Plasma TNFα, ammonia and total bile acids may indicate treatment outcome in patients with elevated blood pressure treated in herbal homes.

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Abstract

Study background: **Ammonia which is a toic substance is converted to less toic urea in the liver for ecretion through the kidney. Pear avocado leaves have be successfully applied in the traditional treatment of elevated blood pressure in Oke- Ogun area of Oyo State, Nigeria. Liver also metabolizes drugs and herbal etracts applied in the treatment of elevated blood pressure. Apart from using pot-treatment blood pressure, plasma levels of inflammatory markers and other biochemical substances may indicate treatment outcome.**

Aims and objectives: **To determine Plasma TNF, Ammonia and Total Bile Acids as anbindication of treatment outcome in patients with elevated blood pressure treated in herbal homes.**

Material and methods: **The subjects studied include thirty patients in ten 1 herbal homes aged years who have just received treatment and thirty age matched hypertensive patients recruited from major hospitals who have never received herbal treatment but have just received treatment in the hospitals. Blood samples were obtained from the patients a day after the completion of the dose and or treatment. Fasting Total Bile Acids, Plasma Urea and Plasma ammonia were determined biochemically by spectrophotometry Plasma TNF HIVp2Ag-Ab, anti-HCV, HBsAg by ELISA, identification of Plasmodium by iemsa Thick blood film techniue and AFB by Ziehl Nelseen staining.**

Results: **There was a significant decrease in plasma TNF and urea, increase in plasma ammonia and TBA following a successful treatment of elevated blood pressure using raw liuid etract of pear avocado leaves p..**
No significant changes was obtained in fasting Total Bile Acids, Plasma Urea and Plasma ammonia in hospital patients after a successful treatment of elevated blood pressure p.. There was a significant decrease in plasma urea, increase in plasma ammonia and fasting TBA in herbal patients compared with the results obtained in the hospital patients after treatments p ..

Conclusion: **Herbal treatment of elevated blood pressure using pear avocado leaves was successful as it lowered the elevated blood pressure and originally increased plasma TNF but could disrupt the liver functions as there was a significant decrease in plasma urea, increase in plasma ammonia and fasting TBA in herbal patients compared with the results obtained in the hospital patients after treatments.**

Keywords: Plasma ammonia, Herbal home, TNFα, Hospital patients, Treatment outcome, Elevated blood pressure, Total bile acids.

Abbreviations:

LAB: Lactic Acid Bacteria; AACCI: American Association of Cereal Chemists International; TPA: Texture Profile Analyses; LV: Loaf Volume; SLV: Specific Loaf Volume; ORAC: Oxygen Radical Absorbance Capacity; GPT: Gluten Peak Tester; MT: Maximum Torque; PMT: Peak Maximum Time; CBP: Chorleywood Bread Method.

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Introduction

The production of ammonia from amino acids and protein including the conversion to urea is the responsibility of the liver while kidneys excrete the urea end product in urine. Any derangement in these processes can cause hyperammonemia [1-6]. Increase in plasma ammonia can be caused by liver failure, viral hepatitis, exposure to hepatoxins, or cirrhosis of the liver; overproduction of ammonia, medication, severe dehydration and small intestinal bacterial overgrowth [6-9].

Pear avocado leaves have be successfully demonstrated in the traditional treatment of elevated blood pressure. Ammonia is converted to urea in the liver while Total Bile acids is a biomarker of liver dysfunction [10, 11].

Herbal treatment does not take into consideration the appropriate dose with respect to the toxicity of the herbal drug. Most of the herbal treatments involve the administration of the crude or raw extract of the herbs which may cause renal and liver damage [10].

Tumor Necrosis Factor alpha (TNFα) is a pro-inflammatory cytokine whose level increases upon inflammation. It is an acute phase protein responsible for cell aptosis and fever as it acts as a pyrogen. Tumor Necrosis Factor alpha (TNFα) has been associated with blood pressure as it has been reported that TNF-α blockade lowered blood pressure [11].

This work was therefore designed to determine To determine Plasma TNFα, Ammonia and Total Bile Acids as an indication of treatment outcome in patients with elevated blood pressure treated in herbal homes.

Ammonia and total bite acid

Study Area: Oke Ogun (Oke Ogun) is a populated northern part of Oyo State in Nigeria. The region is located at an elevation of 188 meters above sea level and its population amounts to 174,152. Its coordinates are 7˚19'60" N and 4˚4'0" E in DMS (Degrees Minutes Seconds) or 7.33333 and 4.06667 (in decimal degrees). Its UTM position is FJ11 and its Joint Operation Graphics reference is NB31-03. The current local time is 08:33; the sun rises at 09:00 and sets at 21:07 local time (Africa/Lagos UTC/GMT+1). The standard time zone for Oke Ogun is UTC/GMT+1.

The major business in the area is farming. There are herbal homes where interested inhabitants receive herbal treatments. There are government owned hospitals and private/faith-based hospitals providing primary, secondary and tertiary healthcare services.

Study population: The subjects include thirty (30) patients in ten (10) herbal homes aged 38 – 67 years who have just received treatment and thirty (30) age matched hypertensive patients recruited from 5 major hospitals who have never received herbal treatment but have just received treatment in the hospitals in Oke Ogun area, Oyo state - Nigeria. Blood samples were obtained from the patients and control a day after the completion of the dose and or treatment. All subjects were negative to TNFα, HIVp24Ag-Ab, anti-HCV, HBsAg by ELISA, Plasmodium by Giemsa Thick blood film technique and AFB by Ziehl Neelsen tests

Treatment of elevated blood pressure

Herbal homes: About 70ml of raw liquid extract of pear avocado leaves was prepared fresh and administered to each patient per oral by the herbal home practitioners on daily basis for at least 14 days until the blood pressure return to normal

Hospitals: The treatment involves the use of drugs like diuretics, alpha-beta blockers and angiotensin converting enzyme inhibitors.

Collection of Blood sample

Five (5) milliliters of fasting venous blood was obtained from each of the subjects a day after the completion of the dose and treatment. The sample was preserved in Lithium heparinized tube. The plasma was extracted for the analysis of plasma total bile acids, urea and ammonia.

Analysis of Biochemical parameters

Total bile acid: This was determined in the Subjects using Fasting Blood Samples by Enzymatic Colorimetric method using the reagent kit of Randox.

Principle: In the presence of Thio-NAD, the enzyme 3-α hydroxysteroid dehydrogenase (3-α HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-α HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405 nm.

Plasma Ammonia by Enzyme Kinetic Method

This was determined in the Subjects using plasma blood Samples by Enzymatic method using the reagent kit of BEACON Diagnostics PVT. Ltd, India.

Principle: Ammonia reacts with –ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD+ in this reaction, which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional plasma ammonia concentration.

Plasma Urea by Urease- Berthelot method

This was carried out using the reagent kit of Randox.

Principle: Urea in the sample is hydrolyzed enzymatically into ammonia and carbon dioxide under the influence of urease. Ammonia reacts with phenol to form indophenol in the presence of sodium hypochlorite and sodium nitroprusside. The intensity of the color formed measured at 490nm is directly proportional to the urea concentration in the sample.

Plasma Total Bile Acids

This was carried out by Enzymatic Colorimetric method using Reagent Kit of Randox.

TNF alpha ELISA

Plasma TNF alpha was determined in the subjects by ELISA using Abcam's kit. This involves binding of antibody to TNFα with its corresponding antigen, the product was made observable and measurable through the addition of enzyme, substrate and chromogen.

Laboratory Identification of Acid Fast Bacilli and Plasmodium spp.,

Laboratory detection of Acid Fast Bacilli and Plasmodium spp., was carried out by Microscopy using Ziehl Neelsen and Geimsha-Thick film methods as described by Cheesbrough[12].

Anti-HCV ELISA assay

This was carried out by a third generation enzyme immunoassay for the determination of antibodies to hepatitis C virus in serum and plasma using the reagent kit of DIA.PRO Diagnostic Bioprobes Srl *Via* Columella, Milano - Italy.

HIV ELISA Test: HIVP24 Antigen and Antibody was detected in the subjects using Genscreen™ ULTRA HIV Ag-Ab Biorad Kit.

The Genscreen™ ULTRA HIV Ag-Ab is an immunoassay technique which is based on the principle of sandwich technique for the detection of HIV antigen and antibodies specific to HIV-1 and/or HIV-2 virus in human plasma.

HBsAg ELISA Test: Hepatitis B surface antigen (HBsAg) test was carried out by using a one step enzyme immunoassay technique of the sandwich type for the detection of HBsAg using the reagent kit of BIO –RAD Raymond Poincare, Marnes La Coquette.

Data analysis: The raw data of this work was subjected to statistical analysis to determine mean, standardrd deviation, student 't'test and probability at 0.05 level of significance using IBM SPSS 23.0.

Ethical Consideration: In addition to the inform consent obtained from each of the subjects, ethical approval was also obtained from the Research and Ethical Committee of Baptist Medical Centre, Saki-Nigeria before the commencement of the work.

RESULTS

There was a significant decrease in plasma urea, TNFα, increase in plasma ammonia and TBA following a successful treatment of elevated blood pressure manifested by a significant decrease in Systolic and Diastolic blood pressure using raw liquid extract of pear avocado leaves ($p<0.05$) (Figures 1 and 2). No significant changes was obtained in fasting Total Bile Acids, Plasma Urea and Plasma ammonia in hospital patients ($p>0.05$) after a successful treatment of elevated blood pressure manifested by a significant decrease in Systolic and Diastolic blood pressure using conventional anti-hypertensive drugs ($p<0.05$) (Figures 1 and 2).

There was a significant decrease in urea , increase in plasma ammonia , TNFα, and fasting TBA in herbal patients compared with the results obtained in the hospital patients after treatments ($p< 0.05$) (Figures 1 and 2).

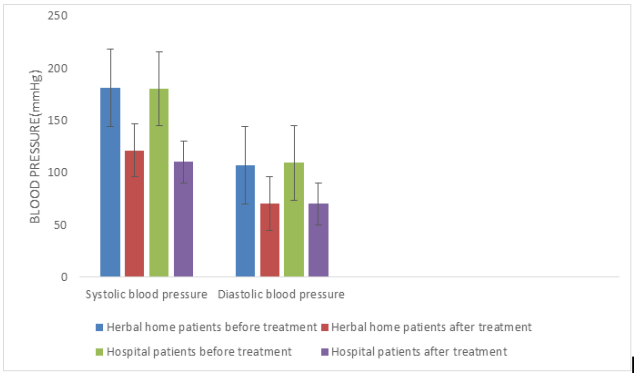

Figure 1: *Chart showing the systolic and diastolic blood pressure in the patients be ore and a ter treatments.*

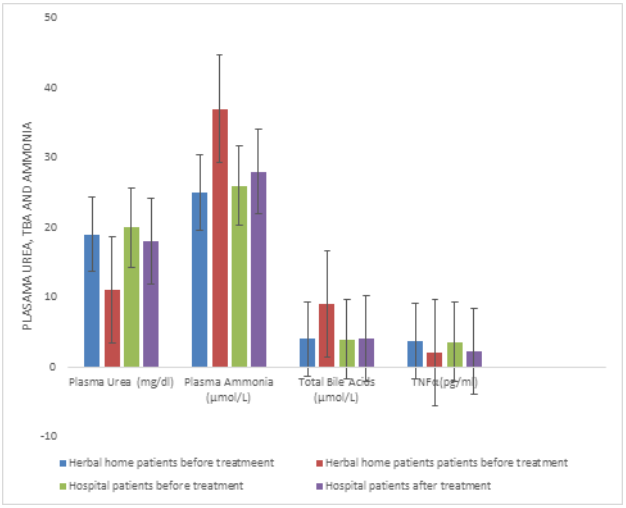

Figure 2: *Chart showing plasma Urea, Fasting Total Bile Acids and Ammonia in the patients be ore and a ter treatments.*

Discussion

There was a significant decrease in plasma urea, increase in plasma ammonia, TNFα, and TBA following a successful treatment of elevated blood pressure manifested by a significant decrease in Systolic and Diastolic blood pressure using raw liquid extract of pear avocado leaves

Decrease in urea could be as a result of defective conversion of ammonia generated from amino acids and protein to urea in the live resulting into hyperammonemia. These biochemical alterations can be associated with liver dysfunction as revealed by increase in total bile acids in the results obtained in this work [3, 14-17].

The above is consistent with the findings of Olaniyan [10] that reported elevated ALT and AST after the administration of raw liquid extract of pear avocado leaves. This implies that the raw liquid extract might be hepatotoxic.

Furthermore, bile acids are produced in the liver from endogenous cholesterol of which the plasma level increases in liver disease [3] .

No significant changes was obtained in fasting Total Bile Acids, Plasma Urea and Plasma ammonia in hospital patients after a successful treatment of elevated blood pressure manifested by a significant decrease in Systolic and Diastolic blood pressure using conventional anti-hypertensive drugs.

There was a significant decrease in urea, increase in plasma ammonia and fasting TBA in herbal patients compared with the results obtained in the hospital patients after treatments. The avocado leaf extract may have hepatotoxic effect compare to the conventional anti-hypertensive drugs thereby affecting normal functions of the liver with respect to the conversion of ammonia to urea for excretion in the urine by renal system [3, 15-17].

In addition hyperammonemia as found in this work after the administration of the leaf extract can be caused by the reduced activity of any of the enzymes involved in the formation of urea from ammonia, reduction in the activities of enzymes that are not part of the metabolic process of urea formation or liver cells dysfunction of cells and drug [4-6]. Genetic defects in one of the enzymes of the urea cycle, decrease production of urea from ammonia [6-9].

There was a significant decrease in plasma TNFα after the treatment of elevated blood pressure in the patients in both herbal homes and hospitals. Tumor Necrosis Factor alpha is a pro-inflammatory cytokine. This can be attributed to the successful treatment outcomes in both herbal and hospital centres because TNF-alpha has been reported to increase Mean arterial blood pressure (MABP) in patients with chronic heart failure (CHF) [18].

Conclusion

Herbal treatment of elevated blood pressure using pear avocado leaves was successful as it lowered the elevated blood pressure and originally increased plasma TNFα but could disrupt the liver functions as there was a significant decrease in plasma urea, increase in plasma ammonia and fasting TBA in herbal patients compared with the results obtained in the hospital patients after treatments.

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