EFFECTS OF AFRICAN BLACK TEA EXTRACT ON RATS

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Abstract：In this investigation, the antioxidant effectiveness of polyphenol compounds, such as theaflavin and EGCG (epigallocatechin gallate) found in African black tea (ABT) extract from Kenya, was examined in rats. The rats were divided into 2 groups, with both groups being exposed to a positive air ion environment for two hours in a day. One group was given distilled water and the other was given ABT water. When ABT group was compared with distilled water group, blood glucose levels and lipid peroxidation levels in cerebellum, cerebrum and blood were significantly decreased. Additionally, 3 groups were studied and results were compared: one group was ABT group; one group was distilled water group; and one group was control group which was given distilled water and placed in an environment where they were not exposed to positive air ions. At the investigation, a lowering effect on lipid peroxidation levels in cerebrum lipid tissue was observed in the ABT group when compared with the distilled water group and the control group. In addition, a significant increase of lymphocyte counts from the spleen tissue was observed in the ABT group. From the data obtained, results of this investigation suggested that drinking ABT has advantageous effects on living bodies.

Introduction

Recently, the increase of positive air ions due to unhealthy environments, such as highly airtight homes, synthetic paint for building materials and home appliances are marked as causes of sick house syndrome.

Positive air ions are largely found in air polluting car exhaust fumes, tobacco smoke and electromagnetic waves from home appliances, and when positive air ions are absorbed into a living body, they reduce the number of beneficial negative air ions by neutralization. Then, neutralized unstable ions in living organisms bind with inhaled oxygen molecules to create harmful active oxygen that promotes oxidation of body tissues.

Also, food additives and pesticides are considered to be health hazards. Active oxygen generated by such alleged health hazards increases lipid peroxide in blood and the brain and oxidizes cells contributing to various illnesses.

Health food has a high public profile now and a wide variety of health food products are now on the market. Among the variety, some have small effects and others have no effect on promoting a healthy body. Thus, it is difficult for consumers to determine which products are truly beneficial. African black tea (ABT) studied in this investigation is known as an effective agent for anti-oxidation, antiviral action, influenza infection prevention, α-amylase inhibition (inhibition of excessive nutrient uptake), liver cancer growth-inhibition in rats, cavity prevention, antibacterial activity and inhibition of food poisoning.

Polyphenol compounds, such as epigallocatechin gallate (EGCG) included in African black tea (ABT), appear to be powerful agents for the antioxidant activity, and this investigation examined its effectiveness on rats.

In this investigation, two groups of rats were prepared in a positive air ion environment. One group was given distillated water while the other was given extract of ABT dissolved in water. Rats in both groups were examined for lipid peroxidation levels in their body tissues, such as the brain, and blood glucose level for data comparison. The result indicated that ABT was effective in lowering peroxidation in cerebrum, cerebellum and blood. It also contributed to lowering blood glucose levels and increasing lymphocyte cells from the spleen, suggesting beneficial effects on health.

Materials and Methods

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Experimental material

ABT leaves of *Camellia sinensis* were extracted by hot water (80℃) and sterilized at 90℃ for 1 hour. Thereafter, the extract was sprayed and dried at a temperature of 200℃.

Table 1 indicates primary components of ABT extract as a result of analysis.

Table 1: Primary components of ABT extract

Constituents	Concentration in extract of ABT 100g
Protein	11.7g
Caffeine	7.0g
Iron	4.36mg
Calcium	54.0mg
Sodium	30.7mg
Potassium	3.99g
Magnesium	265mg
SOD activity	$4.1x10^5$ unit/g
Tannin	30.7g
EGCG	19g
Zinc	103mg
Selenium	11μg

Experiment 1:

Lipid peroxide and glucose level measurement

Ten 7-week-old male Wister rats were bred in the same environment in order to regulate their body weight. The animals were divided into two groups of five with similar changes in body weight: group A (exposed to positive ions), group B (exposed to positive ions and given ABT).

Group A and group B were exposed to positive air ions for 2 hours a day for 3 months.

Ten rats were put in a corrugated plastic box (920×380×360) [mm]. The number of positive ions in the box was several million/cm^3. After completion of exposure, their body weight was measured, group A was given 15g per day of powdered feed and distilled water, and group B was given the same feed and ABT. The ABT concentration was 0.15%.

Experiment 2:

Lipid peroxide and lymphocyte measurement

Fifteen 7-week-old male Wister rats were bred in the same environment in order to regulate their body weight. The animals were divided into three groups of five with similar changes in body weight: group A (exposed to positive ions), group B (control), and group C (exposed to positive ions and given ABT).

Group A and group C were exposed to positive air ions for 2 hours a day for 49 days, and group B was made a control group.

Ten rats were put in a corrugated plastic box (920×380×360) [mm]. The number of positive ions in the box was several million/cm^3. After completion of exposure, their body weights were measured, groups A and B were given 15g per day of powdered feed and distilled water, and group C was given the same feed and ABT. The ABT concentration was 0.3%.

Procedure

1. Rats were weighed to determine their state of health.
2. Two groups of rats were subjected to a stay in their assigned environments for two hours a day. The process was continued for several months.
3. After the end of each process, the rats were fed with 15g of powdered food. Distilled water or ABT was administered *ad libitum*.
4. Blood samples were drawn from the rats' tails before feeding to determine glucose and lactic acid levels.
5. After completing the experiment, blood, livers, brains and spleens were removed from the rats. The brains were divided into cerebellum, brain stem and cerebrum. Thiamine levels in cerebellum, brain stems and cerebrum were measured. Peroxidation levels were measured in the cerebellum, brain stems, cerebrum, livers and blood. The spleens were then passed through a stainless wire mesh (#200). Splenocytes were obtained using Lympholyte-Mouse (Cedarlane Laboratories, Hornby, Ontario, Canada) [1] and red blood cells were removed by hypotonic lysis in ammonium chloride. Then lymphocyte level was counted.
6. Results were presented by mean and standard deviation (SD). Differences between groups were evaluated by ANOVA followed by a Scheff's test.

Experimental Conditions

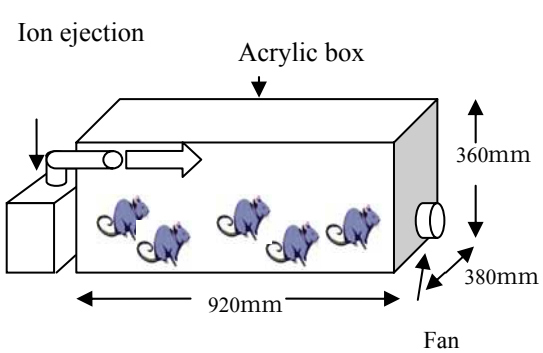

Figure 1: Diagrammatic illustration of the ion ejector

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An ion ejector, which can generate several million positive ions per 1 cm^3, was used. The positive ions were irradiated into a transparent plastic box (920mm×380mm×360mm). A net was set on the bottom of the box to prevent the rats from producing vitamins in the body. A fan was set in the wall to ventilate the box.

Blood lactic acid level measurement

Lactate-Pro Sensor (Arkray Inc.) was used to measure lactic acid levels in the blood.

Blood glucose level measurement

A Dexter-Z Sensor (Bayer Medical Inc.) was used to measure blood glucose levels.

Peroxidation level measurement in blood and brain lipids.

The measurement was based on the TBA Method using a spectrophotometer.

Thiamine level measurement

The measurement was based on the Thiochrome Fluorescent Method using a fluorescence spectrophotometer.

Lymphocyte counting in the spleen

Numbers of living cells were counted by the trypan blue cell exclusion assay, and ultimately, 5×10^6/ml concentration of lymphocyte suspension was prepared.

Results

Rat body weight change during the experiment

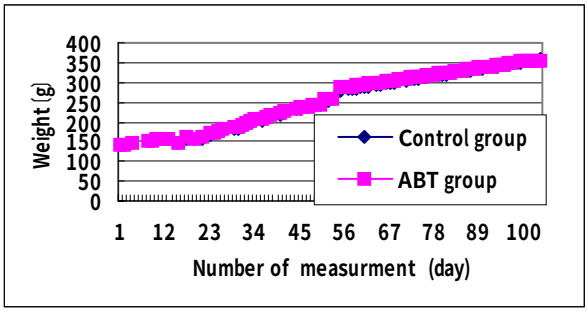

Figure 2: The average body weights of the rats during the experiment

Figure 2 shows the change of averaged body weights of 5 rats in the ABT group and the control group during the experiment. Any influence of the treatments on the state of health of the rats was not significant, because the average body weights in the two groups changed in the same way.

Blood Lactic acid level

Figure 3 shows average values and standard deviations of lactic acid levels of 5 rats in each group 3 months after the start of the experiment.

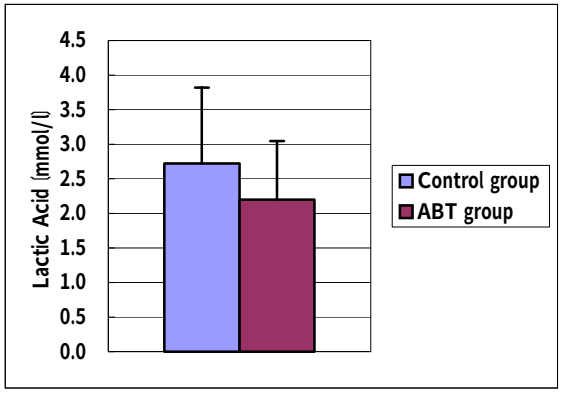

Figure 3: Lactic acid levels in the blood

As shown in Figure 3, the average lactic acid level in the ABT group was lower than the average in the control group. But there was no significant difference between the two groups.

Blood glucose level

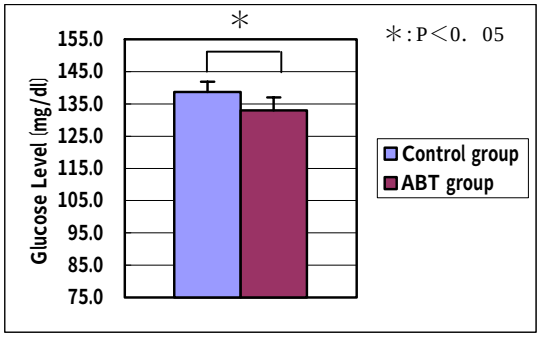

Figure 4: Glucose levels in the blood

Figure 4 shows average values of glucose levels and standard deviation of the 5 rats in ABT group and control groups over 3 months. As shown in Figure 4, the average glucose levels in the ABT group were significantly lower than the average in the control group.

Thiamine levels in tissues

Figure 5 shows average value and standard deviation of thiamine levels of 5 rats in each group in brain tissue.

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As shown in Figure 5, the average thiamine levels in the brain stem in the ABT group was significantly higher than the average in the control group.

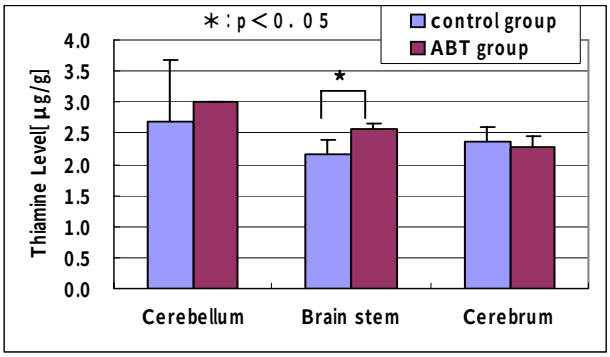

Figure 5: Thiamine levels in brain tissue

Peroxidation levels in the brain, liver and blood

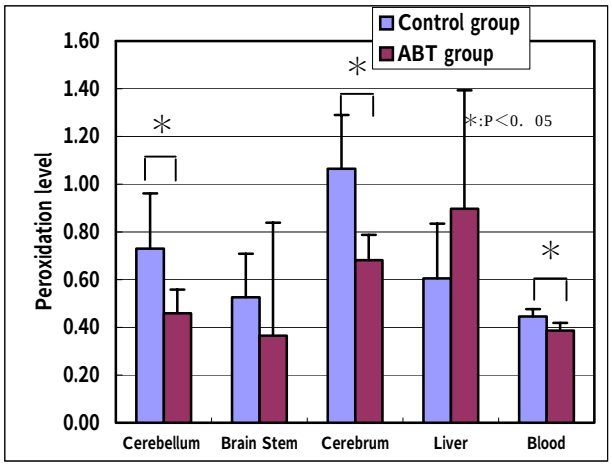

Figure 6: Peroxidation levels in tissues under different ion environments

Figure 6 shows average value and standard deviation of peroxidation levels in the cerebellum, brain stem, cerebrum, liver and blood of the 5 rats in each group, respectively.

As shown in Figure 6, in the average levels of peroxidation in the cerebellum, cerebrum, and blood in the control and ABT group, the latter was significantly lower. ($P < 0.05$). In the brain stem and liver, a significant difference was not observed.

Peroxidation levels in the brain

Figure 7 shows average peroxidation levels in tissues of 5 rats under different ion environment.

As shown in Figure 7, the peroxidation levels in the cerebrum of rats given ABT were significantly lower than that in rats in the positive ion group and control group.

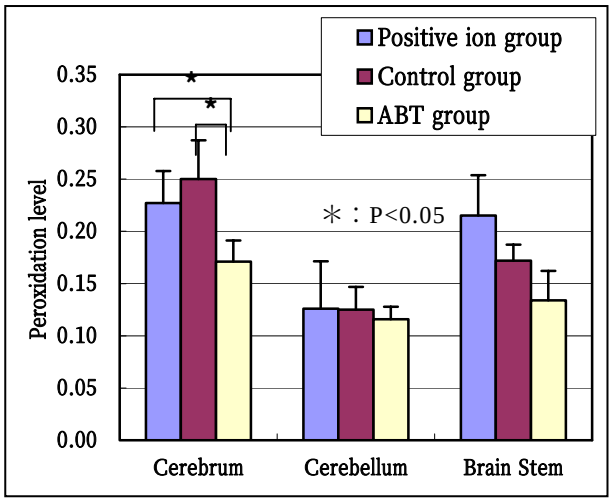

Figure 7: Peroxidation levels in tissues under different ion environments

Lymphocyte levels isolated from the spleen

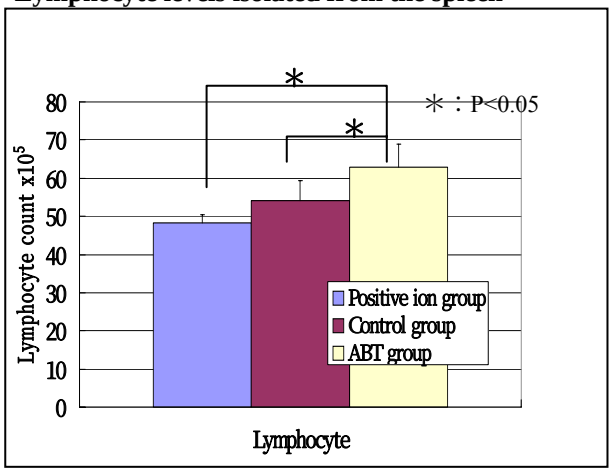

Figure 8: Number of lymphocytes isolated from the spleen

Figure 8 shows average value and standard deviation of lymphocytes count isolated from the spleen of 5 rats in each group.

As shown in Figure 8, the lymphocyte count in the ABT group was significantly higher than that in the positive ion group and control groups.

Discussion

Previous studies have suggested that when rats were placed in the positive air ion environment created by a positive air ion ejector, hepatic vitamin B_1 (thiamine) levels were significantly decreased while lactic acid and brain lipid peroxidation levels were significantly increased. Likewise, exposures to the positive air ion environment significantly increased blood glucose levels compared to the control group.

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Correspondingly, previous studies have suggested that when rats were placed in the negative air ion environment created by a negative air ion ejector, hepatic vitamin B_1 levels were significantly increased when compared to the control group, while lactic acid, brain lipid peroxidation levels and blood glucose levels were significantly decreased when compared to the control group.

In addition, previous study results confirmed that in the negative air ion environment, lymphocytes were increased while helper T cells and killer T cells were significantly increased.

Previous studies have demonstrated that when rats were exposed to a positive air ion environment using a positive air ion ejector, the vitamin B_1 levels in their livers were significantly decreased and lactate acid levels together with brain lipid levels were significantly increased in comparison with non-exposed control groups. Studies also have revealed that blood glucose levels were significantly increased in a positive air ion environment.

In this investigation, extract of ABT was used to test its effectiveness: it has been reported that ABT contains a large amount of polyphenol which is about 770 times more than that in red wine. In addition, ABT is known for its effect in lowering sugar absorption in the intestine.

From this perspective, the following results were revealed by this investigation on rats.

No weight change was observed between the positive air ion exposure group and the control group, indicating that rats in both conditions were in good state of health.

Even with the positive air ion exposure, lipid peroxidation levels were decreased in blood, cerebellum and cerebrum in the ABT group when compared with the control group, indicating strong effects of active oxygen removal performances of the ABT.

An interesting variation recognized as a result of this investigation was that negative air ions lowered lipid peroxidation in brain stems while ABT did the same. The brain stem is responsible for parasympathetic and the automatic nervous system and cerebrum is mainly responsible for memory. From this fact, ABT may be effective against cognitive impairment.

Furthermore, increased vitamin B_1 levels in brain stems may imply sense organ activation since sensory receptors are grouped in the hypothalamic area of the brain stem.

In addition, significant increase of lymphocytes in parasympathetic nerves might lead to beneficial activities of peroxide inhibition, cell excretion and secretion. This cell activation might increase insulin secretion resulting in decreased blood glucose level.

The results of this investigation significantly revealed the beneficial effects of long-term ABT intake on living body.

Conclusion

In this experiment, the long-term consumption of ABT lowered blood glucose levels and lowered peroxidation of cerebral lipids in rats. Also, the increased number of lymphocytes isolated from the spleen suggested there were advantageous effects of ABT on the immune function.

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