Corresponding Author: K.N. Agbafor, Department of Biochemistry Ebonyi State University, Abakaliki, Nigeria.
Analgesic is derived from two Greek words which means ‘without pain’. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics which reversibly eliminate sensation and include paracetamol, the non-steroidal 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 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 [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 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 Na₂CO₃·5H₂O was dissolved in 260mls of distilled water. 4g of CuSO₄·5H₂O 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 (sodium 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 clot. It was centrifuge at 300xg for 10mins and the serum separated from the plasma with the aid of a pasteur pipette.

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

**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).
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 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 underlying 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.

**REFERENCES**