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Ameliorative Effect of Aqueous Leaf Extract of Tectona Gondis on Egg Yolk Induced Heart and Blood Vessels Damage in Adult Wistar Rats

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Abstract: *The aim of this study was to determine the effect of aqueous extract of Tectona gondis leaf on egg yolk induced heart and blood vessel toxicity in adult wistar rats. A total of 35 (thirty five) adult wistar rats weighing between 200 g to 270 g were divided into 5 groups of seven rats per group. Group A rats were placed on normal diet only while Group B rats received 300 mg/ kg body weight / day (BWT/D) of egg yolk. Group C rats received 300mg / kg BWT/D of Tectona gondis leaf. Group D rats received 300 mg/ kg BWT/D of egg yolk and 300mg / kg BWT/D of T. gondis leaf Group E rats received 300 mg/ kg BWT/D of egg yolk and 500mg / kg BWT/D of T. gondis leaf and all the dosage were given for 30 days via orogastric administration. The biochemical result revealed that group B showed marked increase in the level of the serum SOD, CAT, cholesterol when compared with the other groups. Histologically, group B showed asevere vascular obstruction and mild perivascular infiltrates of chronic inflammatory cells, while group A,C,D and E revealed normal bundles of myocardial fibers and coronary artery. It can then be concluded that theaqueous leaf extract of T. gondis was able to reverse the vascular obstruction of the coronary artery caused by the egg yolk.*

Keyword: *Tectona gondis, Superoxide dismutase, Blood vessel, Catalase, Cholesterol, Heart.*

I. Introduction

Nature has been very kind to the inhabitant of the world by making the earth to be habitable, but due to the insatiable nature of man, the natural habitat and food intake of human race have therefore being polluted by environment factors.*Tectona grandis* commonly known as teak or sagon (Family of Verbenaceae), is a native to the South and Southeast Asia, and has been used for both therapeutic and commercial purposes[1].The leaves, the barks and the roots of*Tectona grandis*which is one of the ancient trees,are themajor medicinal componentsof thetree. In traditional medicine, a wood powder paste has been used against bilious headache and swellings[2]. Roots are useful in the treatment of urinary system-related troubles, and oil from the flower promotes hair growth and is also useful in the treatment of scabies[3;4;5]. Several classes of phytochemicals like alkaloids, glycosides, saponins, steroids, flavonoids, proteins and carbohydrates have been reported in *T. grandis*[6]. The leaf tenare is shiny above, hairy below, with vein network, broadly oval with shortly pointed or blunt tip and taping base[7]. A wood powder paste has been used against bilious headache and swellings[8].*Tectona grandis* has been known to possess antibacterial activity[9], anti inflamatory activity[10], cytotoxic activity[11] ,gastroprotective[12], antioxidant activity[13], anti-haemolytic activities[14].

The treatment of hypercholesterolemia is aimed at reducing the risk of heart or cardiovascular disease[15].However,cholesterol content of chicken egg has continued to receive considerable attention, since the cholesterol level in egg yolk is of great concern to people who are trying to lower their dietary intake of cholesterol[16.17;18].

The aim of this study is to determine the effect of *T. gondis* on egg yolk induced cardiac toxicity in adult wistar rat

II. Materials and Methods

Plant Material and Extraction

Fresh leaves of the plant *Tectona grandis* were collected from a farm in Ikhin town in Owan East L. G. A. of Edo State, Nigeria, andwas authenticated at the Department of biology and biotechnology (botany), University of Benin, Benin City.

The fresh leaves were shade-dried for two weeks, after which it was ground into powder form in the department of pharmacognosy, university of Benin. The powder was subsequently soaked in distilled water for 48 hours at room temperature. The mixture was then filtered into a conical flask with a whatman filter paper.

The filtrate obtained from the process was evaporated at a temperature of 30 degree Celsius for ten hours to produce a gel-like extract which was stored in a refrigerator. Upon administration, appropriate concentration of the extract was made subsequently by diluting the extract with distilled water to a concentration of 300mg/kg per body weight of the animals

Experimental design

Atotal of 30 adult albino wistar rats weighing about 180 - 250g were obtained from the animal house, department of Anatomy, University of Benin, Benin city. The rats were kept in well-aerated plastic cages in the animal house.

Each of the cages comprises six wistar rats, and were allowed to acclimatize within the laboratory environment for a period of two weeks before the commencement of other experimental procedures.They rats were given free access to feed and clean drinking water *ad libitum* from the period of acclimatization till the animals were sacrificed.

The animals were grouped into six groups of five rats each.

Group A animals served as control that were administered with normal feed and water.

Group B animals were treated daily with oral administration of 300 mg/kg body weight egg yolk only. **Group C** animals were treated with oral administration of 500mg/kg body weight of aqueous extract of *T. gondis* leaves.

Group D animals were treated with oral administration of 300mg/kg body weight of egg yolk and 300mg/kg of *T. gondis* leaves.

Group E animals were treated daily with oral administration of 500mg/kg body weight of *T. gondis* and 300mg/kg body weight of egg yolk.

After 30 days of administration of the egg yolk and the extract, the animals were sacrificed and their organs were harvested, the blood samples of each animals were also obtained for biochemical analysis.

Before the animals were sacrificed, they were first placed in a plastic container with cotton wool immersed in chloroform. This was done in order to anaesthetize the animals before sacrifice. Afterwards, the animals were dissected through a mid-line abdominal incision using surgical blade and a forceps to expose the relevant organs within their cavities. Their blood samples were first drawn using 5ml syringe into their various sample bottles, before their organs were harvested

The harvested organs were transferred into an organ bottle containing 10% of formal saline for fixation. Afterwards, dehydration was carried out by passing the tissue through an ascending grade of alcohol (which are; 70%, 90% and absolute) respectively. The tissues were first made to remain in a 70% alcohol for 2 hours. It was then transferred to a 90% alcohol and allowed to stay for 18 hours (overnight), and then 100% alcohol which was changed twice for hours each. Clearing was then carried out using xylene. The tissues were immerse in xylene for two hours to completely remove the alcohol, subsequently, infiltration was carried out on the tissues in an oven using molten paraffin wax at temperature range of 56 degree Celsius, after which embedding was carried out using an embedding mould. The molten paraffin was poured into the embedding mould and infiltrated tissues were placed in it, and the molten paraffin was allowed to cool to form a tissue block. Thereafter, the edges of the block was trimmed out and mounted on a wooden block holder. Section was carried out using a rotatory microtome. The tissue was clipped to the microtome and sectioned at thickness of 5microns. Sections were made out in ribbons and were placed in 20% alcohol for spreading of the tissue. The tissue ribbons were cut out and floated in a water bath at a temperature of 30 degree Celsius. The sectioned tissues were placed in xylene for 7 minutes to remove paraffin wax from the tissues. Hydration was carried out and stained with haematoxylin and eosin dyes. The tissues were also cleared in xylene. Eventually, the tissues were mounted a slide with a cover slip using glue or albumen to seal it, before they were mounted and viewed under the microscope.

Biochemical assays: Blood serum was used to determinesuperoxide dismutase (SOD)[19], Catalase (CAT)[20].Cholesterol level was estimated by standard procedure using an autoanalyser with a recognized biochemical kit.

Statistical analysis: Biochemical parameters were evaluated for statistical significance. Data are expressed as the mean ± SD. The data were analyzed by analysis of variance (ANOVA) followed by least square difference using the Statistical Package for the Social Sciences (S.P.S.S. 21). The analysed data were represented with tables. The level of significance was set at $P<0.05$).

III. Results

A Table Showing The Biochemical Results Of The Experimental Groups

Parameter	Group A	Group B	Group C	Group D	Group E
CAT (µm/L)	156.80 ± 4.88	278.30 ± 4.72*	160.70 ± 3.68	175.70 ± 2.39*	172.30 ± 2.89*
SOD ng/ml	58.00 ± 2.25	117.80 ± 2.81*	56.33 ± 1.75	74.33 ± 1.96*	64.33 ± 1.89
CHOLES mg /dl	144.00 ± 6.93	277.20 ± 6.93*	152.50 ± 3.10	183.70 ± 1.82*	177.00 ± 2.85*

*P <0.05 indicates significant difference when group A is compared with the other groups.

The mean concentration of SOD and CAT and cholesterol in rats treated with egg yolk only were significantly elevated (117.80 ± 2.81, 278.30 ± 4.72 and 277.20 ± 6.93respectively)when compared with other group (P <0.05)

Comparison of mean SOD, CAT and cholesterol levels of treated rats in all groups B, C, and D with controls were found to be statistically significant (P<0.05).

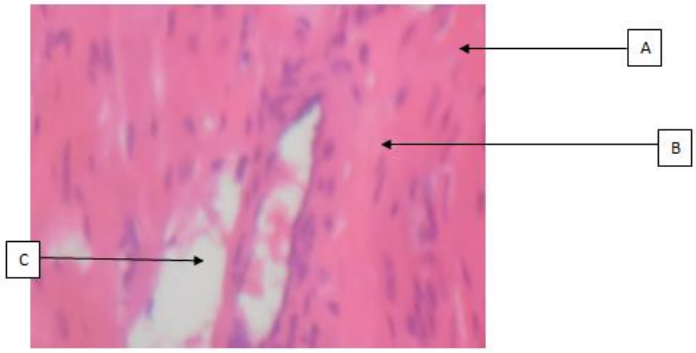

Plate 1. Control: Rat heart composed of A, bundles of myocardial fibres, B, coronary vessel and C, interstitial space (H&E x 100)

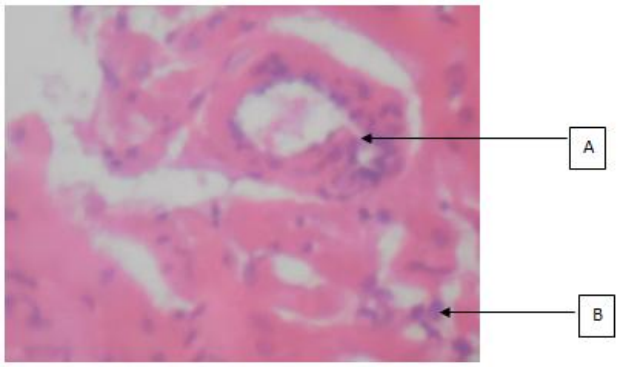

Plate 2: Rat heart given egg yolk only showing A, severe vascular obstruction and B, mild perivascular infiltrates of chronic inflammatory cells (H&E x 100)

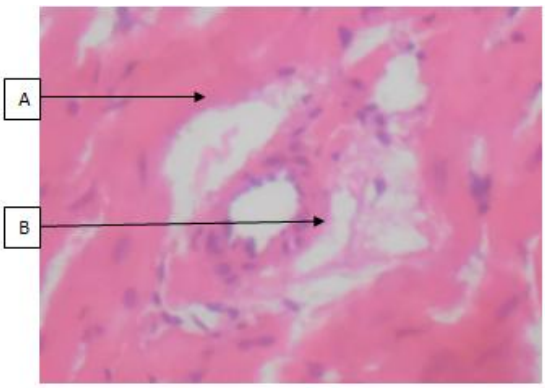

Plate 3: Rat heart given low dose Extract only showing A, normal bundles of myocardial fibres and B, coronary artery (H&E x 100)

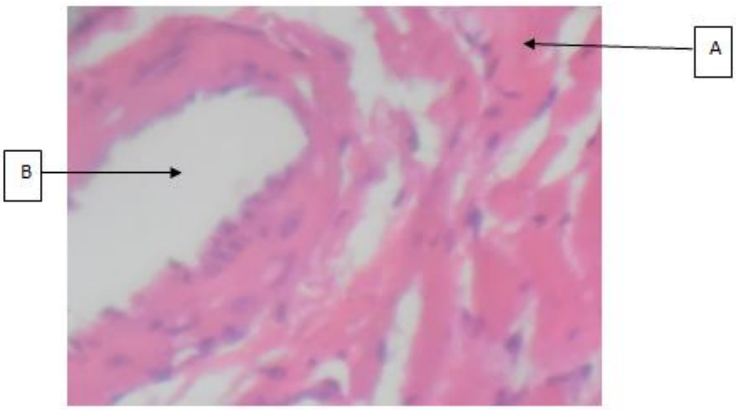

Plate 4: Rat heart given low dose Extract plus egg yolk showing A, normal bundles of myocardial fibres and B, coronary artery (H&E x 100)

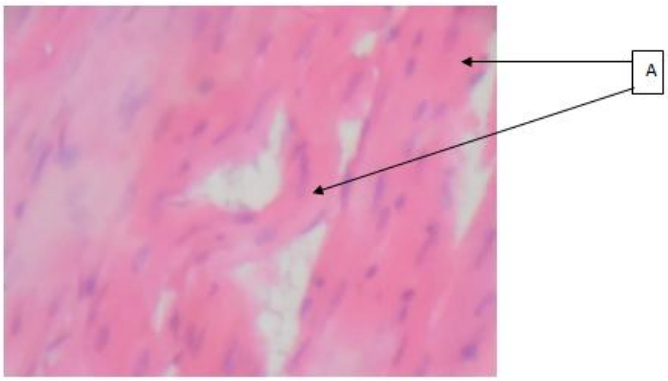

Plate 5: Rat heart given high dose Extract plus egg yolk showing A, normal myocardial architecture (H&E x 100)

Plate1is the control groupof the study and the micrograph reveala heart that is composed of normal bundles of myocardial fibers, coronary vessel and interstitial space

Plate 2 shows the histology of the liver in group B where the rats were fed with 300mg / kg of egg yolk only and the micrograph reveal a severe vascular obstruction andmild perivascular infiltrates of chronic inflammatory cells

Plate 3 shows the histology of the liver in which the rats in the group were given high dose of 500mg / kg of T. gondisand the micrograph reveal normal bundles of myocardial fiber and coronary artery.

Plate 4 shows the histology of the liver in which the rats in the group were administered with low dose of 300mg / kg of T. gondis and 300mg / kg of egg yolk and the micrograph reveal normal bundles of myocardial fiber and coronary artery.

Plate 5 shows the histology of the liver in which the rats in the group were given high dose of 500mg / kg of T. gondis and 300mg /kg of egg yolk and the micrograph reveal normal bundles of myocardial fiber and coronary artery.

IV. Discussion

In this present study, 200mg /kg body weight of egg yolk was given to each group (group B, group D and group E animals), to raise the cholesterol level of the blood of the above mentioned groups of animals. On the heart of the wistar rats, the group B rats fed with egg yolk only manifested a severe vascular obstruction, the medial intima was hypertrophied in such a way that it extended and cut off the lumen, there by forming a double lumen.(i.e. an artery now having two lumen). Some inflammatory cells were also seen around the lumen (Plate 2). The egg yolk caused serious injury to the medial intimal of the blood vessel supplying the heart causing obstruction. The clinical implication of that obstruction is that it can reduce blood to the myocardium,thereby leading to ischemic damage of the heart muscles which can cause myocardial infarction.

The group C administered with low dose of the extract showed a normal architecture of the myocardial muscle and a coronary vessel (plate 3).This histological finding therefore showed the non- toxic nature of *Tectonagondis* at this particular dosage and why people like taken it in the traditional way. The group administered with both low and high dosage of the extract was able to prevent the thickening of the medial intimal of the heart blood vessels (plate 4 and 5). This results revealed the ameliorative strength of *Tectona*

gondis microscopically via the help of its flavonoids and saponin. The outcome of this study is inaccordance with previous work done[21].Kushwah et al concluded that *Tectona gondis* leaf possesses antioxidant properties and the phytochemical screening also showed that the extract have constituent that are needed for its antioxidant activities.

Statistically,only group B showed a significant increase in SOD, CAT and cholesterol level in comparison with the control group.But the other four treatment groups did not show any statistical significance when they are compared with the control group. This results therefore confirmed the oxidative and hypercholeresteremic effect of the egg yolk in the heart. *Tectona gondis* inhibit the generation of reactive oxygen species pathway in the heart thereby reducing the release of hydroxyl,superoxide and hydrogen peroxide groups that are detrimental to the system by causing cell membrane damage and therefore cell death. This resultis in line with earlier work done[22].

V. Conclusion

Both the low dose and high dose of the extract were able to provide an affordable measure of protection to the blood vessel supplying the heart and reversing the severe vascular obstruction of the coronary artery caused by the egg yolk.

VI. Recommendation

After detailed observation of *Tectona grandis* as documented by different researchers on it's effects, it is highly recommended commercially for treatment of heart related diseases associated with hypercholesterolemia.

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