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Glutathione Peroxidase Activity in Albino Rats Treated With Starcimol Extra (Analgesics)

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Abstract: *The use of analgesic in the treatment/management of pains has been shown to produce several side effects. This research examined the effect of starcimol Extra on the glutathione peroxidase activity in albino rats. Twenty albino rats used in this research were separated and grouped into four (A, B, C and D), five rats per group. Groups A, B and C were treated orally with 22.5, 45.0 and 90.0mg/kg body weight of Starcimol Extra solution for seven (7) successive days, while group D served as the control. There was a decrease in body weight of the treated animals unlike the control which increased in body weight. There was increase in feed and water intake of the treated rats relative to the control. There was significant decrease (P< 0.05) in glutathione peroxidase activity of the treated animals compared to the control. There was no significant difference (P>*

0.05) between the total protein concentrations of the treated animals and the control. These effects of Starcimol Extra solution were found to be dose-dependent. The adverse effects of oral administration of Starcimol Extra may include lowering the antioxidant ability of the body.

Keywords: *Starcimol Extra, glutathione peroxidase, analgesic and diseases.*

I. Introduction

Pain is an unpleasant feeling often caused by intense or damaging stimuli, such as stubbing a toe, burning of fingers, putting alcohol on a cut. In medicine pain relates to a sensation that hurts, you feel discomfort, distress and distress and perhaps agony, depending on the severity of it. Pain can be steady and constant, in which it becomes an ache. It might be a throbbing pain- a pulsating pain. The pain could have a pinching sensation, or a stabbing one (Merskey and Bogduk, 2000).

Pain may be Acute, in which case it can be intense and short-lived. Acute pain may be an indication of an injury. When the injury heals the pain usually goes away. Chronic pain is also a type of pain in which its sensation lasts much longer than the acute pain. Chronic pain can be mild or intense (Harris, 2012).

Pain can be nociceptive in which case the specific pain receptors are stimulated. These receptors sense temperature (hot/cold). Vibration, stretch, and chemicals released from damaged cells. Nociceptive pain can be grouped into somatic pain, which is felt on the skin, muscle, joints, bones and ligaments. And visceral pain, which is felt in the internal organs and main body cavities. Also pain can be non-nociceptive which is divided into Nerve pain and sympathetic pain. Nerve pain comes from within the nervous system itself. People often refer to it as pinched nerve or trapped nerve, while sympathetic pain occurs generally after a fracture or a soft tissue injury of the limbs (Harris, 2012).

In pharmacology, a drug is a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being. Drugs may be prescribed for a limited duration or on a regular basis for chronic disorders. Recreational drugs are chemical substances that affect the central nervous system, such as opioids or hallucinogens. They may be used for perceived beneficial effects on perception, consciousness, personality, and behavior. Some drugs can cause addiction and/or habituation (Hals, 2003).

Analgesic is any drug that relieves pain 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 an anesthetic. Analgesics may be classified into anti-inflammatory drugs - which alleviate pains by reducing local inflammatory responses and the opioids- which act on the brain. An anesthetic drug is not an analgesics as some people may think, an anesthetic drug is a drug that causes a reversible loss of sensation. They contrast with analgesics which relieve pain without eliminating sensation. Furthermore it can be said that anesthetic drugs makes an animal or person unable to feel anything especially pain (Hals, 2003).

A Starcimol Extra is an analgesic drug which has paracetamol 500mg and caffeine 30mg. It has a NAFDAC registration number of A4-0335. An analgesic also known as a pain killer is any member of the group of drugs used to achieve analgesia-relief from pain. The word analgesic is derived from two Greek words which means ‘without pain’. Analgesic drugs act in various ways on the peripherial and central nervous systems. They are distinct from anesthetics which reversibily eliminate sensation and include paracetamol, the non-steriodal

anti-inflammatory drugs(NSAIDS) such as the salicylates and opioid drugs such as morphine and opium (Harper, 2001). Glutathione (GSH) is a tripeptide with a gama peptide linkage between the amine group of the cysteine which is attached by normal peptide linkage to a glycine and the carboxyl group of the glutamate side chain. It is an antioxidant preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Eastwood, 2005).

For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxidases such as lipid peroxidase. Peroxidase can contain a heme co-factor in their active sites, or alternately redox active cysteine or selenocysteine residues (Miller, 2001).

II. Aim And Objectives

The adverse effects of analgesics have been widely reported. The aim of this research project is to study the effects of Starcimol Extra on the glutathione peroxidase of the albino rat. This was achieved by measuring the concentration of glutathione peroxidase and Total protein concentration in the serum of the albino rat.

III. Materials And Method

Biological samples
20 Adult male albino rats

IV. Methods

Collection of Samples
Collection of Rats

Twenty adult male albino rats were purchased from the animal house of the university of Nigeria, Nsukka and were transported to the animal house of Ebonyi state university, Abakaliki.

Collection of Drug Samples

A packet of starcimol extra was bought from the pharmacy of Ebonyi State University Teaching Hospital located at the Enugu/Abakaliki express road.

Preparation of Samples (Drug Solution)

10 tablets of starcimol extra weighing 5.3g were put in a well sterilized Beaker and 500ml of distilled water was added to it. The tablets were allowed to dissolve to form a drug solution. The drug solution was stored in a refrigerator.

Animal Handling and Treatment

The albino rats were grouped into four groups in steel cages, labeled A-D, each containing five albino rats.

The rats were allowed to feed (grower's mash and water) for one week (7 days) for acclimatization before treatment commence.

Animal Grouping

The rats were placed in four groups A, B, C and D each group containing four rats.

Measurement of Weight of Animals

The weight of the animals was measured daily, using weighing balance and was used to determine the actual volume of plant extract to be administered.

Administration of Drug Solution to the Rats

The rats were treated orally for the period of seven (7) consecutive days with the drug solution as follows;

Group A: 22.5mg of drug solution to 1kg of body weight of rats.
Group B: 45mg of drug solution to 1kg of body weight of rats.
Group C: 90mg of drug solution to 1kg of body weight of rats.

Collection of Blood Samples from the Animals

After treatment, the animals were fasted overnight and under bio-anethesia using chloroform the animals were killed. Blood samples were collected by cardiac puncture into a sterile container.

Preparation of Working Reagents

2, 4 Dintrophenylhydrazine Reagent(4g/l)

Dinitrophenyldrazine was dissolved in a HCL acid. The mixture was filled and stored in a brown bottle in a refrigerator.

Bovine Serum Albumin Standard Solution

20mg albumin was dissolved in 200ml distilled water to give a concentration of 1mg/ml.

Thiourea Solution (7g/l)

Thiourea solution was prepared by dissolving 10g of crystalline thiourea in 100ml of 50% ethanol solution. The solution was stored in the refrigerator.

Copper Reagent (6g/l)

1.5% copper (ii) sulphate was prepared by weighing 1.5g crystalline copper(ii) sulphate dissolved in distilled water.

2x Lowry Concentrate

20g $NaCO_3$ was dissolved in 260mls of distilled water, 0.4g of $CuSO_4.5H_2O$ dissolved in 260mls of distilled water and 0.2g sodium potassium tartarate dissolved in 200mls of distilled water. These were mixed to form the copper reagent.

Preparation of Serum

3ml of blood was collected from the animal in sterile specimen bottles and allowed to cloth. It was centrifuge at 300xg for 10mins and the serum separated from the plasma with the aid of a pasteur pipette.

Determination of Protein Concentration

Lowry method of protein assay (1951) as modified by Hartree (1972) was used in total protein assay.

Principle

Under an alkaline condition, divalent copper ion forms a complex with peptide bond and it is reduced to a monovalent ion. Monovalent copper and the radical groups of tyrosine, tryptophan and cysteine react with phenol reagent to produce an unstable product that becomes reduced to molybedenum or tungsten blue. The absorbance of the coloured compound was measured at 750nm.

Procedure

The protein concentration was determined according to Lowry (1951) using bovine serum albumin as standard. 0.1ml of serum sample were mixed with 5ml of incubation mixture, and mixed after 10 minutes, 0.5ml of folin reagent were added and mixed after 30 minutes. The absorbance was read at 750nm against the reagent using spectrophotometer and the protein concentrations were obtained from the standard curve.

Determination of Glutathione Peroxidase

Paglia and Valentine method of glutathione peroxidase assay (2001) was used.

Principle

The method according to Paglia and Valentine (2010) was used. This method uses the principle of oxidation of NADPH to NADP+ which is accompanied by a decrease in absorbance at 340nm. This assay is an indirect measure of the activity of Glutathione peroxidase. Oxidized Glutathione produced upon the reduction of an organic peroxidase by glutathione is recycled to its reduced state by the enzyme glutathione reductase.

Methods

Blood samples of the rats were centrifuge at 100xg for 10mins and the serum collected. The serum was added to a solution containing Glutathione, Glutathione reductase and NADPH. The enzyme reaction was initiated by adding tert-butyhydroperoxide and the absorbance at 340nm was recorded. The rate of decrease in the absorbance was directly proportional to the glutathione peroxidase activity in the sample.

V. Statistical Analysis

Resulting data were represented as meant so statistical data was analyzed by student's t-test. The $p < 0.05$ was considered statistically significant.

VI. Results

Physical Observation

There was an obvious increase in physical activities of the albino rats after administration of the drug solution. An increase in food and water intake was also noticed after administration of the drug solution as compared with the control.

Changes In The Weight Of The Rat During The 7days Of Treatment.

The changes in the average weight of the rats during the seven (7) days of treatment are explained in table 1. A linear decrease occurred in the test groups (A-C), while D, which is the control gained weight. The reduction in the treated group also varies among the groups. That is the weight reduction was dependent on their dose

Table 1: Changes in the average weight of the rats during 7 days of treatment

Days	Group A	Group B	Group C	Group D
1	100.00 ± 3.75	100.00 ± 6.00	100.00 ± 3.25	100.00 ±2.10
2	100.00 ± 2.25	100.00± 6.00	80.25 ± 4.67	100.00± 2.10
3	93.75 ± 4.29	81.25 ± 5.23	74.32± 3.26	105.69± 4.26
4	93.75 ± 4.29	76.50 ± 5.00	70.00 ± 2.75	107.50± 3.10
5	81.12 ± 2.36	75.50 ± 4.75	67.01 ± 1.67	107.50 ± 3.10
6	80.31 ± 1.76	74.62 ± 3.76	63.24 ± 1.02	108.37± 5.00
7	73.25 ± 5.73	72.75 ± 3.96	50.31 ± 1.00	108.76 ± 5.23

Values are the mean weight ± standard deviation (S.D)
Group A: 22.5mg of drug solution to 1kg body weight of rat
Group B: 45 of drug solution 1kg body weight of rat
Group C: 90mg of drug solution to 1kg body weight of rat
Group D: Rats in group D were treated with distilled water (control)

Glutathione Peroxidase Activity And Total Protein Concentration In The Serum Of Albino Rats After 7 Days Of Treatment With Starcimol Extra

The changes in the glutathione peroxidase activity and total protein concentration of the animals after seven (7) consecutive of treatement with the drug solution are summarized in the table 2 below. In the glutathione peroxidase activity, a linear decrease occurred in the test groups (A-C), while D, the control was normal. In addition, the total protein concentration, both the test groups and the control were stable.

Table 2 Glutathione Peroxidase Activity And Total Protein Concentration In The Serum Of Albino Rats After 7 Days Of Treatment With Starcimol Extra

GROUP	ENZYME ACTIVITY (U/L)	TOTAL PROTEIN (Mg/L)	SPECIFIC ENZYME ACTIVITY
A	85.32 ± 3.45^{b}	0.63 ± 0.04^{a}	136.98 ± 12.68^{a}
B	64.52 ± 4.20^{a}	0.52 ± 0.07^{a}	$125..81 \pm 9.87^{a}$
C	47.84 ± 6.34^{a}	0.43 ± 0.01^{a}	109.67 ± 13.50^{c}
D	118.57 ± 8.65^{c}	0.76 ± 0.02^{a}	156.81 ± 13.98^{b}

All values are mean ± standard deviation.
Values in the same column having different superscript are significantly different.

VII. Discussion

The actual biochemical mechanism underling the observed increase in physical activity, food and water intake cannot be stated at this level of research. However, the observation maybe as a result of the chemical constituents of the drug solution administered to the rats. This observation is in line with that made by Paglia and Valentine (2001), when they treated guinea pigs with a solution Anadin Extra was attributed to caffeine which was in constituent of the drug solution. Caffeine is a central nervous and metabolic stimulant. It is used recreationally and medically to reduce physical fatigue and to restore alertness when drowsiness occurs. It produces increased wakefulness, faster and clearer flow of thought, increased focus and better general body coordination. Caffeine can also improve sprint and endurance when used by an athlete (Broe, 2014).

Increase in appetite has been indicated as one of the side effects of Paracetamol. (Reimann, 2004). Paracetamol cause these effects possibly by distorting the general body system of the organism into which they are introduced. The distortion may be as a result of enzyme inhibition or stimulation.

The reason behind the decrease in the average body weight of the rat relative to the control is still not partially understood. Therefore, further research is needed to ascertain the exert biochemical mechanism. However, these decrease in body weight maybe ascribed to a toxic effect of the drug solution. Some researchers have reported a similar observation on treating laboratory animals with various analgesic based on Paracetamol. For instance, Ahmad (2010) made the same observation on Albino rats after treating with an Aspirine solution. In the same vein, Broe (2014) also reported a decrease in body weight of Albino rats after treating them with a solution of Anadin Extra.

The protein analysis carried out on the serum revealed no significant difference ($p>0.05$) between the test groups (A-C) and the control, group D. The chemical components of the drug solution may play no significant rule in the degradation and synthesis of proteins. Further, the biochemical event responsible for this observation cannot be stated at this level of research. However, this observation is in line with that made my Oko (2012), when he treated Albino rats with a solution of Emzor Paractamol. According to him, there was no significant difference in the protein concentration between the treated animal and the control. Paracetamol has been reported to have low significant effect in the total protein concentration of laboratory animals. (Kent 2000).

The exact biochemical event for the observed significant decrease in the serum glutathione peroxidase is not yet known but the chemical constituents of the drug solution may have contributed to this mechanism. For instance, Oko (2007) also made the same observation on Albino rats after treating them with a solution of Emzor Paracetamol. According to him, the decrease in serum glutathione peroxidase of Albino rats treated with Emzor Paracetamol was attributed to the chemical constituents of the drug solution. The reactive metabolite, N-acetyl-p-benzoquinone imine produced by Paracetamol is toxic and produces free radicals in the body (Kent,2004).

VIII. Conclusion And Suggestion

The observations made in this research have suggested that Starcimol Extra solution may produce free radicals in the body which is the major cause of the Aging process. This can be shown by the decrease in glutathione peroxidase activity of the animals treated with the drug solution. This findings are however speculative, since the drug solution contain other chemical constituents. The identity of the exact chemical constituent of the drug solution responsible for these observations is a subject of further investigation. In the same vein, it is recommended that the mechanism by which the drug solution decreases glutathione peroxidase level should be studied.

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