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Effect of Ethanolic Leaf Extract of *Kalanchoe pinnata* on Serum Creatine Kinase in Albino Rats

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The effect of ethanolic extract of fresh leaves of *Kalanchoe pinnata* on the level of cretatine kinase was investigated using albino rats. The extract with ethanol yielded 7.80%. The albino rats were treated with doses of 200mg/kg and 400mg/kg body weight of the extract for seven days. Results showed that there was a slight decrease in physical activities and body weight of all the animals treated with the ethanolic extract compared to the control. There was a significant ($P > 0.05$) increase in serum creatine kinase activity in albino rats treated with the ethanolic extract than the control. Within the groups treated with the ethanolic extract, Group A (400mg/kg body weight) was significantly ($P > 0.05$) higher than Group B (200mg/kg body weight). This increase value of creatine kinase activity could encourage the supply of energy needed for muscular contraction

Keyword: *Kalanchoe Pinnata*, Creatine Kinase, Ethanolic, Albino Rats, Serum

1. Introduction

Traditional medicine represents a part of Nigeria's heritage and deserves greater recognition of its importance to our history[1]. The retention of medicinal practices and traditions is evident mainly in the rural communities. To a lesser extent, this is also evident in urban communities. With modern technology, medicine has moved from a purely traditional phase to high-technological production of synthetic chemicals and the extraction of chemicals from plants to produce drugs. Several of these drugs are derived from the plants that form the basis of traditional medicine[1]. It was the use of certain plants for medicinal purposes by people like the Maroons that prompted scientists to study these plants in order to ascertain the usefulness of their components for the production of drugs[2]. The importance of traditional medicine has gained support from the World Health Organization and other international body that have been actively promoting it on a global level. This made such an impact that the herbal medicine market has been revitalized and seems to be a new option rather than an afterthought for the treatment of various ailments and diseases[3].

Medicinal plants are plants in which one or more of their organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[4]. The World Health Organization consultative group that formulated this definition stated also that such a description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as

medicinal but which have not yet been subjected to a thorough scientific study.

Kalanchoe pinnatum that belongs to the Crussulaceae family is a smooth skinned more or less erect fleshy herb[5]. It grows up to about 1-2m tall. Its lower leaves are simple and the upper complex in formation. Its flowers are light green (and turn yellowish) and dull brownish red.

The mucilaginous leaves of the plant are pressed or boiled and its juice used for relief of stomach pains; the mucilage is also thought to be useful in the healing ulcers. The pulp of the crushed leaves is also used as a poultice that is applied over the stomach and the crushed leaves are applied to the forehead to relieve headaches. It also has been used for hypertension and treatment of sore feet, but generally it is most commonly used for treating the common cold[6]. The leaves are boiled in water and the extract is given as a sedative for asthma and palpitation[7].

Creatine kinase (CK), also known as phosphocreatine kinase (or CPK) is an enzyme that is mostly predominant in the skeletal and cardiac muscle that plays an important role in providing energy for contraction in skeletal and cardiac muscles[8]. The normal function of creatine kinase in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. Phosphocreatine is burned as a quick source of energy by cells[9].

Creatine kinase catalyzes the following reaction:

Creatine kinase is widely distributed and seems to be primarily concerned with ATP regeneration. Creatine kinase may represent 10-20% of muscles cytoplasmic protein[8].
The enzyme is dimeric and exists as three isozymes: MM (muscle), MB (hybrid), and BB (brain). Names indicate major tissue of origin. Since the MB isozyme has its highest concentration in heart muscle, its level in serum has diagnostic value[10]. Most investigation work has been on the soluble enzyme from rabbit muscle (There is also an insoluble CK associated with mitochondria).

However, the normal function of creatine kinase is not as relevant, in this case, as what happens to CK when muscle is damaged. During the process of muscle degeneration, muscle cells break often and their contents find their way into the bloodstream. Because most of the creatine kinase in the body normally exists in muscle, a rise in the amount of creatine kinase (CK) in the blood indicates that muscle damage has occurred, or is occurring[10].

2. Materials and Methods

2.1 Animals

Twenty four male albino rats were obtained from animal house in the faculty of Basic Medical Sciences, Delta State University, Abraka. The animals were divided into three different groups – A, B and C of eight animals per group and kept in a room illuminated for twelve hours per day at room temperature. The weights of the animals were measured on daily basis to determine the actual volume of the extract to be given to the animals. Oral administration was used throughout the experiment.

2.2 Preparation of Leaf Extract

Fresh leaves of *Kalanchoe pinnatum* was collected from Abraka in Ethiope East Local Government Area of Delta State. The method of extraction used by Duncan[11] was adopted. 200g of the fresh leaves of *Kalanchoe pinnatum* were cut/sliced into small pieces and soaked in 500ml of ethanol for 48 hours. 435ml of the dark-green solution was obtained after decantation. The ethanolic extract was then evaporated by heating on a water bath to get a jelly-like residue. 2g of the extract was dissolved in 20ml of distilled water given rise to 0.1g/ml concentration which is due or required to administer to the rats.

2.3 Administration of Extract to Animals

The albino rats were fed with poultry feed and water for five days for acclimatization, the first group (Group – A) of the rats were fed with

poultry feed, water and an oral administration of the organic extract of *Kalanchoe pinnatum* leaves with a dose of 400mg/kg body weight daily for seven days. The second group (Group – B) were fed with poultry feed, water, and an oral administration of the organic extract of *Kalanchoe pinnatum* leaves with a dose of 200mg/kg body weight daily for seven days. The third group (Group – C) were fed with poultry feed and water only for seven days.

2.4 Collection of Blood from Animals

After seven days of administration of the extract to the albino rats, they were starved for a day (24 hours). Subsequently, blood samples were collected from them (albino rats) after decapitation. The blood samples were collected into sterile specimen bottles free of anti-coagulant. The blood was centrifuged at 3000 Xg for 10 minutes and allowed to stand. The supernatant (serum) was decanted.

2.5 Determination of Creatine Kinase Activity

The method of Duncan[11], as described by Saha[12] was adopted. 2.5ml of substrate (0.4M glycine + 0.03M creatine phosphate + 0.062M K_2CO_3); pH adjusted to 8.9 with NaOH and 0.1ml of sample were mixed and incubated at 30°C for 2 minutes. Absorbance was read 0, 1, 2 and 3 minutes at 340nm against the blank.

3. Results

Table 1: Percentage Yield of Extraction of Fresh Leaves of *Kalanchoe pinnatum*

Plant Part	Weight of Plant Before Extraction (g)	Weight of Plant After Extraction (g)	Weight of Dried Extract (g)	Percentage Yield (%)
Leaves	200.00	184.00	15.60	7.80

Table 1 above shows that the extraction carried out with ethanol yielded 7.80%. This low value suggests that most of the chemical components of *Kalanchoe pinnatum* are only slightly soluble in ethanol.

Table 2: Average weight(g) of animals during seven days of extract administration and a day of starvation

Days	Group A	Group B	Group C (Control)
1	82.50 ± 5.00	88.75 ± 4.79	95.00 ± 10.80
2	82.50 ± 5.00	87.50 ± 5.00	93.75 ± 9.81
3	82.50 ± 5.00	87.50 ± 5.00	91.25 ± 11.09
4	82.50 ± 5.00	86.25 ± 2.50	91.25 ± 11.09
5	82.50 ± 5.00	85.00 ± 7.07	91.25 ± 11.09
6	81.25 ± 4.79	85.00 ± 4.08	90.00 ± 10.80
7	81.25 ± 4.79	85.00 ± 4.08	90.00 ± 10.80
8	72.50 ± 5.00	77.50 ± 5.00	81.25 ± 14.46

Values are mean ± standard deviation

From Table 2 above, it was observed that the average weight of the animals in the test groups is not significantly different from that of the control group.

Table 3: Average enzyme activity and average enzyme specific activity in serum after seven days of administration of extract

Animal Group	Time of incubation (min)	Average Enzyme Activity (Mmol/hr/ml)	Average Enzyme Specific Activity (Mmol/hr/ml/protein)
A	1	44.37 ± 8.51	0.22 ± 0.00
	2	45.91 ± 2.60	0.23 ± 0.00
	3	47.91 ± 0.69	0.24 ± 0.00
B	1	41.27 ± 3.87	0.21 ± 0.00
	2	43.85 ± 2.60	0.22 ± 0.00
	3	43.68 ± 3.95	0.22 ± 0.00
C	1	19.61 ± 3.95	0.09 ± 0.00
	2	20.64 ± 4.46	0.10 ± 0.00
	3	20.64 ± 3.73	0.10 ± 0.00

NB: values are mean ± standard deviation

4. Discussion

The extraction of the fresh leaves of *Kalanchoe pinnatum* with ethanol yielded 7.80% (Table 1). This low value suggests that most of the chemical components of the leaves are only slightly extractable with ethanol. During the administration of the ethanolic extract of fresh leaves of *Kalanchoe pinnatum* to the albino rats, there was a general decrease in food and water intake. The mechanism is yet unknown. However, it could be due to metabolic upset in the albino rats treated with ethanolic extract. The slight general decrease in physical activities and

body weights (Table 2) of the rats administered with the ethanolic extract could be as a result of reported decrease in food and water intake by the rats (Table 2).

The mean creatine kinase specific activity in the serum of the test groups were found to be significantly (P>0.05)) higher than those of the control group. This increase in mean specific activity of creatine kinase in serum of the test groups may be as a metabolic response in association with the ingestion of the extract. However, physiological factors and some disease states like myocardial infarction may lead to a fluctuation in the activity/specific activity of the enzyme because of the leakage of creatine kinase into the bloodstream when a muscle is damaged[13].

Moreover, the activities of creatine kinase in serum were found to be significantly (P>0.05) higher in the albino rats treated with a dose of 400mg/kg body weight of the extract than those treated with a dose of 200mg/kg body weight. This suggests that the extract appear to elicit higher effect at higher doses. However, the actual; dose that could elicit higher effect has not been established. The precise mechanism of the extract is not yet well understood.

These increased values of creatine kinase activity in the albino rats treated with the ethanolic extract could encourage the supply of energy (ATP) needed for muscular contraction and relaxation, which in the case of asthma brings about dilatation of the constructed smooth muscles of the bronchi. It was reported in an *in vivo* study (with rats and guinea pigs) that the extract was able to protect against asthma by selectively blocking histamine receptors in the lungs and this could be altering (increased) the activity of creatine kinase[14].
Creatine kinase is found predominantly in the heart, brain and skeletal muscle and when the total creatine kinase level is substantially elevated and it usually indicates injury or stress to one or more of these organs[15]. This could be responsible for the significant (P>0.05) increase of creatine kinase in the test groups than the control group.

Raised level of creatine kinase has serious implications for animals administered with the organic extract. Such elevations are in cases of both myocardial infarction and other cardiovascular diseases[13]. The elevation of creatine kinase makes the muscle a target of suspicion as this is the usual pattern in cases of cardiovascular toxicity caused by toxic agents[16].
The use of crude extract limits the extent conclusions could be drawn. Thus, only speculative conclusions were made. The use of purified extract from the leaves and other plant parts is worthwhile. It is therefore suggested that a low dose of the plant extract should be used as medicine having known the adverse effect of the plant to the consumers.

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