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Creatine Kinase Activity In Albino Rats Administered With Ibuprofen (ANALGESIC)

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Abstract: *Ibuprofen, 2-(4-isobutylphenyl) propanoic acid is an analgesic used in the treatment of different types of body pains. The effect of the drug on creatine kinase activity in albino rats was investigated. The twenty (20) albino rats were divided into five groups (A, B, C, D and E) with four rats each. Group A, B, C and D were treated orally with 0.4, 0.8, 2.4 and 3.6mg/kg body weight of Ibuprofen drug sample respectively for seven consecutive days while group E acted as the control. The average body weight decreased in the test groups while control group gained weight. The treatment of animals with the sample did not produce any significant difference in total protein concentration of the treated and control groups. The creatine kinase levels in the serum of the treated groups were significantly higher than in the control. The effect of creatine kinase level was found to be dose dependent.*

Keywords: *Ibuprofen, creatine kinase, total protein and analgesic.*

I. Introduction

According to World Health Organization (1966), drug is any substance taken in order to explore or modify physiological or pathological state for the benefit of the recipient.

Drug use is as old as mankind itself. Human beings have always had a desire to eat or drink substance that make them feel relax, stimulated or euphoric. Human have used drugs of one sort or another for many years. Iodine was used at least from the time of early Egyptians, narcotics from 4000BC and medical use of marijuana has been dated to 2737BC in China (Baldwin, 2000).

As time went by, home remedies were discovered and used to alleviate aches, pains and other ailments, most of these preparations were herbs, roots, mushroom and fungi. They could be eaten, drunk, rubbed on the skin, inhaled to achieve the desire effect. The term Analgesics (colloquially known as painkiller) is any member of the diverse groups of drugs used to relieve pain and to achieve analgesia (Green, 2001).

This is derived from Greek word an-, "without," and algia, "pain"

Analgesic drugs act in various ways on the peripheral and central nervous system. They include paracetamol (acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDS) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol and various others.

Analgesic is a drug that relieves pains selectively without blocking the conduction of nerve impulses, markedly altering sensory perception, or affecting consciousness. This selectivity is an important distinction between an analgesic and anesthetic

Analgesics may be classified into two types:

Anti-inflammatory drugs which alleviates pains by reducing local inflammatory responses and the opiod which act on the brain. The opiod analgesics were once called narcotic drugs because they can induce sleep. The opiod analgesics can be used for either short term or long term relief of severe pain. In contrast, the anti-inflammatory compounds are used for short term pain relief and for modest pain, such as that of headache, muscle strain, bruising and arthritis (Floyd, 2013).

Pain can be defined in many ways. It is usually described as a distressing sensation in the body system or, an unpleasant or hurtful sensation resulting from the stimulation of nerve endings by noxious stimulus/stimuli resulting to gross tissue damage. However, pains can also be explained as a mental or emotional suffering or torment. Various daily activities and actions may cause pain and aches like headache, muscle cramp or a pinch from someone or engaging in strenuous exercises that can lead to muscle aches. Occasionally, pain also occurs through serious injuries and illnesses such as sore throats (Backonja and Rowbotham, 2011).

Ibuprofen ($C_{13}H_{18}O_2$) is a painkiller which is available over the counter, without a prescription. It is one of a group of painkiller called non-steroidal anti inflammatory drugs (NSAIDS). It is a derivative of propionic acid and can be used to:

- Ease wild to moderate pains- such as toothache, migraines and menstrual period pains.

- Control of fever (high temperature. also known as Pyrexia). For example when someone has a flu (influenza)
- Ease pain and inflammation (reduces swelling) caused by rheumatic disease (conditions that affect the joints) and musculoskeletal disorders. (conditions that affect the bone and muscles) such as rheumatoid arthritis and osteoarthritis.
- Ease pains and swelling caused by sprains and strains, such as sports injury,

Ibuprofen is made by many different companies under many different brand names and in a wide range of forms, including:

Tablets or caplets, gels sprays and liquids. In some products, ibuprofen is combined with other ingredients e.g. it is sometimes combined with a decongestant (a medicine for a blocked nose) and sold as a cold and flu remedy for e.g. Sudafed.

Ibuprofen works as a painkiller by affecting chemicals in the body called prostaglandins. Prostaglandins are substance released in response to illness or injury. They cause pain and swelling (inflammation). Prostaglandins that are released in the brain can cause a high temperature (fever). The painkilling effect of ibuprofen begins soon after a dose is taken, but the anti-inflammatory effect is weak and will take longer to begin. It can sometimes take up to 3weeks to get the best results and ibuprofen should not be used to treat conditions that are mainly related to inflammation. Ibuprofen can cause side effects such as nausea and vomiting.

Ibuprofen should be avoided by people with certain health conditions such as a current or recent stomach ulcer or a history of bad reaction to NSAIDS. It should be used with caution by older people and people with certain health conditions, including asthma or kidney or liver problems. Ideally pregnant woman should not take Ibuprofen unless recommended by a doctor. But Ibuprofen appears in breast milk in small amounts, so its unlikely to cause harm to your baby while breastfeeding (Schmitt and Barton, 2005).

Creatine Kinase is an enzyme that catalyses the following reaction.

ATP + creatine < = > ADP + Phosphocreatine.

Creatine kinase is an enzyme or type of protein that is found in several tissue types of the human body, including the heart, muscle and the brain. The function of this enzyme is to catalyze the conversion of creatine to phosphocreatine by applying itself in the consumption of adenosine triphosphate and the generation of adenosine diphosphate, and it is a reversible reaction. Adenosine triphosphate is a vital source of energy in biochemical reactions. In the skeletal muscle, the brain and the heart or all tissue that swiftly use up adenosine triphosphate. Phosphocreatine acts as an energy reservoir for the quick regeneration of adenosine triphosphate (Richard , 2003).

Aims and Objectives

This research was designed to determine the cardiovascular effect of ibuprofen in albino rats by measuring creatine kinase activity in its cardiovascular system using ibuprofen analgesics.

II. Materials And Methods

Collection of Albino Rats

Twenty albino rats were purchased from the Biochemistry Department of the University of Nigeria Nsukka (U.N.N) and were transported down to Abakaliki, to the Biochemistry Laboratory of Ebonyi State University, Abakaliki.

Collection of drug sample

Ibuprofen drug was bought from Jabera pharmacy, Abakaliki, Ebonyi State.

Preparation of Drug Sample

8g of Ibuprofen was dissolved in 300ml of distilled water to obtain concentration of 26.67mg/ml

Animals (Rats) Handling and Treatment

The animals were allowed for days to acclimatize and were placed in five (5) groups which consist of four (4) rats each. All the animals were given water and growers mash.

Animal grouping

The animals were grouped in five cages, four rats per group and were labeled (A, B, C, D and E). All animals were given water and growers mash and also were acclimatized for seven days prior to commencement of administration.

Measurement of the Weight of the Animals

The weight of the animals was taken daily using a weighing balance. The results obtained were used to monitor weight changes and the volume of the sample to be administered to each of the animals.

Administration of Drug Sample

The samples were administered orally to the animals using 2ml syringe. The animals in group A, B, C, D were given 0.4, 0.8, 2.4 and 3.6mg/kg body weight respectively, while the animals in group E (control) were given distilled water and growers mash.

Collection of Samples from the Animals

The animals were starved for 24 hours after seven days of treatment with the drug sample and their blood samples were collected by cardiac puncture into a sterile bottle. The sterile bottle was free of anticoagulants.

Preparation of Reagents

Copper Reagent

Copper reagents were prepared by mixing the solution of 20g of Sodium Carbonate in 260ml of distilled water, solution of 10.4g of Copper Sulphate ($CuSO_4$ $5H_2O$) in 20ml of distilled water and 0.2g of Potassium Carbonate titrated with 20ml of distilled water.

1% SDS Solution

1g of SDS was dissolved in 100ml of distilled water

Lowry's Concentrate

The reagent was prepared by mixing 30ml of copper reagent, 10ml of SDS solution and 10ml of 1M solution of NAOH.

Substrate Buffer

0.4M glycine was mixed with 0.03M creatine phosphate and followed by the addition of 0.062M potassium carbonate (K_2CO_3) and the pH was adjusted to 8.9 with sodium hydroxide NAOH.

0.2N Folin Reagent

10ml of 2N folin reagent was mixed with 90ml of distilled water. This solution is stable for several months at room temperature if stored in an amber bottle.

Protein Determination

Protein levels in the serum were determined by the method of (Lowry, 1951) using bovine serum albumin as a standard 0.4ml of serum was mixed with 0.2ml of 2x Lowry concentrates and incubated at 37°C for 10minutes before adding 0.2ml of 0.2N folin reagent. The mixture was incubated further at 37°C for 30 minutes. Absorbance was read at 750nm against a blank using a spectrophotometer.

Determination of Creatine Kinase activity

Assay reaction

(1) This reaction can be either be kept on a room temp or 37°C. Prepare enough of the reconstituted reagent for each sample to be tested according to the scheme. Each sample requires 100µL of reconstituted reagent.

Reagent	Volume
Assay butter	100µL
Substrate solution	10µL
Enzyme mix	1µL

2. Transfer 11µL of water into one well (Blax) and 100µL of water plus 10µL of 96 well plate.

3. Transfer 10µL of sample into seprated weel. Add 100µL of the reconstituted reagent to each sample well and tap plate to mix.

4. Incubate the sample at either room temperature or 37°C after 0 mins and take the initial absorbance measurement at 340nm.

Note CK is fully activated within 20mins by the glutathione present in the substrate solution.

5. Continue to incubate the plate at room temperature or 37°C for 20 additional minutes, measure the absorbance (A_{340}) final.

Statistical Analysis

Data were expressed as mean and standard deviations. The mean and standard deviations obtained were subjected to statistical analysis using analysis of variance (ANOVA) statistical significance was assumed at $p<0.05$.

III. Results

Physical Observation

The rats in group A, B, C and D showed decrease in physical activities as well as in feed and water intake while the animals in the control group showed otherwise.

Average Weight (g) of Animals during Seven Days of Ibuprofen Sample Administration

The result of average weight (g) of albino rats treated with ibruprofen for seven days is shown in table 1. There was a significant decrease in the average weight of the treated animals in groups A and B when compared to group E that showed no-significant decrease in weight.

Day	Group A	Group B	Group C	Group D	Group E
1	154.00±33.20	131.25±18.79	93.75±18.80	106.25±18.78	112.50±25.00
2	125.00±36.20	131.25±18.79	93.75±18.88	118.75±18.75	108.75±57.50
3	125.00±36.20	100.00±25.00	87.50±25.00	81.25±18.75	106.25±62.50
4	112.50±12.50	106.25±37.50	100.00±25.00	87.50±25.00	93.75±62.50
5	100.00±25.00	87.50±37.50	100.00±25.00	100.00±0.00	93.75±18.75
6	118.75±18.75	106.25±37.50	87.50±25.00	81.25±37.50	100.00±50.00
7	106.25±18.75	105.25±50.00	100.00±12.50	87.50±25.00	100.00±50.00

Table 1: Mean weight changes ±standard deviation

All values are mean ± standard deviation

Table 1 shows that the mean values of the test groups are significantly decreased unlike that of the control group.

Average Protein Concentration, Average Enzyme Activity and Average Specific Enzyme activity in Serum of Albino Rats after Seven Days of Administration of Sample and a Day Starvation.

Table 2 shows the result on enzyme activity, specific enzyme activity and total protein concentration. The protein concentration of test groups did not differ significantly from that of the control. Conversely, there was a significant difference between the specific enzyme activity of the treated groups and the control.

Table 2: Average Protein Concentration, Average enzyme Activity and Average Specific Enzyme Activity

Animal groups	Average enzyme activity (mmol/ml/protein)	Average protein concentration (mg/ml)	Average Specific enzyme activity (µ/l/mg/ml)
A	173.79±5.63	0.43±0.07	404±22.08
B	190.71±5.29	0.39±0.01	492.64±23.88
C	220.22±15.19	0.33±0.01	600.36±36.57
D	277.81±4.80	0.25±0.02	530.05±74.83
E	170.07±7.40	0.55±0.04	247.67±23.28

Values are mean ± Standard deviation

Table 2: Above shows that the average enzyme activity and average specific enzyme activity in the serum of the test group were found to be higher than those of the control group while the protein concentrations of the test animals were lower than that of the control.

IV. Discussion

Administration of ibuprofen sample resulted in a slight decrease in physical activities of animals in groups A, B, C and D compared to the control. The actual biochemical mechanism behind this reduction in agility is still not known at the level of this research. However, it could be as a result of metabolic upset in general body mechanism as a result of the administration of the sample. This observation is in line with that made by Murphy (2000) in his administration analgesics to mouse.

There was also reduction in food and water intake by animals administered with the sample. The actual biochemical reason for this loss of appetite is not clearly known at the stage of this research, and however some drugs have shown to reduce appetite (Murphy, 2000).

There was also a reduction in food and water intake by the animals administered with the sample. The actual biochemical reason for this loss of appetite is not clearly known at the stage of this research, and however some drugs have shown to reduce appetite (Murphy, 2000).

The average body weight of the animals treated with the sample decreased significantly during the days of administration while the control group showed no significant difference and the reason for this is a subject for further biochemical research. The actual mechanism to support the loss of weight is not yet known but it may be due to the reported decrease in food and water intake caused by metabolic responses of animals. This is consistent with work of Murphy and Peger (2007).

The protein analysis carried out on the serum revealed no significant difference ($p<0.05$) in average protein concentration levels between the test groups and the control. The samples may have little or no effect regulating or controlling the rate of synthesis and degradation of protein. this is in line with the work of Pager (2007).

The creatine kinase activities in serum of Albino rats in the treated groups were found to be significantly higher ($p<0.05$) than those of the control group. The actual biochemical mechanism for this is still unknown at this level of research. However, many analgesics have been reported to have adverse effect on the enzyme level. The high level of creatine kinase is the serum of the treated animals might be due to cardiovascular toxicity which could be as a result of physiological response in association with the injected sample and it was also found to be dose dependent. Similar observation was made by Okazaki (2003).

The average specific enzyme activities in serum of albino rats in the test groups were found to be significantly higher ($p<0.05$) than those in the control group. This could also be a result of metabolic response in association with the ingestion of drug samples. Also among the treated groups, the specific creatine kinase activities were significantly higher ($p<0.05$) in the groups dependently of the dosage. This could be as a result of the leakage of the enzyme from damaged tissues. 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 blood stream when a muscle is damaged (Murphy, 2000).

Agarwal and Anki-Badu (1999) also reported the importance of creatine kinase in the diagnosis of myocardial infarction. They found out that the measurement of the serum concentration of creatine kinase by the creatine kinase test provide information about the security of the damage. Myocardium damage facilities the blood stream, hence leading to a rise is serum creatine kinase.

Enzyme assay for clinical diagnosis depend on the measurement of the catalytic activity of the enzymes and not on the concentration of the enzyme test provide information about the security of the damage. Myocardium damage facilities the blood stream, hence leading to a rise in serum creatine kinase.

Enzyme assay for clinical diagnosis depend on the measurement of the catalytic activity of the enzyme and not on the concentration of the enzymes assay condition such as temperature or physiological conditions. Therefore, measurements must however be optimized and standardized to give reliable and reproducible result. The serum activity of a intercellular enzyme is cleared from blood stream as well as when the cell injury subsides (Murphy, 2000).

Raised levels of creatine kinase have serious implication for the animals administered with drugs samples such elevation are in cases of both myocardial infarction and other cardiovascular disease (Murphy, 2000).

V. Conclusion

The observation made in this research indicates that Ibuprofen can be used for both treatment and prevention of different forms of pains but it has been found to raise the level of creatine kinase. In consideration of the issue that has been discussed in this research, care must be taken and the doses strictly maintained.

References

[1]. Backonja, A. A. and Rowbotham, C. E. (2011). Neurotherapy and pain associated with it. Pharmacological Therapy, **107(5):** 1 - 40.

[2]. Baldwin, E. R. (2000). *An Introduction to Comparative Biochemistry*. Cambridge University Press, New York, 270-279.

[3]. Body, R. P., Pemberton, P. N. and Jones, N. C. (2011). Low Soluble P-section may Facilitate Early Exclusion of Acute Myocardial Infarction. *Clinical Chemical*, 52: 614-618.

[4]. Bosh, F. R. (2008). The Contributions of Paul Ehirlich to Pharmacology. A tribute on the occasion of the centenary of his Noble Prize. Pharmacology, 82(3): 171-179.

[5]. Catella-Lawson, M. M. (2001). Interaction of Central Analgesic, Tramadol with the uptake and release of 5-OH triptamine in rat brain *invitro*. *British Journal of Pharmacology*.

[6]. Conagham, P. G. (2012). "A turbulent decade for NSAIDs update on current concept of classification, epidemology, and toxicity". *Rheumatology international*, 32(6):1491 - 502.

[7]. Dave, G. E. (2004). Mechanism of action of analgesic. Clinical Chimica. Acta, 514-518.

[8]. Fendrick, A. M. (2002). Assessing the gastrointestinal complications of over-the-counter analgesics. *American Journal of Family Practice Recertification*. 27:9-15.

[9]. Floyd, E. O. (2013).Analgesics and Pain. Annual Review of Public Health, 198(3): 90-101.

[10]. Garcia, B. V. Rodgriguiez and Jick, H. H. (2004). Comparism of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *Journal of Medical Sciences*.England 343 (21): 1520-8.

[11]. Glaxosmithkline (2009). Ibuprofen, an example of anti-inflammatory analgesics, definition and type of analgesics. *Glaxosmithkline Journal*, 347-376.

[12]. Graham, O. L. and Scott, L. T. (2005). Drug used in treatment of interstitial cystitis. Drug treatment in urology. 6th Edition. John and sons international press, United Kingdom.
[13]. Green, D. E. (2000). *Mechanism of Biological Oxidation*. Cambridge University Press, New York, 456-467.
[14]. John, W. P. (2008). *Medicinal Role of Analgesics Education*. Wiley and Sons Reference Guide to Symptoms., Common illness and Healthy Development 2nd ed. Bantam Books, New York, 553-568.
[15]. Johnstone, T. O. (2001). *Intramuscular injection and creatine kinase levels*. Cambridge University Press, 20: 30-40.
[16]. Kingston, U. W. (2008). Irish Contribution to the Origins of Analgesics. *Irish Journal of Medical Science*, 177(2): 87-92.
[17]. Kubacka. W. H., Sherry, O. P., and Thomas, F. U. (2006). Adverse interaction of Ibuprofen with anti-coagulants. 567-99
[18]. Larson, E. (2007). *Community Factors in the Development of Analgesics*. Annual Review of Public Health, 28: 435-447.
[19]. Lindblad, W. J. (2008). Considerations to Determine if a natural product is an effective wound- healing agent. *International Journal of Lower Extremity Wounds*, 7(2): 74-81.
[20]. Limbrid, L. E. (2004). The Receptor Concept: a continuing Evolution. *Molecular Literature*, 4(6): 326-336.
[21]. Millington (2011). *A review on Opioid Analgesics*. Bantam Books, New York, 2(5):20-28.
[22]. Mogensen, J. P. and Marylene, V. C. (2004). Clinical relevance of Creatine kinase in the heart muscle of a mature albino rats. *Cell* 128(6): 1037-1080.
[23]. Murphy, A. M. (2002). Transgenic Mou. se Model of Stunned Myocardium. *Science Journal*, 287(1): 488-491.
[24]. Pelezer, M. J. and Porter, J. R. (2004). An Introduction to Analgesics. *Journal of Medical Biochemistry*, 2(435): 17-21.
[25]. Peterson, E. T. (2013). Effects of non-steroidal anti-inflammatory drugs. *Irish Journal of Medical Science*, 23: 45-50.
[26]. Pi, Z. O., Gua, X. A. and Guan, J. P. (2011). Differentiation of Cardiosphere Derived Cells into a mature cardial linage using biodegradable poly (N-isoprophylacrylamide) Hydrogels. Biomaterials, 3220-3332.
[27]. Rainsford, K. D. (2003). Discovery, mechanisms of actions and safety of ibuprofen. *International Journal of Clinical Practice*, 20(5): 3-8
[28]. Richard, P. L. (2003). Hypertrophic Cardiomyopathy Distribution of Diseases, Genes, Spectrum or Mutation and Implications for a molecular Diagnosis Strategy. Circulation. *Science Journal*, 107(2): 22227-2232.
[29]. Russell, R. (2007). *Adjuvant analgesics: Definition and types of analgesics*. Bantam Press, New York, 30: 20-40.
[30]. Scheiman, J. M., and Fendrick, A. M. (2007). Summing the risk of NSAID th(1): therapy. Lancet. 369.1580-1.
[31]. Schmit, L. and Barton, D. (2005). Your Child's Health. The parents one-step Reference Guide to symptoms. Common Illness and Healthy Development, 2nd ed., Bantam Books, New York, 553-568.
[32]. Sorensen, H. T., Mellemkjaer, L. L., Blot, W. J., and Olsen, J. H. (2000). Risk of upp gastrointestinal bleeding associated with the use of low-dose aspirin. *American Journal of Gastroenterology*. 95 (55): 158-169.
[33]. World Health Organization (1966). An Introduction to Drugs. Cambridge University Press, New York. 10-14.