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ANTI-INFLAMMATORY (IL-6, IL-10, IL-2 AND IL-4.) ACTIVITIES OF *SOLANECIO BIAFRAE* (WÒRÒWÓ) IN RABBITS INDUCED WITH INFLAMMATION OF THE LIVER USING ACETAMINOPHEN OVERDOSE

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ABSTRACT

Background: *Solanecio biafrae* (Wòròwó) contains phytochemicals, specifically antioxidants such as vitamins with effective health benefits as it is evidenced in the use of the vegetable in traditional treatments. Acetaminophen is an anagelsic which people used irrationally that could lead to drug abuse and inflammation of the liver in overdoses or prolong administration, notwithstanding the normal therapeutic dose is safe. **Materials and Methods:** Fifteen rabbits of the same sex (male) with weight ranging from 1.0 -1.4 Kg were classified into control (n=5) given water and normal meal alone through out the period of investigation and experimental groups such as B1 (n=5) ; B2; were the B1 rabbits that were given 400mg/kgBW supplement of ethanolic extract of *Solanecio biafrae* (Wòròwó) after acetaminophen overdose for 7 days; C1(n=5) these were rabbits given 2,500mg/kgBW of acetaminophen before the aqueous extract of *Solanecio biafrae* (Wòròwó) while C2; were the C1 rabbits that were given 400mg/kgBW supplement of aqueous extract of *Solanecio biafrae* (Wòròwó) after acetaminophen overdose for 7 days**.** Plasma Aspartate Transaminase (AST/SGOT) and Alanine Transaminase (ALT/SGPT) were measured by spectrophotometry while the Interleukins 2, 4, 6 and 10(IL-2, IL-4, IL-6 and IL-10)were determined in the plasma of the rabbits by ELISA. **Results:** There was a significantly higher mean plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in rabbits given 2,500mg/kgMW of acetaminophen than in control rabbits and then when these rabbits were given the supplements of ethanolic and aqueous extract of *Solanecio biafrae* (Wòròwó) following the administration of 2,500mg/kgMW of acetaminophen($p<0.05$). **Conclusion:** There was an increase in plasma AST, ALT, IL-2, IL-4, IL-6 and IL-10 after a 7 day overdose of acetaminophen due to possible drug induced hepatotoxicity, inflammation and liver damage which was significantly reduced when the rabbits were given the ethanolic and aqueous extracts *Solanecio biafrae* (Wòròwó). Consequently, the extract could be a good supplement at reducing drug-induced hepatotoxicity/hepatitis.

KEYWORDS: *Solanecio biafrae.*

INTRODUCTION

Solanecio biafrae (Èfó Wòròwó) is a common vegetable plant in South-Western Nigeria. It has some nonscientific traditional health benefit claims such as treatment of diseases like cough, heart troubles, to relieve rheumatic pain, food allergies and localized oedemas. And also used for rituals to ward off smallpox. Phytochemical constituents of *Solanecio biafrae* leaves per 100 g dry matter: include; crude protein 12.3 g, crude dietary fibre 11.8 g, Ca 342 mg, P 39 mg, Fe 52 mg. Leaves of purple-stemmed types contain per 100 g dry matter: crude protein 11.6 g, crude fibre 10.5 g, Ca 320 mg, P 46 mg, Fe 53 mg , and less than 0.1 g/100 g fresh leaves of terpenoids, majorly sesquiterpene germacrene D. The leaves also contain Vitamin A, B (Folic acid, niacin, thiamin, riboflavin), C, E, Tecopherols and Flavonoids.[1,2].

Drug-induced hepatitis is inflammation of the liver that could be caused by the administration of drugs. Some anti-inflammatory drugs such as ibuprofen, diclofenac, and naproxen, may also cause drug-induced hepatitis.[3,4] Inflammation forms part of the complex biological and generic response of body tissues to harmful stimuli, like pathogens, damaged cells, drugs , or irritants. It is a protective immune response which involves ; immune cells, blood vessels, and molecular mediators to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair.[5.6]. It is a mechanism of innate immunitywhich is a non-specific immune response. Signs of inflammation include; heat, pain, redness, swelling, and loss of function. Metabolism and detoxification of drugs take place in the liver.[3,4]

Acetaminophen (*N* -acetyl-p-aminophenol (APAP)) is a common pain reliever accessible by the community which could be abused by individuals through self-medication. Recommended doses are safe but high doses or overdoses of acetaminophen can cause potentially fatal hepatitis/ liver damage. Acetaminophen toxicity is the foremost cause of acute hepatitis/ liver failure.[7,8]

Immunological cytokines include those that enhance cellular immune responses as type 1 (TNFα, IFN-γ, etc.), and those that favors antibody responses as type 2 (TGF-β, IL-4, IL-10, IL-13, etc.) Interleukin 2 (IL2) is secreted by lectin- or antigen-stimulated T cells to regulate the growth and differentiation of T cells and certain B cells. It stimulates growth and differentiation of T cell response. It can be used in immunotherapy to treat cancer or suppressed for transplant patients. Has also been used in clinical trials (ESPIRIT,Stalwart) to increase CD4 counts in HIV positive patients. It found in Th1-cells.[9-11]. Interleukin 4 (IL4) is found in Th2 cells, just activated naive CD4+ cell, memory CD4+ cells, mast cells, macrophages Interleukin 4 (IL4) is produced by CD4+ T cells primarily to provide help to B cells in the proliferation T and B cells including differentiation of B cells, synthesis of IgG1 and IgE. It also plays an important role in allergic response (IgE). It promotes adhesion of lymphocytes.[9-11]. Interleukin 6 (IL6) is found in macrophages, Th2 cells, B cells, astrocytes and endothelium. Interleukin 6 (IL6), known as B-cell stimulatory factor-2 (BSF-2) and interferon beta-2, is essential in the differentiation of B cells into immunoglobulin-secreting cells (plasma cells) for the production of antibody. It induces myeloma/plasmacytoma growth and nerve cell differentiation. It also differentiates hematopoietic stem cells. It also induces acute phase reaction, hematopoiesis, differentiation and inflammation [9-11]. Interleukin 10 (IL-10) is a protein that produces cytokines and inhibits Th1 cytokine production (IFN-γ, TNF-β, IL-2). It is involved in the activation of B cells and stimulation of Th2 cells. It is found in monocytes, Th2 cells, CD8+ T cells, mast cells, macrophages and B cell subset.[9-11].

MATERIALS AND METHODS

Study area

Animal house of Achievers University, Owo-Nigeria equidistant between Nigeria Federal capital territory-Abuja and former Federal capital-Lagos. It has a Latitude: 6.98575 , Longitude: 5.27103 and Time Zone: UTC+1, Africa/Lagos.

Study population

Rabbits were bought from Oja Ikoko-a major market in Owo and were identified and confirmed having same sex (male) by the Department of Biological Sciences, Achievers University, Owo-Nigeria. This include 15 rabbits with weight ranging from 1.0 -1.4 Kg grouped as follows:

Group A: Five rabbits weighing 1.2 ±0.1 Kg fed with normal meal and water were studied as control group A.

Group B_1: Five rabbits weighing 1.3 ±0.1 Kg given normal meal with water and 2,500mg/kgBW of acetaminophen for seven days

Group B_2: Five rabbits weighing 1.1 ±0.1 Kg given normal meal, water and 400mg/kgBW of ethanolic extract of *Solanecio biafrae* (Èfó Wòròwó) for another seven days.

Group C_1: Five rabbits weighing 1.3 ±0.1 Kg given normal meal with water and 2,500mg/kgBW of acetaminophen for seven days

Group C_2: Five rabbits weighing 1.1 ±0.1 Kg given normal meal, water and 400mg/kgBW of aqueous extract of *Solanecio biafrae* (Èfó Wòròwó) for another seven days.

Preparation of the *Solanecio biafrae* (Èfó Wòròwó) Extracts

Solanecio biafrae (Èfó Wòròwó) were plucked from major farms in and around Owo-Nigeria. They were identified by the Department of Biological Sciences. *Solanecio biafrae* (Èfó Wòròwó) were air dried for 14 days Ethanolic and aqueous extraction was carried out by soaking 50g of powers of *Solanecio biafrae* (Èfó Wòròwó) into 500ml of each of ethanol and sterile distilled water for 24hours. Following the report of Das *et al.*[12] that solvent to sample ratio of 10:1 (v/w; solvent to dry weight ratio) has been used as ideal. Each extract was filtered through Whatmann filter paper No.1 and filtrates concentrated at room temperature in order to reduce the volume. Further concentration and drying by volume extraction was carried out using rotary evaporator and stored in refrigerator prior to use. Four hundred milligramme of the extract powder was dissolved in 2ml of distilled water for administration.

Blood specimen

Blood samples were collected from the veins lining the ear of the rabbits after each treatment into lithium heparinized bottles for the estimation of plasma concentrations of IL-6, IL-10, IL-4 and IL-2.

Determination of Biochemical Parameters

Plasma concentration of IL-6, IL-10, IL-4 and IL-2 were measured in the control and the experimental rabbits by ELISA using the reagent kits of MyBioSource.

Rabbit Interleukin 2 (IL2) ELISA.

Principle of the Assay: The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Interleukin 2 (IL2). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Interleukin 2 (IL2). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain

Interleukin 2 (IL2), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Interleukin 2 (IL2) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Rabbit IL-4 ELISA

Principle of the assay: This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to IL-4. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for IL-4 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain IL-4, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm +/- 2 nm. The OD value is proportional to the concentration of IL-4. You can calculate the concentration of IL-4 in the samples by comparing the OD of the samples to the standard curve**.**

Rabbit IL-6 ELISA

Principle of the Assay: This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to IL-6. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for IL-6 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain IL-6, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm +/- 2 nm. The OD value is proportional to the concentration of IL-6. You can calculate the concentration of IL-6 in the samples by comparing the OD of the samples to the standard curve.

Rabbit IL-10 ELISA

Principle of the Assay: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-10 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-10 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Method of Data analysis

The results obtained was subjected to statistical analysis using SPSS 18.0

Ethical Consideration

The rabbits were treated and sacrificed in line with the ethical guideline as provided by Research and Ethical Committee of the Department of Medical Laboratory Science, Achievers University, Owo-Nigeria.

RESULTS

The results obtained in the rabbits showed no significant difference in the plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits given aqueous and ethanolic extracts compared with the results obtained from the control rabbits($p>0.05$). However, there was a significantly higher difference in the plasma value of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits given 2,500mg/kgBW of acetaminophen than the control rabbits with $p<0.05$. There was also a significantly lower difference in the plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits given 400mg/kgBW of ethanolic and aqueous extracts of *Solanecio biafrae* (Wòròwó) for seven days than when these rabbits were given 2,500mg/kgBW of acetaminophen for seven days before the administration of the extracts($p<0.05$). There was no significant difference in the plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits supplemented with aqueous extract of *Solanecio biafrae* (Wòròwó) compared with those given ethanolic extract of *Solanecio biafrae* (Wòròwó) for seven days after the administration of 2,500mg/kgBW of acetaminophen($p>0.05$).

Table 1: Mean and Standard deviation of plasma AST, ALT and anti-inflammatory cytokines in the rabbits before and after the administration of Acetaminophen and the extracts

Parameters	Group A Rabbits (Control)	Group B1	Group B2	Group C1	Group C2
IL-2 pg/ml,	2.2 ± 0.5	4.3±0.2	2.0±0.1	4.5±0.3	1.8±0.1
IL-4 pg/mL	2.1 ±0.1	4.9±0.3	2.0±0.5	4.8±0.3	2.2±0.5
IL-6 pg/mL	1.2±0.2	4.3±0.4	1.9±0.2	4.4±0.3	1.9±0.2
IL-10 pg/ml	4.2 ±0.6	6.7±0.2	3.8±0.3	7.0±0.4	3.9±0,3
AST (IU/L)	9.0±2.0	19.0±1.0	7.0±1.0	20.0±2.0	8.0±1.0
ALT (IU/L)	4.5±1.0	15.0±2.0	6.0±2.0	13.0±2.0	6.0±1.0

Table 2: Comparative analysis of the plasma AST, ALT and anti-inflammatory cytokines in the rabbits before and after the administration of Acetaminophen and the extracts

		B1 vs A	B2 vs A	C1 vs A	C2vs A	B1 vs B2	C1vs C2	B2 vs C2
IL-2	't'	-3.90	0.39	-3.94	0.74	10.29	8.53	1.41
	'p'	0.03*	0.37	0.03*	0.26	0.005**	0.007**	0.15
IL-4	't'	-8.85	0.19	-8.54	-0.20	4.97	4.46	-0.28
	'p'	0.006**	0.43	0.006**	0.43	0.02*	0.02*	0.40
IL-6	't'	-6.93	-2.47	-8.88	-2.47	5.37	6.93	0.00
	'p'	0.01*	0.07	0.006**	0.07	0.02*	0.01*	0.5
IL-10	't'	-3.95	0.6	-3.88	0.44	8.04	6.2	-0.24
	'p'	0.03*	0.31	0.03*	0.35	0.008**	0.01*	0.42
AST (IU/L)	't'	-4.47	0.89	-3.89	0.45	3.79	5.37	-0.32
	'p'	0.02*	0.23	0.03*	0.35	0.03*	0.02*	0.39
AST (IU/L)	't'	-4.70	-0.67	-3.80	-1.06	3.18	3.13	0
	'p'	0.02*	0.29	0.03*	0.20	0.04*	0.04*	0.5

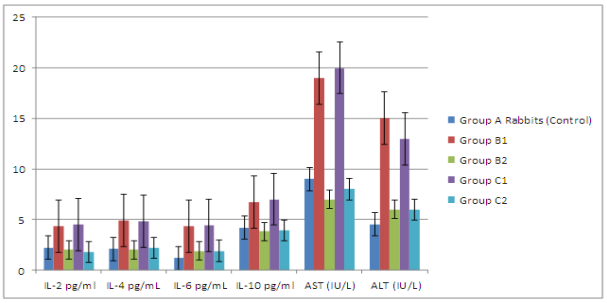

Figure 1; Comparative analysis of the plasma AST, ALT and anti-inflammatory cytokines in the rabbits before and after the administration of Acetaminophen and the extracts

DISCUSSION, CONCLUSION AND RECOMMENDATION

There was a significantly higher difference in the plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits given 2,500mg/kgBW of acetaminophen without any supplement of either the aqueous or the ethanolic extract of *Solanecio biafrae* (Wòròwó) than the control rabbits that were neither given 2,500mg/kgBW nor *Solanecio biafrae* supplement.

This finding could be attributed to hepatotoxicity resulting into inflammation and liver damage as a result of acetaminophen overdose. Acetaminophen is commonly used as oral analgesics and antipyretics. Appropriate therapeutic doses are safe but hepatotoxicity can occur after overdose or when misused. Acetaminophen toxicity is now more common than viral hepatitis as the most common cause of acute hepatic failure and is the second most common cause of liver failure requiring transplantation.[7,8] Acetaminophen is converted to nontoxic- water-soluble compounds form in the liver by conjugation in the liver .These are eliminated in the urine. In acute overdose over a prolonged period, metabolism by conjugation becomes saturated, and excess N -acetyl-p-aminophenol (APAP) is oxidatively metabolized by the CYP enzymes (CYP2E1, 1A2, 2A6, and 3A4) to the hepatotoxic reactive metabolite known as N-acetyl-p - benzoquinoneimine (NAPQI). N-acetyl-p - benzoquinoneimine (NAPQI) has a short half-life as a result it rapidly forms a conjugate with glutathione (a sulfhydryl donor) and excreted through the renal system.[13-16]. Excessive formation of NAPQI or a reduction in glutathione stores, consequently, NAPQI will covalently binds to the cysteinyl sulfhydryl groups of hepatocellular proteins to form NAPQI-protein adducts. This causes an ensuing cascade of oxidative damage and mitochondrial dysfunction.[13-16] The subsequent inflammatory response propagates hepatocellular injury and death. Necrosis primarily occurs in the centrilobular (zone III) region, owing to the greater production of NAPQI by these cells. As a result of the anti-inflammatory cytokines are excessively produced and released into the circulation hence an increase in the plasma values [7,8].

There was also a significantly lower difference in the plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in the rabbits given 400mg/kgBW of ethanolic and aqueous extracts of Solanecio biafrae (Wòròwó) for seven days than when these rabbits were given

2,500mg/kgBW of acetaminophen for seven days before the administration of the extracts. Reduced plasma values of AST, ALT, IL-2, IL-4, IL-6 and IL-10 in rabbits supplemented with aqueous and the ethanolic extract of *Solanecio biafrae* (Èfó Wòròwó) could be associated to significant reduction in excessive formation of NAPQI, hepatotoxicity, formation of NAPQI-protein adducts that can cause an ensuing cascade of oxidative damage and mitochondrial dysfunction as a result of the administration of aqueous and ethanol extract of *Solanecio biafrae* (Èfó Wòròwó) owing to the phytochemical and the antioxidant constituents such as terpenoids, majorly sesquiterpene germacrene D,. Vitamin A, B (Folic acid, niacin, thiamin, riboflavin), C, E, Tecopherols and Flavonoids that prevent oxidative reactions as a result of acetaminophen overdose resulting into hepatotoxicity and inflamatory response [13-16]. Consequently it will lead to decrease in inflammatory response and liver damage which will progressively return the raised plasma concentrations of IL-2, IL-4, IL-6 and IL-10 to about normal. The leaves also contain Vitamin A, B (Folic acid, niacin, thiamin, riboflavin), C, E, Tecopherols and Flavonoids.[1,2].

Elevated serum level of IL-2 and IL-6 have also been reported by Antonia *et al.,* 2014 and including IL-10 by Hoda *et al.*,[17] in patients with chronic hepatitis/liver disease which is consistent with the findings of this work. However, Antonia *et al.*,.[18] reported a decrease in IL-10 in patients with chronic hepatitis which does not agree with the finding of this work. Acording to Antonia *et al.*,[18]: IL-10 plays an anti-inflammatory role in the immune system because it inhibits the production of proinflammatory cytokines and limits T cell activation and differentiation. Due to its immunoregulatory action, it has been assumed that inadequate levels of IL-10 can determine long-term escape of pathogens from immune control and give rise to persistent infection. Elevated IL-10 in this work could be associated with the initial acute phase response by the body system to produce IL-10 in excess to be able to carry out its normal immunological response which include inhibition of proinflammatory interleukin and other proinflammatory agents as stated above by Antonia *et al.*, [18].

Plasma increase in AST and ALT in the rabbit given 2500mg/kgBW of acetaminophen without *Solanecio biafrae* (Èfó Wòròwó) supplement could be linked with the fact that overdoses and prolong uses of acetaminophen could result into liver inflammation and damage as a result of drug induced hepatotoxicity. This finding agrees with the report of Watkins *et al.*, 2006 who reported elevated Aminotransferase in healthy adults receiving 4 grams of acetaminophen daily in a randomized controlled trial AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate (Hayashi *et al.*, 2003; Gaze, 2007).

Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate (Hayashi *et al.*, 2003; Gaze, 2007). This could also be attributed to the fact that ALT and AST are indices of liver inflammation though ALT is more specific than AST as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma. This is because ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle (Hayashi *et al.*, 2003; Gaze, 2007).

Decrease in Plasma ALT and AST in the experimental animal given 400mg/kgBW of aqueous and Ethanolic extract of *Solanecio biafrae* (Èfó Wòròwó) for 7 days after they have been given 2500mg/kgBW of acetaminophen for 7 days could be associated with the phytochemical and specifically the antioxidant constituents of *Solanecio biafrae* (Èfó Wòròwó) leading to reduction in hepatotoxicity, inflammation and possible liver damage (Adebooye, 2000; 2001)..

CONCLUSION AND RECOMMENDATION

This study revealed an increase in plasma AST, ALT, IL-2, IL-4, IL-6 and IL-10 after a 7 day overdose of acetaminophen due to possible drug induced hepatotoxicity, inflammation and liver damage which was significantly reduced when the rabbits were given the ethanolic and aqueous extracts *Solanecio biafrae* (Wòròwó). Consequently, the extract could be a good supplement at reducing drug-induced hepatotoxicity/hepatitis.

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