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Glutathione Peroxidase Activity in Albino Rats Treated with Forpain® (Analgesics)

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Abstract: The use of analgesics in the treatment/management of pain has been shown to produce several side effects. This research examined the effect of Forpain® on the glutathione peroxidase activity in albino rats. Twenty albino rats used in this research were separated into five groups (A, B, C, D and E), four rats per group. Groups A, B, C and D were treated orally with 7.57, 15.14, 30.28 and 45.42mg/kg body weight of Forpain® solution for seven days, while group E 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 a decrease in feed and water intake of the treated rats when compared with the control. There was a significant decrease ($P<0.05$) in glutathione peroxidase activity of the treated animals when 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 Forpain® solution were found to be dose-dependent. The adverse effects of oral administration of Forpain® may affect the antioxidant system of the body.

Key words: Forpain® · Glutathione peroxidase · Analgesics and pain management

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 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 and pulsating pain. The pain could have a pinching sensation or a stabbing one [1].

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 [2].

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 [3].

An 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 [4]. An anesthetic drug is not an analgesic 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 make an animal or person unable to feel anything especially pain [5].

A Forpain® is an analgesic drug which has paracetamol 500mg and caffeine 30mg. 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

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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 reversibly eliminate sensation and include paracetamol, the non-steriodal anti-inflammatory drugs (NSAIDS) such as the salicylates and opioid drugs such as morphine and opium [6].

Glutathione (GSH) is a tripeptide with a gamma 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 [7]. 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 [8].

Glutathione peroxidase (GPx) (EC 1.11.1.9) is a type of enzyme that serves as a cellular antioxidant. It reduces the peroxide group to a relatively un-reactive alcohol group, using glutathione as the reducing agent and thus protects the cell from oxidative damage. Glutathione peroxidase 1 (GPx1) is the most abundant, found in the cytoplasm of nearly all mammalian tissues and whose preferred substrate is hydrogen peroxidase. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydro-peroxidases. It is found nearly in every mammalian cell, although at much lower concentration. So far, eight different isoforms of glutathione peroxidase (GPx 1-8) has been identified in mammals) [9].

Furthermore, N-acetyl-p-benzoquinone imine, which is a reactive cytochrome P450 metabolite formed by paracetamol has been found to be toxic to the body system and therefore affects the concentration of glutathione peroxidase in the serum negatively. In this way, paracetamol toxicity lowers the concentration of glutathione peroxidase in the serum due to the ability of these animals to efficiently recover glutathione depleted as a result of paracetamol metabolism [10].

There are some factors that affect glutathione peroxidase in the body which include; selenium supplementation (in diseased patient), alcohol, pesticides, diet and drugs: example analgesics [11].

Aim and Objectives: The adverse effects of analgesic drugs have been widely reported. The present research investigated the effect of Forpain® on the glutathione peroxidase in albino rats.

MATERIALS AND METHODS

Twenty (20) 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 Sample: Drug sample (forpain tablet) was bought from Nic-Joy Pharmacy located at Presco Campus, Abakaliki, Ebonyi State, Nigeria.

Preparation of Samples (Drug Solution): Ten tablets of forpain weighing 5.3g were put in a 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 Grouping: The twenty (20) albino rats were placed in five different cages, each containing four rats. The cages were labeled A, B, C, D and E, with each cage containing animals of similar weights.

Measurement of Weight of Animals: The weights of the animals were measured daily (every morning), using weighing balance and was used to determine the actual volume of drug to be administered.

Administration of Drug Solution to the Rats: The rats were treated orally for the period of seven consecutive days with the drug solution as follows:

Group A: 7.57 mg/kg body weight of drug solution.

Group B: 15.14 mg/kg body weight of drug solution.

Group C: 30.28 mg/kg body weight of drug solution

Group D: 45.42 mg/kg body weight of drug solution

Collection of Blood Samples from the Animals: After treatment, the animals were fasted overnight and under anesthesia using chloroform and blood samples were collected from the animal by cardiac puncture into a sterile container.

Preparation of Working Reagents

GPx Assay Buffer: 3ml of assay buffer is diluted with 27ml of HPLC-grade water, to give a final assay which is stored at 4°C.

Gpx Sample Buffer: 2ml of sample buffer concentrate is diluted with 18ml of HPLC-grade water to give a final sample buffer which is stored in 4°C. it is used to dilute GPx control and GPx sample prior to assaying

Bovine Erythrocyte GPx (control): 10µl of GPx is diluted with 490µl of diluted sample buffer and kept on ice.

Gpx Co-substrate Mixture: Co-substrate mixture contained in each vial of the kit is the GPx co-substrate mixture which contains lyophilized powder of NADPH, glutathione and glutathione reductase are added to 6ml of HPLC grade water to give a reconstituted reagent which is kept at 25°C while assaying and stored at 4°C

2x Lowry Concentrate: 20g $Na_2CO_4 5H_2O$ was dissolved in 260mls of distilled water. 4g of $CuSO_4.5H_2O$ dissolved in 20mls of distilled water and 2g sodium potassium tartate was dissolved in 20mls of distilled water. The resultant solutions were mixed to give a solution containing 30ml of copper reagent, 10ml of SDS (dodium dodecyl sulfate) and 10ml of NaOH.

Folin Reagent: 0ml of 2N folin reagent was mixed in 90ml of distilled water. The solution is made stable for several months at room temperature if stored in an amber bottle.

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 pasterum pipette.

Determination of Glutathione Peroxidase Activity: Paglia and Valentine (2001) [12] method of glutathione peroxidase assay was used.

Principle: 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.

Method: This method is based on the procedure outlined below:

In background or Non-Enzymatic Wells, 120µl of Assay Buffer and 50 µl of co-substrate mixture were added to the three wells. In postive Control Well (bovine erythrocyte Gpx), 100µl of Assay Buffer, 50µl of Co-substrate Mixture and 20µl of dilute GPx (control) were added to three wells. In the Sample Wells, 100µl of Assay buffer, 50 µl of Co-substrate Mixture and 20µl of sample were added to three wells. The reaction were initiated by adding 20µl cumene hydroperoxide to wells been used. Quickly note the time the reacted was initiated. Carefully shake the plate for a few seconds to mix. The absorbance was read once every minute at 340nm using a plate reader to obtain at least 5 time points.

$$\text{Gpx activity} = \frac{\Delta A_{340}/\text{min}}{0.00373\mu M^{-1}} \times \frac{\text{Volume of Sample}}{\text{Volume of Reaction}} \times \text{sample dilution} = \text{nmol/min/ml}$$

Determination of Total Protein Concentration: Lowry method of protein assay (1951) [13] was used in total protein determination.

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 procedure was carried out using bovine serum albumin as standard. 0.1ml of serum was mixed with 5ml of incubated Lowry concentrate (at 37°C). After 10 minutes, 0.2ml of 2N folin reagent were added and mixed and incubated for additional 30 minutes at room temperature. After incubation, absorbances were read at 750nm against a blank reagent using spectrophotometer. The protein concentrations were obtained from the standard curve.

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.

RESULTS

Physical Observation: During the seven days treatment, there was an obvious decrease in physical activities, feed and water intake of the albino rats after administration of the drug solution. However, there was a decrease in the weight and physical activities example; movement in treated animals in respect to control (group E).

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

Days	Group A	Group A	Group C	Group D	Group E
1	100.10 ± 5.75	100.26 ± 5.90	100.01 ± 5.25	106.05 ± 6.24	100.07 ±5.70
2	97.75 ± 4.25	92.15 ± 3.03	94.25 ± 3.67	100.10 ± 5.75	101.12± 5.83
3	95.25 ± 4.01	91.25 ± 2.98	92.32± 3.26	97.22 ± 4.02	105.69± 6.26
4	93.75 ± 3.29	88.50 ± 2.87	89.60 ± 2.91	91.32 ± 3.00	107.50± 7.15
5	91.12 ± 2.94	85.50 ± 2.65	87.01 ± 2.80	87.24 ± 2.83	109.50 ± 7.96
6	86.31 ± 2.76	84.62 ± 2.36	83.24 ± 1.20	85.31 ± 2.55	112.37± 8.20
7	83.25 ± 2.13	82.75 ± 1.92	80.31 ± 1.074	83.00 ± 2.01	115.76 ± 9.23

Values are the mean weight ± standard deviation (S.D), n=4

Group A: 7.57 mg/kg body weight of drug solution.

Group B: 15.14 mg/kg body weight of drug solution.

Group C: 30.28 mg/kg body weight of drug solution

Group D: 45.42 mg/kg body weight of drug solution

Group E: Rats in group E were treated with distilled water (control)

Table 2: Glutathione Peroxidase Activity and Total Protein Concentration in the Serum of Albino Rats after 7 Days of Treatment with Forpain

Group	Enzyme Activity (U/L)	Total Protein (mg/l)	Specific Enzyme Activity(U/L/Protein)
A	104.18 ± 2.20^{a}	0.48 ± 0.02^{a}	$213.71 \pm 2..21^{a}$
B	93.46 ± 7.37^{a}	0.45 ± 0.04^{a}	$208..65 \pm 6.48^{a}$
C	74.42 ± 6.64^{b}	0.40 ± 0.03^{a}	185.98 ± 6.88^{b}
D	61.86 ± 6.45^{b}	0.27 ± 0.02^{a}	172.52 ± 3.02^{b}
E	118.03 ± 8.65^{c}	0.46 ± 0.61^{a}	247.58 ± 8.65^{c}

All values are mean ± standard deviation.

Values in the same column having different superscript are significantly different.

Changes in the Weight of the Rat During the 7 Days of Treatment: The changes in the average weight of the rats during the seven days of treatment were explained in table 1. A linear decrease occurred in the test groups (A-D), while group E, which is the control, gained weight. The reduction in the treated group also varied among the groups. That is the weight reduction was dose-dependent.

Glutathione Peroxidase Activity and Total Protein Concentration in the Serum of Albino Rats after 7 Days of Treatment with Forpain: The change in the glutathione peroxidase activity and total protein concentration of the animals after seven consecutive days of treatment with the drug solution are summarized in the table 2. The level glutathione peroxidase decreased significantly ($p<0.05$) in group A-D when compared to the control, while there was no-significant difference ($p>0.05$) between the total protein concentration in group A-D and the control.

DISCUSSION

The actual biochemical mechanism underling the observed decrease in physical activity, feed and water intake cannot be stated at this level of research. The decrease in the physical activity of the treated animals in group A-D were more significant ($p>0.05$) when compared to control (group E). 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) [12], when they treated guinea pigs with a solution of Anadin Extra. The effect of this 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 [14].

Some researchers have reported a similar observation on treating laboratory animals with various analgesics containing paracetamol. For instance, Ahmad (2010) [15] made the same observation on albino rats after treating them with an aspirine solution. In the same vein, Broe (2014) [16] also reported a decrease in body weight of Albino rats after treating them with a solution of starcimol Extra.

The reason behind the decrease in the average body weight of the rat relative to the control is still not partially understood, but it could be as a result of the doses given to the rats since the side effects of the drugs solution includes vomiting, coughing, diarrhea etc, especially when taken in high doses. Thus, group A received the smallest dose, 7.57mg/kg, group B, C and D received 15.14, 30.28 and 45.42mg/kg respectively. The highest dosage 45.42mg/kg which was given to group D experienced the highest weight loss, 7.57mg/kg was given to group A, which experienced the lowest weight loss, while the control, group E animals were not given any drug solution and thus experienced an increase in body weight. Similar observations have been reported by Ahmad (2010) [15] from his researches on effect of ibuprofen on antioxidant levels.

The activity of serum glutathione peroxidase in the test group A-D animals showed a decrease ($p<0.05$) when compared to control, group E. the enzyme activity of animal in group A (treated with 7.57mg/kg drug solution) decreased slightly below the enzyme activity levels of animals in group E, while that of group D (treated with 45.42mg/kg drug solution) decreased well below the activity levels of group E animals. This decrease might be found to be dose dependent. For instance, Oko (2012) [17] also made the same observation on Albino rat after treating them with a solution of Emzor Paracetamol. According to him, the decrease in glutathione peroxidase of Albino rat treated with Emzor Paracetamol was dose dependent.

The total protein analysis carried out on the serum revealed no-significant difference ($p>0.05$) between the treated groups (A-D) and the control, group E, which shows that the chemical components of the drug solution may play no significant role in the degradation and synthesis of proteins. However, this observation is in line with that made by Oko (2012) [17], when he treated guinea pigs with a solution of aspirine. According to him, there was no significant difference in the protein concentration between the treated animal and the control.

CONCLUSION

The observations made in this research have suggested that Forpain 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, we recommend that the mechanism by which the drug solution decreases glutathione peroxidase level should be studied.

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