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Evaluation of hematological and biochemical parameters of the heat-stress rats treated with Abutilon indicum aqueous extract

Ghasaq S. Mshary and Zainab Y. Kadhim Department of Physiology, chemistry & pharmacology/ College of Veterinary Medicine/ AL- Muthanna University/ Iraq

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*Corresponding author: Ghasaq S. Mshary.Email address:

ghassaq51@gmail.com

Abstract

The first shrubs of Abutilon indicum plant is approved to have therapeutic actions as a broad medicine for various diseases. This study intends to evaluate the anti-stress, hematopoietic and biochemical effects of orally administrated

Abutilon indicum aqueous extract (Malvaceae) on male albino rats that exposed to environmental stress. The Abutilon indicum plant was collected from Bsia area/ Al Muthanna governorate and identified in the Botany Department / Al-Muthanna University. The Abutilon indicium prepared as an aqueous extract. A thirty Wister albino rats used in this study and divided into 3 groups after one-week of acclimatization, each group with 10 animals. These group were: negative control group (GI) administered with normal saline, the positive control (GII) exposed to heat and humidity stress and treatment group (GIII) exposed to heat and humidity stress and treated by 100mg/kg B.W Abutilon indicum extract for 28 days. Blood samples collected from the heart of each animal after euthanasia. The results of this study revealed high significant hematological features in the treated group (GIII) with decreased of ALT, AST and urea parameters. Moreover, RBCs, WBCs and lymphocytes percentages were also increased in (G III) in compare to (GII) that showed a marked increase in the enzymes AST, ALT and urea due to environmental stress. In conclusion, this study approved the immune stimulant effects of Abutilon indicum extract promoted by its flavonoids content. The authors recommend doing another study on A indicium to recognize its active ingredient that improves the immunity of the animals supported with histological studies using different doses of plant extract.

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Key words: Abutilon indicium, Heat-stress, Hematological, Anti-stress.

Introduction

Abutilon indicum is one of the important species along with 150 species of Abutilon genus (Ojezele and Abatan, 2009). The plant is extended principally in the

tropical and sub-tropical area of the world, and broadly mature in India, Pakistan, and Bangladesh (Abdul et al., 2010). A number of medicinal plants are belonging to family Malvaceae like Abutilon indicum (Mohite et al., 2012) and constituent a large family of trees, shrubs, and herbs (Saldanha et al., 1996; Atkinson and Eisenbarth, 2001). Each plant has some compounds that have pharmacological significance. The chemical structure of Abutilon indicum reveals several compounds like alkaloids, carbohydrates, saponins, gum and mucilage, fixed oil, phenolic compounds, tannins, terpenoids, flavonoids, organic acid, sterols triterpenoids, glycosides, proteins, coumarins, vanillic, fumaric acid, caffeic acid, sesquiterpene lactones, steroids and essential oil (Jelena et al., 2006). The leaves, seeds and roots of the plant have extensively used for the treatment of a variety of diseases. It showed activity as antidiabetic with least toxicity (Kaushik et al., 2010). Moreover, a mixture of biological components of the plant could be used to treat many ailments (Pieme et al., 2006). This herbal plant is also used for wounds healing, antitumor, antioxidative, antifungal, antiproliferative and antimicrobial (Singh and Mendhulkar, 2015). The A indicum (aqueous and ethanol extracts leaves) had diuretic property (Chang et al., 2008). Alkaloids, tannins, phenolic compounds, and flavonoids are the actives in therapeutic of kidney and stomach troubles. Also, it uses as anti-inflammatory, and its steroids content, also help in modifiable the immune reaction (Roshan et al., 2010). The immune modulatory activity of an ethanolic and aqueous extract of A. indicum leaves was ascribed by Flavonoid content on Albino mice.

The hematological parameters are considered as one of the health indices and have a diagnostic importance in the routine clinical health estimation (Dashputre and Naikwade, 2010). The exposure of the animal to the abnormal heat is one example of circumferential stress conditions (Dodd *et al.*, 1997). Heat stress had been reported to affect almost every organ in the body (Hoppe *et al.*, 2008; Helman *et al.*, 2007). Heat stress is the causes of severe physiological dysfunction that may result diseases like coronary artery disease, cancer, and diabetes mellitus (Steenland, 1996). However, cold stress had shown a reduced in the total WBC count (Sundaresan *et al.*, 1990). Moreover, some researcher described Leucopenia in stress animals (Selye, 1950; Fraser *et al.*, 1975). Review of literature revealed no publication on the properties and effects of *A. indicum* in Iraq. Therefore, this study designed to determine the hematopoietic and biochemical effects of the orally administrated *Abutilon indicum* extract on albino male rats that exposed to heat stress.

Materials and methods

Preparation of extracts

Abutilon indicum was collected from Bsia area/ Al- Muthanna governorate and identified in the Botany Department / Al-Muthanna University. The Parts of the plant washed with tap water and rinsed with sterile distilled water for three times, followed by air dried. The plants were cut into pieces and kept for one week in room temperature at shade-dried. Later on, the fine powder prepared according to (Krisanapun *et al.*, 2009). A 10g of plant powder was dissolved in 100 ml distal water for 24 hours on the hot plate with starring. Later on, the solution filtrated through filter

paper (what-man No .1). Then, the extract centrifuged at 3000 rpm for 10 min, and the filtrate transferred to sterile bottle and kept in the refrigerator until use.

Experimental animals

Thirty male albino rats, around eight weeks old were used in this study. The rats were provided by the animal house/College of Veterinary Medicine (AL-Muthana University) and kept under standard condition (fed pellet diet and given water ad libitum). The animals divided randomly into three treatment groups ten rats\ each group as follow:

- **First group** (GI) negative control/ administered orally with Normal saline (0.9%, NaCl) every day for a period of 28 days.
- **Second group**(GII) positive control/ exposed to heat stress (which exposed to high temperature 39°C for 4 hours daily by using electric heaters and 75-80% humidity for 4 hrs (12.00-04.00 pm) daily for 28 days.
- **The third group** (GIII) / exposed to heat and humidity stress treated by 100mg/kg B.W of *Abutilon indicum* extract along 28 days.

Ethical approval

This study was approved by research and animal ethical committee in College of Veterinary Medicine (AL-Muthana University).

Hematological and biochemical analysis

All rats were anesthetizing by intraperitoneal injection of a combination of Ketamine drug (50 mg/Kg body weight) plus Xylazine drug (6.8 mg/kg body weight). The male rats sacrificed according to Harwood, (1963). Blood samples were collected by cardiac puncture using a disposable syringes and kept in the anticoagulant tubes for hematological investigation and without anticoagulant tubes for biochemical tests. An hematological autoanalyzer was used to determine different hematological parameters, (red blood cells (RBCs) count, total white blood cells (WBCs) count and lymphocytes %). The serum separated from each sample and Transaminases (AST and ALT) and urea were estimated.

Statistical analysis

All the values are expressed as mean \pm S. D. Data of the experiment were analyzed using one way analysis of variance (ANOVA). The value (P \leq 0.05) was considered statically significant.

Results

All animals in control group revealed normal hematological and biochemical parameters. The animals in third group(GIII) revealed elevation in hematological indices including RBCs, WBCs and lymphocyte with means 8.096 ± 0.060 , 10.866 ± 0.202 and $56.100 \% \pm 0.152$ respectively. All mice in the second group (GII) revealed

decrease in RBCs, the WBCs count and lymphocytes percentage with means 2.99 ± 0.04 , 2.033 ± 0.088 and 32.00 ± 0.577 respectively (Table.1). The results of the biochemical indices (ALT, AST and urea) revealed decrease of the values of the third group (GIII) with means 22.00 ± 0.91 , 217.25 ± 1.10 and 46.50 ± 0.94 respectively (Table.2). All animals in (GII) revealed elevation in AST, ALT and urea with means 34.50 ± 0.64 , 226.50 ± 1.55 and 70.75 ± 0.85 respectively (table.2).

Table .1: shows the effects of aqueous extract of on blood parameters in different treatment groups

Parameters	GI Negative control N=10	GII positive control Heat stress only N=10	GIII Heat stress treated by A.indicum 100mg\Kg N=10
RBC (million/cubic mm)	4.903 ± 0.2016 ^b	2.99±0.04°	8.096 ± 0.060 ^a
WBC (×109/l)	7.266± 0.233 ^b	2.033 ± 0.088°	10.866± 0.202a
Lymphocytes (%)	38.600±0.305 ^b	32.00±0.577°	56.100± 0.152a

A, b, c: means on the same row with different superscripts are represent significant difference compared with control (P \leq 0.05). Data expressed as Mean \pm S.D.

N (number of animals).

Table.2: Shows the effect of aqueous extract on the biochemical tests in the different treatment groups

Liver enzymes	GI Negative control N=10	GII positive control Heat stress only N=10	GIII Heat stress treated by A.indicum 100mg\Kg N=10
(IU\L)ALT	20.18± 0.30°	34.50 ± 0.64^{a}	22.00 ± 0.91 ^b
(IU\L)AST	98.4± 0.17°	226.50 ± 1.55^{a}	217.25 ± 1.10^{b}
Urea (mg/dl)	36.2 ± 1.1°	70.75 ±0.85 ^a	46.50 ± 0.94^{b}

A, b, c: means on the same row with different superscripts are represent significant difference compared with control ($P \le 0.05$). Data expressed as Mean \pm S.D. N (number of animals) =10, ALT=Alinine transaminase, AST = Aspartate aminotransferase.

Discussion

The present study showed that *A. Indicum* had favorably modified the immune reaction profile in male rats. This effects might be due to its steroid content (Roshan, 2010) with significant increase in total RBCs, WBCs counts and the lymphocyte percentage in GII. These elevations in these parameters might be resulted from the

antioxidant action of A. indicum extract that stimulated the erythropoesis and hematopoietic system. This results are in agreement with other researcher that approved the reduction of anemia (Seetharam et al., 2002). The decline in RBC count referred to the destruction of the mature RBCs and decrease in the level of erythropoiesis. However, anemia may occurred from some other conditions such as respiratory diseases that lead to decline in oxygen- carrying capacity of the blood. The reduction of the amount of oxygen delivered to the tissues may be increased the breakdown of RBCs and lead to stimulate the hematopoietic stem cells of the bone marrow. The stress considers as one of the causes that lead to decrease the survival of red blood cells in the blood. The heat exposure approved to has a principal diminution on the levels of red blood cell counts and to reduce the WBC count in experimental rats and laying hens (Mashaly et al., 2004) and growing female rabbits (Ondruska et al., 2011). The first defense mechanism against infection in the body is the lymphocytes (Kaladhar, et al., 2014). The reduction in WBCs count might indicated to the inefficacy or disorder in the function of the immune system. The exposure of the experimental animals to the heat stress led to reduce in the total WBC count and reductions in the percentage of Lymphocyte count in compare to the treatment group. These results are compatible with others (Joseph et al., 1991).

The results of this study also revealed the changes in the biochemical parameters. The GII showed elevation in the level of AST and ALT enzymes which might be from liver damage. Moreover, the extensively increased of blood urea in this group is a good indicator for kidney disorder and nephrotoxicity and is compatible with previous study (Ojezele and Abatan, 2009). The decreased of ALT, AST and urea in GIII might occur from the actions of phenolics compound in the plant extract. These compounds have a supportive on the kidney troubles and anti-inflammatory action.

In conclusion, the results of this study revealed that the oral administration of the *abutilon indicum* extract help in the improvement of the immune-stimulatory activities and boost the hematological parameters and promote health. The aqueous extract may be correcting some abnormality in hematological parameters. The authors recommend more future studies that including the extraction of different chemical compounds and evaluate its activities in the in vitro and in vivo studies.

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