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Free radical scavenging activities of anthocyanin flavonoid

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Abstract

Despite its nutritional content and huge application in the food, pharmaceutical and cosmetic industries, the use of anthocyanin remains restricted due to the difficulty in its extraction process. In this study, we examined the antioxidant effects of anthocyanin (one of the numerous flavonoids known in modern enthno-medicinal practice) on some serum free radicals in wistar rats. Twenty (20) adult wistar rats of between 100 – 250 g were procured and housed in the animal house of the Ambrose Alli University, Ekpoma, Edo State. After two (2) weeks of acclimatization, the animals were then grouped into five groups of four (4) rats each (n=4). Control (Group I) rats were fed normal rat chow and water *ad libitum*, whereas, group II rats received 100 mg/kg body weight (bw.) of anthocyanin extract. Groups III, IV and V rats also received 200 mg/kg bw., 300 mg/kg bw., and 400 mg/kg bw of anthocyanin extract respectively for a period of four weeks, using the oro-gastric cannula post prandial. For each week, body weights were obtained, and serum assayed for changes in the levels of anti-oxidant enzyme activities [Catalase (CAT), Malondialdehyde (MDA), Glutathione Peroxidase (GPx) and superoxide Dismutase (SOD)] using appropriate biochemical procedures. Obtained results were subjected to statistical comparisons using the one way analysis of variance (ANOVA). Tukey post hoc tests were also performed (where necessary) to ascertain the source(s) of mean differences between groups. We found a statistically significant decrease (at $p < 0.05$) by week in serum CAT, MDA, GPx and SOD levels with the administration of test substance. This also proved to be dose-dependent as increasing dose cause an increase in the enzyme activities over the period of administration. Body weight changes were also duration and dose dependent. Anthocyanin therefore showed potency in improving serum antioxidant enzyme activities. We recommend similar studies on other body tissues than blood.

Keywords: Anthocyanin; Serum; Anti-oxidants; Body weight

1. Introduction

Over the last century, researchers have focused on the application of herbs in the control of numerous ailments and their associated complications. In recent times though, Anthocyanin, one of the known numerous flavonoids in modern enthno-medicinal practice has raised a growing interest and beneficial health effects [1].

Anthocyanin (of the Greek anthos = flower and kianos = blue) is a common plant pigment that is harmless and useful in natural water-soluble colorant [2, 3], giving shiny orange, pink, red, violet and blue coloured plants their characteristic colour. Though numerous reports have implicated its medicinal importance as antioxidant, anti-neuronal, anti-cancer,

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and anti-diabetic agent [4, 5], its major use has recently been emphasized in cancer treatment [6], as food additive for nutritional purpose, and as a biological active substance [7].

Geographically, there is a plethora of natural occurrences of anthocyanins across the globe, with key differences seen in the number of hydroxylated groups between them, their bond type, nature and sugar chemistry [7]. Currently, over 500 species of anthocyanins and 23 anthocyanidins, active component of anthocyanins have been reported [7, 8], with only six being the most common in vascular plants [9].

Anthocyanins and anthocyanidins reportedly have great antioxidant activities than vitamins C and E [10]. Through the donation of phenolic hydrogen atoms, they capture and scavenge free radicals [11]; giving them their much reported anti-carcinogenic properties [12]. A linear correlation has also been reported between the antioxidant capability of anthocyanins in blackberries, red raspberries, black raspberries and strawberries; all of which have been documented to pose huge scavenging activity on reactive oxygen species that are generated as metabolic by-products [13].

On the cardiovascular system, strong evidences exist that anthocyanin containing grape juice have potent antioxidant function, protecting the heart against heart attack by increasing the permeability and strength of the capillaries, and inhibiting thrombopoeisis, as well as nitric oxide (NO) production to facilitate vasodilatation. Current investigated the effect of anthocyanin on serum antioxidant marker enzymes, using wistar rats as experimental model. Study also attempted to evaluate the durational effects of anthocyanin on body weight changes in wistar rats.

1.1. Aim of Study

This study examined the free radical scavenging activities of anthocyanin flavonoid on wistar rats. Specifically, the study;

- Determined the effect of administration of varying doses of anthocyanin on elected serum antioxidant enzyme activities [Catalase (CAT), Malondialdehyde (MDA), Glutathione Peroxidase (GPx) and superoxide Dismutase (SOD)]
- Determined the effect of administration of varying doses of anthocyanin on Body weight
- Investigated the durational effect of anthocyanin administration on serum antioxidant enzyme activities and body weight changes

2. Material and methods

2.1. Study Design

Twenty (20) adult wistar rats of between 100 – 250 g were procured and housed in the animal house of the Ambrose Alli University, Ekpoma, Edo State. After two (2) weeks of acclimatization, the animals were then grouped into five groups of four (4) rats each (n=4). Control (Group I) rats were fed normal rat chow and water *ad libitum*, whereas, group II rats received 100 mg/kg body weight (bw.) of anthocyanin extract. Groups III, IV and V rats also received 200 mg/kg bw., 300 mg/kg bw., and 400 mg/kg bw of anthocyanin extract respectively for a period of four weeks, using the oro-gastric cannula post prandial, for each week, body weights were determined, and serum obtained and subjected through biochemical procedure to determine the levels of antioxidant enzyme activities [Catalase (CAT), Malondialdehyde (MDA), Glutathione Peroxidase (GPx) and superoxide Dismutase (SOD)].

2.2. Anthocyanin Extraction

By way of reported extraction methods in available literatures, Using the solvent extraction method; being the most common method for extraction of diverse compounds found in fruits, including flavonoids, anthocyanin was extracted by grinding, drying or lyophilising pineapple and mango fruits after soaking in solvent extraction [14]. The extraction process was conducted with weak acid media (formic or acetic acid) to avoid their water loss (hydrolysis); this extraction method was however non-selective for anthocyanins only as it co-extracted a great number of other compounds, such as sugars or organic acids [15]. Consequently, outcome of the extraction process was subjected through purification techniques in order to isolate the anthocyanin of interest.

2.3. Anthocyanin Purification

The purification of anthocyanin to separate it from other constituent compounds (after extraction) was performed with the spectrophotometric method, using the spectrophotometer. By principle, because of the spectral characteristics of

anthocyanin, the technique provides very useful qualitative and quantitative information. Our procedure followed those of Giusti and Wrolstad (2001) and Wrolstad *et al.* (2005) [16].

2.4. Sample Collection

At the end of test period, Blood samples were collected from the animals by cardiac puncture, using needle and syringe. Collected blood was then centrifuged, and serum obtained for biochemical assays on antioxidant enzyme activities

2.5. Biochemical Assay

2.5.1. Determination of Superoxide Dismutase (SOD) Activity

The SOD activities in these tissues were determined by the method of Misra and Fridovich, (1972) [17]. An aliquot (0.04mls) of the supernatant was added to 5mls of 0.05M carbonate buffer (Ph 10.2) equilibrated in the spectrophotometer for 2-3 minutes. The reaction was then initiated by the addition of 0.6mls of freshly prepared adrenaline as substrate to the buffer-supernatant mixture which was quickly mixed by inversion and the absorbance taken. The reference cuvette contained 5ml of the carbonate buffer, 0.6ml of the substrate and 0.4ml of distilled water. The increase in absorbance of 420nm due to the adrenochrome formed was monitored every 30 seconds for 120 seconds. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome during 120 seconds.

2.5.2. Determination of Catalase Activity

The method of Cohen *et al.*, (1970) was adopted [19 and 20]. Aliquots of the homogenate supernatant (0.05ml) were added into ice cold test tubes while the blank contained 0.05ml distilled water. The reaction were initiated by adding sequentially, at fixed interval, 5ml of cold 30nM hydrogen peroxide and was mixed thoroughly by inversion. The test samples and the blank were taken one at a time, and 7ml of 0.01M potassium permanganate was added which was mixed twice by inversion and absorbance at 480nm. It was read within 30-60 seconds. The spectrophotometer standard was prepared by adding 7ml of 0.01M phosphate buffer with pH 7.0 and 1ml of 6M - Tetraoxosulphate (VI) acid solution. The spectrophotometer was zeroed with distilled water and the activity of enzymes was estimated.

2.5.3. Determination of Peroxidase Activity

The assay was based on the method of Junqueira, and Carneiro (2004) in which 0.4ml of the sample homogenate was added into clean test tubes, follow the addition of 5ml phosphate buffer and then 5ml hydrogen peroxide which was subsequently followed by 3ml of distilled water [19]. Finally the addition of 5ml of pyrogallol and the absorbance was taken at 330nm. The blank was prepared by the addition 0.5ml of phosphate buffer, follow by 5ml of hydrogen peroxide. 3ml of distilled water was then added and finally, pyrogallol which was used to zero the spectrophotometer before taking the absorbance of the test

2.5.4. Determination of Malonyldialdehyde (MDA) Activity

Lipid peroxidation was estimated in terms of thiobarbiturate acid reactive species using malonyldialdehyde (MDA) as standard by the method of Beuge and Aust, (1978) [20]. 1.0ml of a sample extract was added with 2ml of the TCB- TBA reagent (15%w/v TCA, 0.375% (W/V) TBA and 0.25N HCl). The contents were boiled for 15minutes, cooled and centrifuge at 10,000rpm to removed precipitate. The absorbance was read at 535nm and the Malonyldialdehyde coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ Cm}^{-1}$.

2.5.5. Determination of Weight

Animal weights were measured in grams (g) using an electronic weighing scale (Cardinal Scale Manufacturing Co., Webb City, MO).

2.6. Ethical Clearance

Ethical approval was sought from the Research and Ethics Committee of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

2.7. Statistical Analysis

Data were represented as mean standard deviation. Statistical analysis was done using student's t test and One-Way Analysis of Variance (ANOVA). Statistics was carried out with a Graph pad prism software (version 8.0). A p-level less than 0.05 was considered as statistically significant.

3. Results

Table 1 Serum Antioxidant Enzyme Activity in Varying Anthocyanin Dose Administration to Wistar Rats.

| Groups (Doses) | Serum Antioxidant Activity | | | |
|-----------------|----------------------------|------------|------------|------------|
| | CAT | MDA | GPx | SOD |
| I (Control) | 11.00±0.22 | 25.02±0.11 | 26.77±0.12 | 25.09±0.12 |
| II | 12.90±0.20 | 27.29±0.17 | 28.49±0.13 | 29.60±0.13 |
| III | 14.20±0.16 | 22.26±0.18 | 21.03±0.13 | 20.16±0.11 |
| IV | 19.70±0.27 | 25.06±0.12 | 23.07±0.19 | 25.06±0.10 |
| V | 24.70±0.18 | 29.73±0.11 | 26.32±0.19 | 22.19±0.25 |
| p-value (ANOVA) | 0.0021 * | 0.0000 * | 0.0039 * | 0.0000 * |

Table 2 Post Hoc (Tukey) test for Source(s) of Difference in Catalase (CAT) Activity for Compared Groups

| Groups | II | III | IV | V |
|-------------|---------|---------|---------|---------|
| | p-value | p-value | p-value | p-value |
| I (Control) | 0.3010+ | 0.0030+ | 0.0000+ | 0.0000+ |
| II | | 0.0020+ | 0.0010+ | 0.0130+ |
| III | | | 0.0061+ | 0.0021+ |
| IV | | | | 0.0000+ |
| V | | | | |

p-value < 0.05 = significant, + = significant increase

Table 3 Post Hoc (Tukey) test for Source(s) of Difference in Malonyldialdehyde (MDA) Activity for Compared Groups

| Groups | II | III | IV | V |
|-------------|---------|---------|---------|---------|
| | p-value | p-value | p-value | p-value |
| I (Control) | 0.0000+ | 0.0400+ | 0.0000+ | 0.0007+ |
| II | | 0.0000- | 0.0060+ | 0.0200+ |
| III | | | 0.0027+ | 0.0001+ |
| IV | | | | 0.0000+ |
| V | | | | |

p-value < 0.05 = significant, + = significant increase, -- = significant decrease

Table 4 Post Hoc (Tukey) test for Source(s) of Difference in Glutathione Peroxidase (GPx) Activity for Compared Groups.

| Groups | II | III | IV | V |
|-------------|---------|---------|---------|----------|
| | p-value | p-value | p-value | p-value |
| I (Control) | 0.0000+ | 0.0000- | 0.0001- | 0.2011#+ |
| II | | 0.0000- | 0.0000- | 0.0010- |
| III | | | 0.0500+ | 0.0000+ |
| IV | | | | 0.0000+ |
| V | | | | |

p-value < 0.05 = significant, + = significant increase, -- = significant decrease, #+ = insignificant increase

Table 5 Post Hoc (Tukey) test for Source(s) of Difference in Superoxide Dismutase (SOD) Activity for Compared Groups

| Groups | II | III | IV | V |
|-------------|---------------------|----------------------|-----------------------|----------------------|
| | p-value | p-value | p-value | p-value |
| I (Control) | 0.0301 ⁺ | 0.0070 ⁻⁻ | 0.1000 ^{#--} | 0.2010 ⁻⁻ |
| II | | 0.0110 ⁻⁻ | 0.0020 ⁻⁻ | 0.0011 ⁻⁻ |
| III | | | 0.2200 ^{#--} | 0.0000 ⁺ |
| IV | | | | 0.0080 ⁻⁻ |
| V | | | | |

p-value < 0.05 = significant, + = significant increase, -- = significant decrease, #-- = insignificant decrease

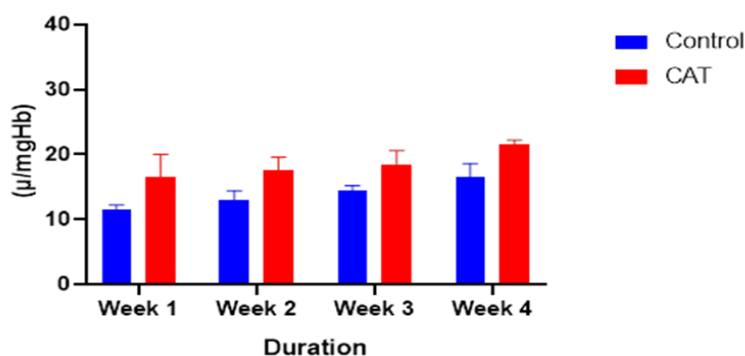


Figure 1 Durational Changes in Catalase Activities in Anthocyanin Administration to Wistar Rats

From above figure, catalase activities increased with increase duration of administration of anthocyanin.

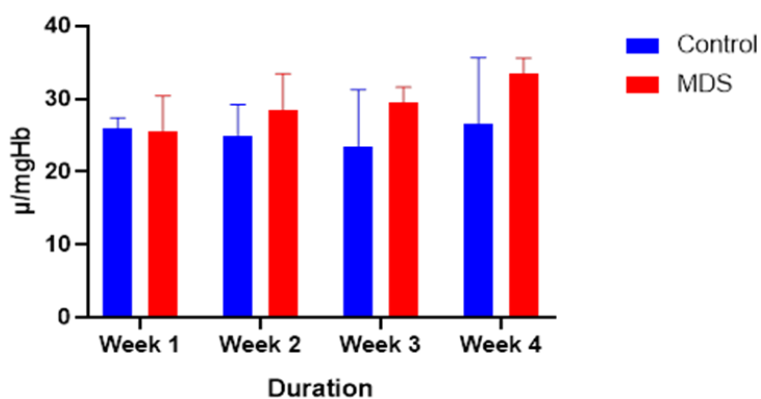


Figure 2 Durational Changes in Malonyldialdehyde (MDA) Activities in Anthocyanin Administration to Wistar Rats

Anthocyanin caused a duration dependent change in MDA activity compared with control group.

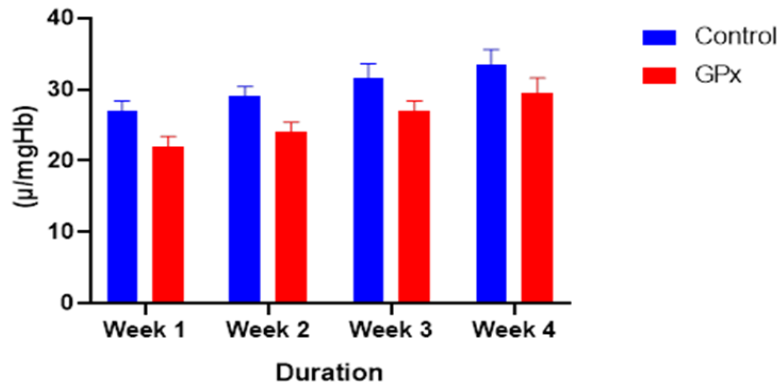


Figure 3 Durational Changes in Glutathione Peroxidase (GPx) Activities in Anthocyanin Administration to Wistar Rats

From above figure, GPx activity was duration dependent in the administration of anthocyanin.

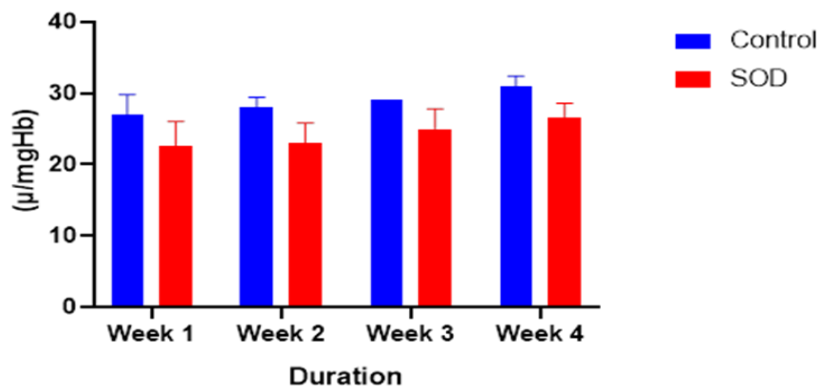


Figure 4 Durational Changes in Superoxide Dismutase (SOD) Activities in Anthocyanin Administration to Wistar Rats

From above figure, SOD activity is seen to increase with increased anthocyanin administration duration.

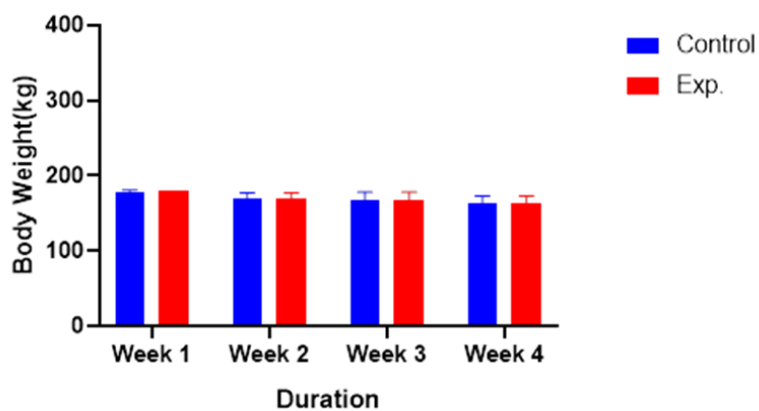


Figure 5 Durational Changes in Body Weight of Anthocyanin Administered Wistar Rats

From above figure, body weight change was duration dependent, though insignificant in the administration of anthocyanin on wistar rats over the test period.

4. Discussion

The proven abilities of plant flavonoids to promote health could be due to phytochemical constituents like anthocyanin, which reportedly boosts the antioxidant defence structure of the body system [11]. In this study, we investigated the effect of anthocyanin on selected serum antioxidant enzyme markers [Catalase (CAT), Malondialdehyde (MDA), Glutathione Peroxidase (GPx) and superoxide Dismutase (SOD)] in wistar rats.

Results from current study showed serum CAT level to significantly increase (at $p < 0.05$) in anthocyanin administered rats compared with control group. This observation was however duration and dose dependent, with average values of 11.00 μmgHb , 12.90 μmgHb , 14.20 μmgHb , 19.70 μmgHb and 24.70 μmgHb for group I through V respectively (Table I, Figure I). This finding corroborates the reports of Nichenametla et al., (2006), who posited that the phytochemical substances of high antioxidant activity include majorly the phenolic flavonoid compounds which anthocyanin apparently falls under [5]. The implication of this is that, in oxidative stress exposed organisms where serum antioxidant marker enzyme depletes, catalase activities can specifically receive a boost (increase) by incremental dose administration of anthocyanin over a period of time.

In a similar development, MDA, GPx and SOD results from current study increased significantly with the administration of increasing doses of anthocyanin to wistar rats over the period of the experiment. To this point, average value ranged from 25.02 μmgHb , 27.29 μmgHb , 22.26 μmgHb , 25.06 μmgHb and 29.73 μmgHb for MDA groups I – V respectively, 26.77 μmgHb , 28.49 μmgHb , 21.03 μmgHb , 23.07 μmgHb , 26.32 μmgHb for GPx activities across groups I-V treatments respectively, and 25.09 μmgHb , 29.60 μmgHb , 20.16 μmgHb , 25.06 μmgHb , 22.19 μmgHb for groups I-V SOD respective activities. The implications of this is that at any instance, durational and/or incremental dose administration of anthocyanin to oxidatively stressed animals may improve MDA, GPx and SOD marker activities to ameliorate the effect of the stress. The possible mechanisms of action of these enzymes is by scavenging and eating up supposed free radicals that are often released as by-products of metabolic activities and / or tissues breakdown resulting from stress. This concurs with the view of Rice-Evans et al., (1996).

Again, figure V of current study shows the effect of varying doses of anthocyanin administration to wistar rats on the body weights (g) of over a period of four (4) weeks of treatment. Here, a significant loss in body weight (g) in all experimental groups (CW fed rats) is observed when compared with control. This implies that treatment with anthocyanin caused a duration and dose dependent increase in body weights (g) of extract treated groups as against control.

5. Conclusion

For most treatment groups I current study, anthocyanin administration on wistar rats caused a significant increasing effect on the serum antioxidant enzyme activities, even though this action was duration and/or dose dependent. Body weight changes in treatment groups were also significantly improved with anthocyanin administration at incremental doses.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest among all the authors.

Statement of ethical approval

Ethical approval was sought from the Research and Ethics Committee of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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