined using a specific Elabscience ELISA kit according to the manufacturer's instructions

RESULTS

The administration of ethylacetate fraction of *Psychotria vogeliana* notably ($p<0.05$) decreased the catalytic activities of ALP, ALT, and AST (Fig. 1-3). Furthermore, a noticeable ($p<0.05$) increase was recorded in total protein and albumin levels of rats that received the fraction against the toxic (Fig. 4-5). The results of the effect on the cytokine levels, and kidney parameters analyzed when compared to the control were presented in (Fig. 6-11). The samples showed significant differences in values ($p < 0.05$).

DISCUSSION

A classic experimental animal model of human rheumatoid arthritis, complete Freund's adjuvant-induced rheumatoid arthritis is widely used to explore anti-arthritic drugs. The mouse model of arthritis produced by collagen antibodies is one of the other models (25), pristine-induced arthritis (26), rat collagen-induced arthritis (27), and Collagen Type II Induced Arthritis (28). Animals vaccinated with CFA have been reported to have a significant inflammatory response following the inoculation which can lead to substantial joint remodeling and edema (29). This medical condition is often accompanied by discomfort during movement of the joint, physical allodynia, and thermal excessive pain.

In this study, administration with CFA-induced rheumatoid and exhibited liver damage showed in noticeable rise in the sera levels of ALP, AST, and ALT. This correlates with previous findings (30). Elevated levels of ALP, AST, and ALT in the blood biochemical findings of the CFA-induced rheumatoid arthritis rat model indicate impairment of the liver, and this is also related to the inflammatory situation (31). This is also in consonance with the study of (32). Patients with RA could have raised levels of liver enzymes (ALT, ALP, and AST) for a variety of reasons, such as the immune-mediated condition that coexists with the RA, the anti-rheumatic medicine, or the patient's illness (33). (34) found that hepatic function aberrations are widespread in individuals with inflammation-related arthritis and are often caused by DMARDs or disease-modifying anti-rheumatic drugs. Considerable increases in serum AST and ALP were seen in the research in rats given CFA; these increases may have been caused by extra-hepatic factors such as myocardial infarction (MI) or bone disorders. Consistent with other studies, the increased serum ALP in arthritic rats indicates the

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existence of ongoing skeletal damage.

Psychotria vogeliana is a herb that is commonly used in conventional medical practices to treat inflammation-related conditions like RA, yet there lack many research to back up this assertion (35). This study evaluated the effect of the ethyl acetate fraction of *Psychotria vogeliana* leaf in CFA-induced arthritic rats. Under the skin pedal injections of 0.1 milliliters of CFA were administered repeatedly and daily to consistently generate experimental arthritic conditions (36). The animals were treated orally with the ethyl acetate fraction of *Psychotria vogeliana* leaf for 28 days at a graded dose of 200mg/kg, 400mg/kg, and 600 mg/kg body weight for Group 4, 5, and 6 respectively, while CFA-induced arthritic group 2 was treated with methotrexate.

In-vivo studies showed that oral arthritic treatments using 200mg/kg, 400mg/kg and 600 mg/kg body weight of the leaf extract respectively notably ($p<0.05$) lowered ALT, AST, and ALP serum levels against the induced group. There is no noticeable ($p>0.05$) change observed among the different doses. ALP, ALT, and AST levels were lowered to the level of the normal control group, having a similar effect as the standard drug (methotrexate). Furthermore, oral arthritic treatments using 200mg/kg, 400mg/kg, and 600 mg/kg body weight of the leaf extract significantly ($p<0.05$) raised albumin in the test groups against the toxic group. This reveals the anti-arthritic impacts of the ethyl acetate fraction of *Psychotria vogeliana* leaf. Thus, plant extract can remedy the liver which is evident in the decreased liver enzyme markers and elevated albumin and globulin. This is in correlation with previous studies of (37).

By activating NF-kB and AP-1, inhibiting the inflammatory-causing activation of enzymes such as cyclooxygenase-2, lipoxygenase, and inducible NO synthase, and activating phase II antioxidant detoxifying enzymes in rats, flavonoids have been shown to have anti-inflammatory properties through molecular mechanisms (38). Pro-inflammatory cytokines/chemokines are inhibited by antioxidants in a variety of cell types, such as peripheral blood mononuclear cells, Jurkat T-cells, and RAW macrophages. Plant extracts' ability to reduce arthritis may be related to the concentrations of carotenoids and saponins they contain (Barbosa-Filho *et al.*, 2016).

The study revealed that by administering CFA, RA was generated in rats in less than ten days. This resulted in a notable decrease in body weight, indicating a rise in the production of inflammatory mediators such as TNF-α and interleukin-1 (39). Because of enhanced protein breakdown by the ubiquitin-proteosome proteolytic pathway, experimental arthritis in arthritic rats may result in muscle wasting and a decrease in body weight (39). The findings are consistent with earlier research by (39), which discovered that a decrease in weight, sickness, fever, exhaustion, diminished appetite, and stiffness in the morning were prevalent complaints among individuals with active rheumatoid arthritis(40). Within 24 hours of treatment, rats with CFA-induced arthritis exhibit anorexia and weight loss (40).

In comparison to the normal group serving as the control, the investigation revealed that the arthritic rats had considerably ($P<0.05$) greater levels of TNF-α, IL-1β, and IL-6. TNF-α stimulates invading cells to proliferate and produce more cytokines, and rheumatoid arthritis rats have higher levels of

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IL-6, an essential inflammatory cytokine (41). The administration of plant extracts reduced the concentration of cytokines to a level similar to that of the normal control group. Nonetheless, until the study's conclusion, the number of untreated rats increased dramatically. This result is consistent with other studies that have shown a noteworthy rise in IL-1β, IL-6, and TNF-α following adjuvant-induced arthritis in mice(42).

The results of the study showed that injecting rats with CFA markedly raised their levels of creatine, urea, and blood urea nitrogen. *Psychotria vogeliana* ethyl acetate leaf extract therapy, however, reversed this tendency. A classic pattern of nephrotoxicity was observed with CFA, characterized by elevated BUN, urea, and creatinine(43). The kidney damage and dysfunction marker creatinine were much lower in *Psychotria vogeliana* ethyl acetate fraction. *Psychotria vogeliana* ethylacetate's antioxidant properties are thought to have a therapeutic impact on kidney indicators and may enhance the glomerular filtration rate.

Inflammation produced by the CFA's effect may be the reason for this study's elevated values. According to earlier research, phenolics and flavonoids reduce the chance of degenerative illnesses linked to oxidative stress and kidney damage (44). The findings of this investigation are consistent with the research of (45), which proposed that *Cuscuta reflexa* exhibited noteworthy kidney-protective and antiarthritic properties. Plant extracts could have an anti-arthritic impact because flavonoids, which have anti-inflammatory properties, are present. This is in line with the findings of (46), however, the precise mechanism is yet unclear. The presence of phytoconstituents like flavonoids and phenols is associated with the protective effect.

Conclusion

Our study demonstrated that *Psychotria vogeliana's* ethyl acetate fraction had anti-arthritic effects in rats with arthritis caused by CFA. In rats given an adjuvant-induced arthritic stimulus, it decreased oxidative stress in the liver tissues, increased albumin levels, and decreased arthritic markers such as TNF-α, IL-1β, and IL-6. Its promise as a therapeutic medication for arthritic and inflammatory disorders is suggested by the fact that the impact was concentration- and time-dependent for the results.

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FIGURES

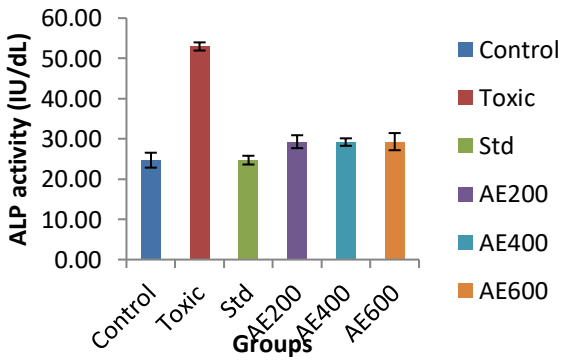


Figure 1. Effect of ethyla cetate fraction of *Psychotria vogeliana* leaf on alkaline phosphatase of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

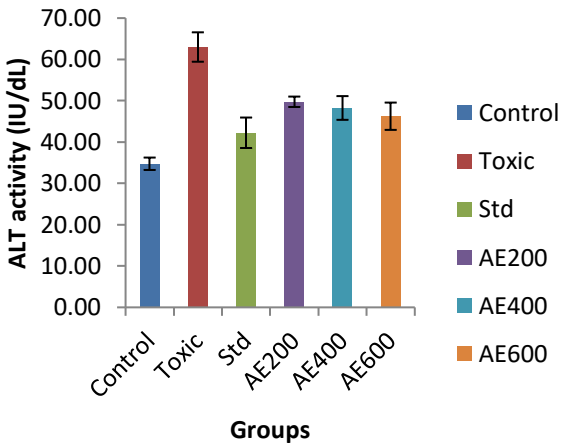


Figure 2. Effect of ethyl acetate fraction of *Psychotria vogeliana* leaf on alanine of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

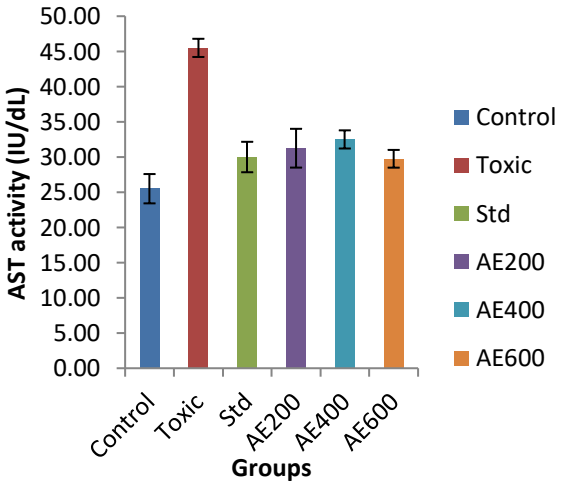
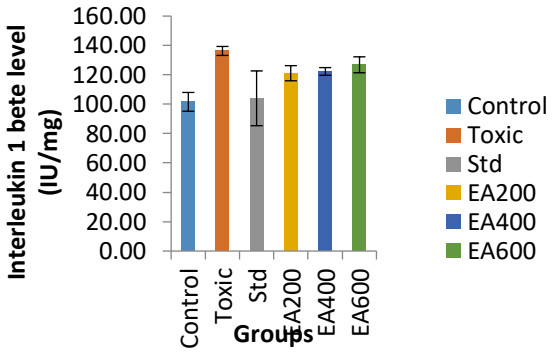


Figure 3. Effect of ethyl acetate fraction of *Psychotria*

vogeliana leaf on aspartate transaminase of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.



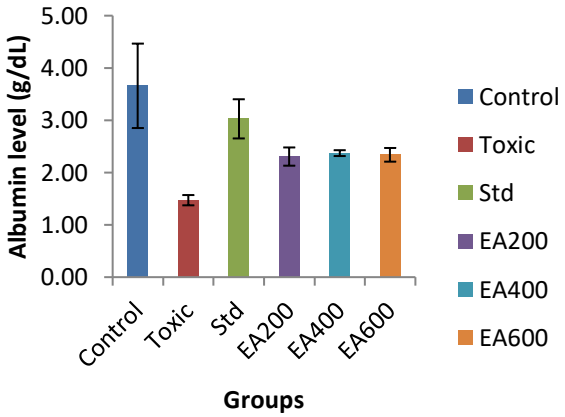


Figure 4. Effect of ethyl acetate fraction of *Psychotria vogeliana* leaf on albumin level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

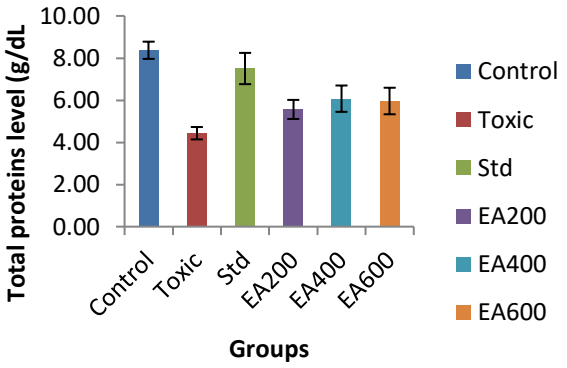


Figure 5. Effect of ethylacetate fraction of *Psychotria vogeliana* leaf on total proteins of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

Figure 6: The effect of *Psychotria vogeliana* ethyl acetate leaf fraction on interleukin 1 beta level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

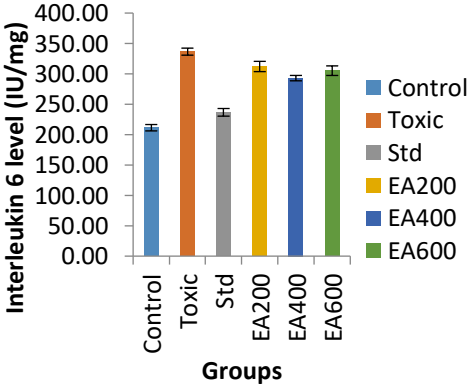


Figure 7: The effect of *Psychotria vogeliana* ethylacetate leaf fraction on intereukin 6 level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

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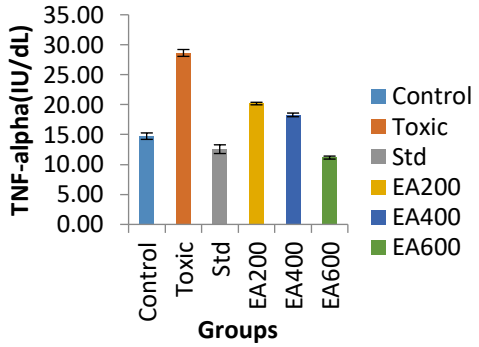

Figure 8: The effect of *Psychotria vogeliana* ethyl acetate leaf fraction on TNF-alpha of CFA-induced rheumatoid arthritis in female albino rats. Data represented as mean ±SD; p<0.05 compared to control group.

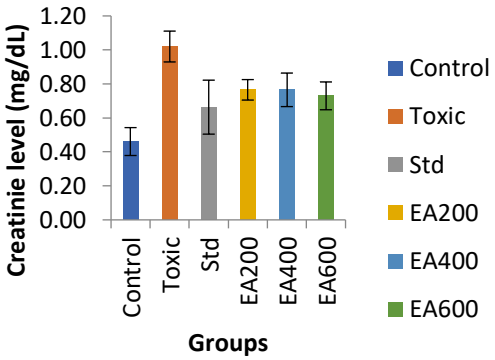

Figure 10: Effect of *Psychotria vogeliana* leaf extract on creatinine level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

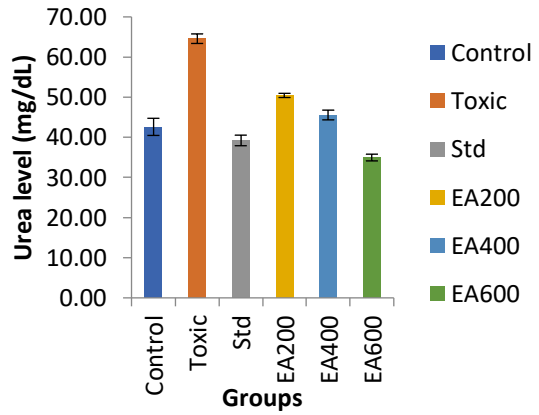

Fig. 9: Effect of *Psychotria vogeliana* leaf extract on Urea level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

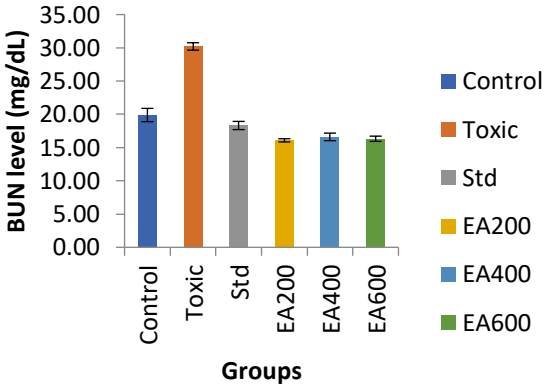

Figure 11: Effect of *Psychotria vogeliana* ethyl acetate leaf extract on blood urine nitrogen BUN level of CFA-induced rheumatoid arthritis in female albino rats. The data (n=8) are displayed as mean ± S.D. At P<0.05, there is a significant difference in the mean values.

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