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Original Research Article

Effect of Ethanolic Extract of Tetrapleura Tetraptera on Some Antioxidant Enzymes in Rats Administered 1, 2-Dimethylhydrazine

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Abstract: This work was aimed at determining the antioxidant activity of an ethanol extract of *Tetrapleura tetraptera* fruits in rats administered DMH. Group 1 (positive control) contains rats without the administration of DMH, extract, or CMC. Group 2 (negative control) received DMH, 1 ml of 1% CMC, and no extract. Group 3 received a low dose of the ethanol extract of *Tetrapleura tetraptera* fruits (100mg/kg) and DMH. Group 4 received a high dose of the ethanol extract of *Tetrapleura tetraptera* (200mg/kg) and DMH. The DMH was administered once a week for four consecutive weeks, while the ethanol extract of *Tetrapleura tetraptera* was administered daily for four consecutive weeks. The result showed that there was a significant increase in the activities of SOD, CAT, and GST in rats administered an extract of *Tetrapleura tetraptera* fruits compared to the negative control group. In general, the introduction of *Tetrapleura tetraptera* to the rats caused an increase in the activity of the antioxidants compared to the negative control. The histopathological profile, however, showed that the DMH did not affect the liver, colon, and small intestine morphology. It could thus be concluded that the ethanol extract of *Tetrapleura tetraptera* fruit has potent antioxidant activity.

Keywords: *Tetrapleura tetraptera,* Antioxidants-Enzymes, DMH.

1. INTRODUCTION

Plants have long been a rich source of medicine that humans have utilized to cure a variety of illnesses (Rafieian-Kopaei & Sewell, 2014). This culturally based healthcare practice of using plants as the medication is typically passed down orally by groups of various cultures (Karunamoorthi *et al.,* 2013). About 80% of people in underdeveloped nations utilize conventional therapies because they are unable to afford the high cost of Western medications and healthcare and because conventional medicines are more suitable from a cultural and spiritual standpoint (Chen *et al.,* 2016). Additionally, the WHO estimates that more than 80% of the world's population relies mostly on traditional medicines made from plants (Hosseinzadeh *et al.,* 2015). This might be a result of the expense of synthetic pharmaceuticals and their adverse effects, as well as the dearth of healthcare services in some parts of developing nations. Compared to synthetic or modern medications, these are generally safer and less expensive (Bahmani *et al.,* 2014). There is a demand for medicinal herbs, and acceptance of them is growing. The poisonous and harmful side effects of conventional and allopathic medications are to blame for the quick rise in population demands, growth in the number of medicinal plants, and decrease in the usage of chemical treatments (Rasool Hassan, 2012). These plants are not equally dispersed globally, and most of their therapeutic herbs are gathered from local animals. Over 50,000 plant species, or more than one in ten, are employed in the production of pharmaceutical and cosmetic products (Huang, 2011; Rafieian-Kopaei & Sewell, 2014). The seeds, roots, leaves, fruit, skin, flowers, and even the entire plant are beneficial components of medicinal plants. According to Rasool Hassan, (2012), these plants are abundant sources of chemicals that can be used to create drugs. The majority of the medicinal plants' active chemicals have physiological impacts on

living things. These active chemicals' direct or indirect therapeutic properties are what made it possible to employ them as medication. The majority of the medicinal plants' active chemicals have physiological impacts on living things. These active chemicals' direct or indirect therapeutic properties are what made it possible to employ them as medication. The components of these plants may interact, and this interaction may be advantageous for both, detrimental for either, or erase the negative effects of both. Compounds from plants can significantly better diseases that are difficult to treat, such as cancer, which is an uncontrolled cell development in our bodies that can be fatal. It causes an imbalance in the body and damages healthy cells. It is among the most serious health issues in both industrialized and developing nations. More than a hundred diseases in humans are influenced by free radicals, including atherosclerosis, arthritis, ischemia and reperfusion damage to several tissues, damage to the central nervous system, gastritis, cancer, and AIDS. Free radicals are scavenged by antioxidants. The Fabaceae family includes the medicinal plant Tetrapleura tetraptera. Among the Yoruba of southwestern Nigeria, it is called "Aridan." It is used to cure a variety of human illnesses in African traditional medicine, such as convulsions, inflammation, rheumatism, arthritis, asthma, and hypertension. According to studies (Ojewole & Adewunmi, 2004), this plant's fruit extract has anti-inflammatory and antioxidant properties.

In this study, rats given DMH were given an ethanol extract of *Tetrapleura tetraptera* fruits to test the fruit's antioxidant capacity.

2. MATERIALS AND METHODS

2.1 Materials

Dried fruit of *Tetrapleura tetraptera.*

2.1.1 Reagents

Carboxyl methyl cellulose (CMC), Diethylether, Sodium chloride and Sodium hydroxide Thiobarbituric acid (TBA), Sodium carbonate, Trichloroacetic acid (TCA), Epinephrine, Sodium bicarbonate, Phosphate buffer (pH 7.0), Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Potassium dichromate acetic acid (1:3 Potassium dichromate and glacial acetic acid respectively), Ethanol.

2.1.2 Apparatus

Beaker, Conical flask, Measuring cylinder, Spatula spoon, Weighing balance (electronic compact scale, model-110c), Refrigerator (Haier Thermocool, Model: HR-1379, Deep freezer, water bath (model no: DK-240), Heparin bottle, Universal bottle, Ice bath, Pin, Cotton wool, Centrifuge (techmel &techmel USA, model: 412B), Centrifuge (Heraenous sepatech centrifuge, model:90-1), Test tubes, Eppendorf tubes, Needles and syringe, Micropipettes, Genesys UV spectrophotometer (model no: G10S UV-Vis), Spectrum lab spectrophotometer (model: S23A), Cages, Dissecting set, Blender and Cannula.

3.2 METHODOLOGY

3.2.1 Source and Preparation of the Plant Material

The dried fruit was purchased from Ajanbadi Market and was identified by Dr. (Mrs) Saraibi, a Botanist in the Department of Botany, Lagos State University, Ojo, Lagos, Nigeria. One kilogram (1kg) of the fresh ripe fruits of *T.tetraptera* was air-dried at room temperature, the dried fruits were washed and grated into coarse particles. The dried fruits were washed and grated into coarse particles. The grated particles (300 g) were macerated cold with 1800ml of ethanol (3 batches) for seventy-two hours (72 hours) at room temperature, filtered and the filtrate was concentrated to dryness at 40°C stored in the refrigerator.

The average weight of the extract

The extraction was done in three batches. 28.28g, 26.11g and 30.16g.

The average = $\frac{28.28+26.11+30.16}{3}$= 28.18g

Result on percentage yield

The total weight of the plant material was 300g.

The % yield =$\frac{average\ weight\ of\ extract}{total\ weight}$×100% =$\frac{28.18g}{300g}$ ×100% = 9.4%.

2.3 Experimental Design

2.3.1 Toxicity Tests

The acute toxicity value (LD50) of *Tetrapleura tetraptera* fruits was determined to know its lethal and non-lethal doses. The acute toxicity (LD_{50}) of fruit extracts of *T. tetrapleura* was determined using OECD 423 guidelines in rats using the oral route. Twenty rats were assigned equally into five well-ventilated plastic cages and administered 2ml of different doses (50, 100, 200, 400 and 1000mg/Kg body weight) of the extracts suspended in 1% CMC.

2.3.2 Experimental Animals

Twenty (20) male Wistar strain albino rats weighing 110-130 g were used in this experiment. The rats, which were purchased from Ratz Matters, were maintained under standard conditions in the animal house for 4weeks before induction. They were housed in plastic cages and fed a high-fat diet produced by local Ratz matters and water ad libitum. The DMH was administered once a week while the ethanol extract of *Tetrapleura tetraptera* was administered daily for four consecutive weeks. The extract was given orally while the DMH was injected into the rats intraperitoneally. Group feeding was done to ensure animals in a group were subjected to the same condition. All the rats were fed with respective treatments, weighed weekly and their cages were disinfected weekly.

The rats were divided into four groups of 5 animals each. The animals were kept fasting overnight prior to each administration. The three groups of animals were placed on different treatments as shown in Table 1.

Table 1

Groups	Dosage
1	No extract, No DMH
2	DMH, No extract
3	DMH, low dose
4	DMH, high dose

2.4 Biochemical Assays

For the biochemical assay analysis, the serum was collected in a plain bottle, checked to avoid coagulation and kept in ice.

2.4.1 Determination of Superoxide Dismutase Activity (SOD)

1 ml of serum sample was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of the substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds was determined by the method of (Misra & Fridovich, 1972).

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5} \quad (1)$$

Where A_0 = absorbance after 30 seconds

A_3=absorbance after 150 seconds

$$\%\text{Inhibition} = \frac{100 \times Increase\ in absorbance\ for\ substrate}{Increase\ in absorbance\ for\ blank} \quad (2)$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

2.4.2 Determination of Catalase Activity

Spectrophotometric determination of H_2O_2. Different amounts of H_2O_2, ranging from 10 to 100 μmoles were taken in small test tubes and 2ml of dichromate/acetic acid was added to each. The addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10 minutes in a boiling water bath changed the colour of the solution to stable green due to the formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made to 3ml and the optical density was measured with a spectrophotometer at 570nm. The concentrations of the standard were plotted against absorbance.

Table 2

Test tube	1	2	3	4	5	6	7
H_2O_2 (ml)	0.05	0.10	0.15	0.20	0.30	0.40	0.50
Dichromate/ acetic acid (ml)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Distilled water (ml)	0.95	0.90	0.85	0.80	0.70	0.60	0.50
H_2O_2 Concentration (μ moles)	10	20	30	40	60	80	100
Absorbance (570nm)	0.049	0.095	0.145	0.195	0.291	0.385	0.484

2.4.2.1 Determination of Catalase Activity of Samples

1ml of a supernatant fraction of the testicular homogenate was mixed with 19 ml of distilled water to give a 1: 20 dilution. The assay mixture contained 4ml of H_2O_2 solution (800µmoles) and 5ml of phosphate buffer, pH 7.0 in a 10ml flat bottom flask. 1 ml of properly diluted serum sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1ml portion of the reaction mixture was withdrawn and blown into 2ml dichromate/acetic acid reagent at 60 seconds intervals. The method described above determined the hydrogen peroxide contents of the withdrawn sample. according to the method of (Robert A. Greenwald, 2018).

Catalase activity was obtained by plotting the standard curve and the concentration of the remaining H_2O_2 was extrapolated from the curve.

H_2O_2 consumed = 800µmoles – H_2O_2 remaining
Catalase activity = (H_2O_2 consumed)/(mg Protein) (3)

2.4.3 Determination of Glutathione-S-Transferase Assay

The medium for the estimation was prepared as shown in table and the reaction was allowed to run for 60 seconds each time before the absorbance was read against the blank at 340nm. The temperature was maintained at approximately 31^0C. The absorbance was measured.

Table 3: Glutathione-S-Transferase Assay Medium

Reagent	Blank	Test
Reduced glutathione (0.1M)	30µl	30µl
CDNB (20mM)	150µl	150µl
0.1M Phosphate buffer, pH 6.5	2.80ml	2.80ml
Cytosol/Microsomes	-	30µl

The extinction coefficient of CDNB = $9.6mm^{-1}$ Cm^{-1}

GSH-S-transferase activity = $\frac{OD/\min \times 1}{9.6} = \frac{0.03ml}{mg} protein$ (4)

2.5 Histopathology

Histopathology examination was carried out at Lagos State Teaching Hospital (LASUTH) Department of Forensic Pathology. The rats were sacrificed with the use of chloroform and the colon, liver and small intestinal were isolated and preserved.

2.6 Statistical Analysis

All data were represented as mean ± standard error of the mean (SEM) and statistical significance was taken for $p< 0.05$. Data were analyzed using one-way analysis of variance (ANOVA), with a GraphPad Prism® software version 5.01 (GraphPad Software, Inc. CA 92037 USA).

3. RESULTS

3.1 Result of LD50

The acute toxicity value (LD50) of *Tetrapleura tetraptera* fruit was found not to be within the range of the concentration of 50, 100, 200, 400 and 1000mg/Kg body weight used in this study since no sign of toxicity was observed. However, 100mg/kg and 200mg/kg doses of fresh blended *Tetrapleura tetraptera* fruit were used in this study.

3.2 Result of Glutathione-S-Transferase Assay

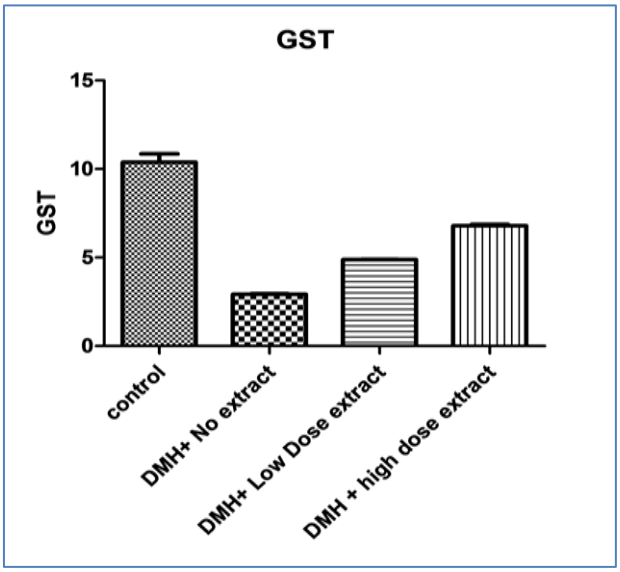

Figure 1: The effect of ethanol extract of Tetrapleura tetraptera on the concentration of GST in the serum. Data are presented as ± S.E.M from 5 rats. Bars with different significance at p> 0.05.

Figure 1 above shows the effect of ethanol fruit of *Tetrapleura tetraptera* on the activity of glutathione-S-transferase in the rat serum. There was a significant difference ($p<0.05$) in the activity of glutathione-S-transferase between animals fed with 100mg/kg of *Tetrapleura tetraptera* fruit and that of control but animals fed with 200mg/kg had their activity significantly increased compared to negative control.

3.3 Result of Catalase Activity

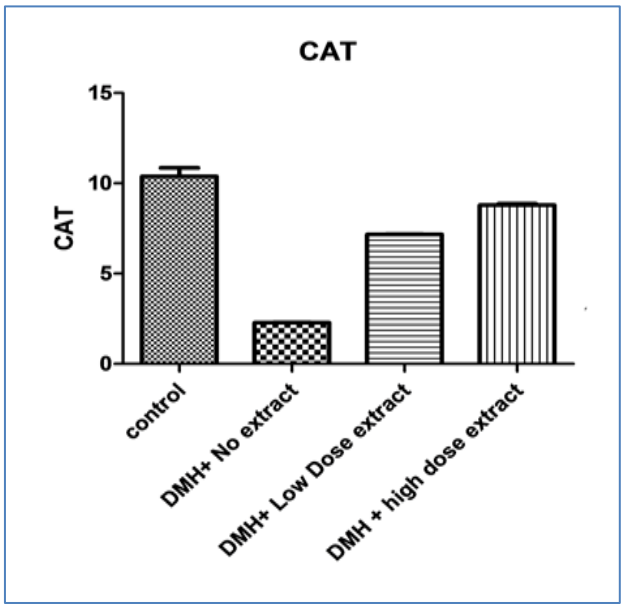

Figure 2: The effect of ethanol fruit of *Tetrapleura tetraptera* on the concentration of CAT in the rat serum. Data are presented as the mean ± S.E.M from 5 rats. Bars with different are significantly different at p<0.05.

Figure 2 above shows the effect of ethanol fruit of *Tetrapleura tetraptera* on the activity of catalase in the rat serum. There was a significant difference ($p>0.05$) in the activity of catalase between animals feed with 100mg/kg of dried *Tetrapleura tetraptera* fruit and that negative control but animals fed with 200mg/kg have their activity significantly increased compared to the negative control

3.4 Result of Superoxide Dismutase Activity (SOD)

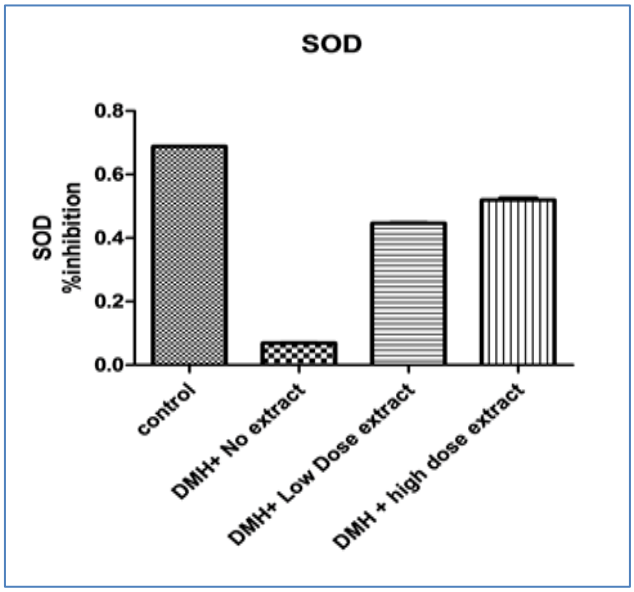

Figure 3: The effect of ethanol fruit of *Tetrapleura tetraptera* on the concentration of SOD in the rat serum. Data are presented as the mean ± S.E.M from 5 rats. Bars with different are significantly different at p<0.05.

Figure 3 above shows the effect of ethanol fruit of *Tetrapleura tetraptera* on the activity of SOD in the rat serum. There was a significant difference (p>0.05) in the activity of SOD between animals feed with 100mg/kg of dried *Tetrapleura tetraptera* fruit and the negative control but animals fed with 200mg/kg have their activity significantly increased compared to the negative control

3.5 Result on Histopathology

Group 1
No treatment
Colon

Figure 4: The histologic sections of the colon show tubular glands and crypts lined by uniform Goblets cells.

Group 2
Colon

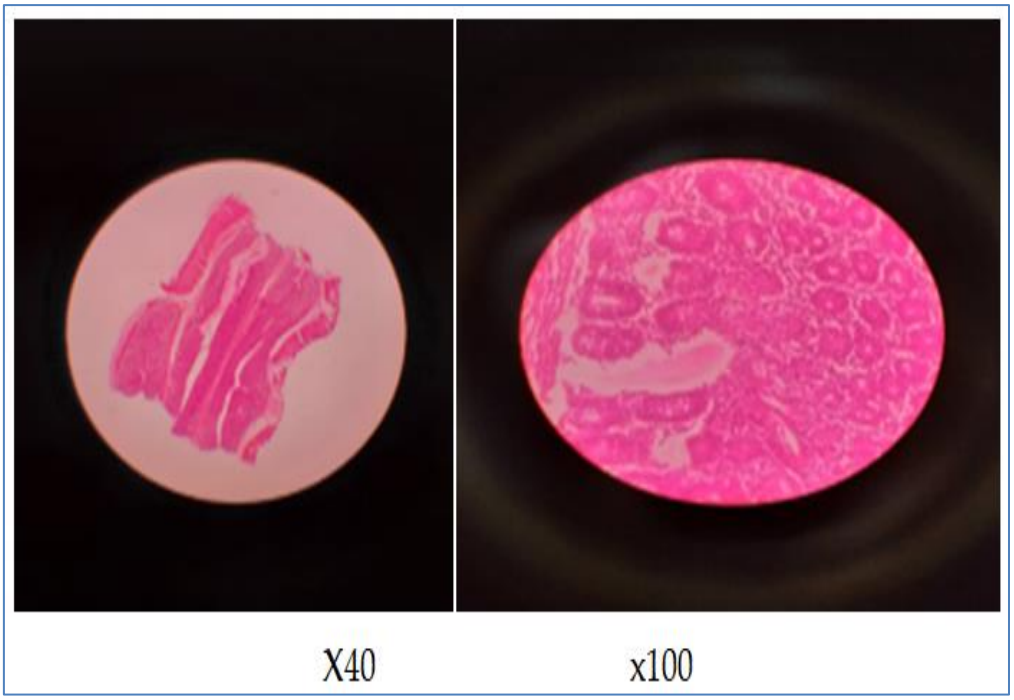

Figure 5: The histologic sections of the colon show tubular glands and crypts lined by uniform Goblets cells.

Group 3
Colon

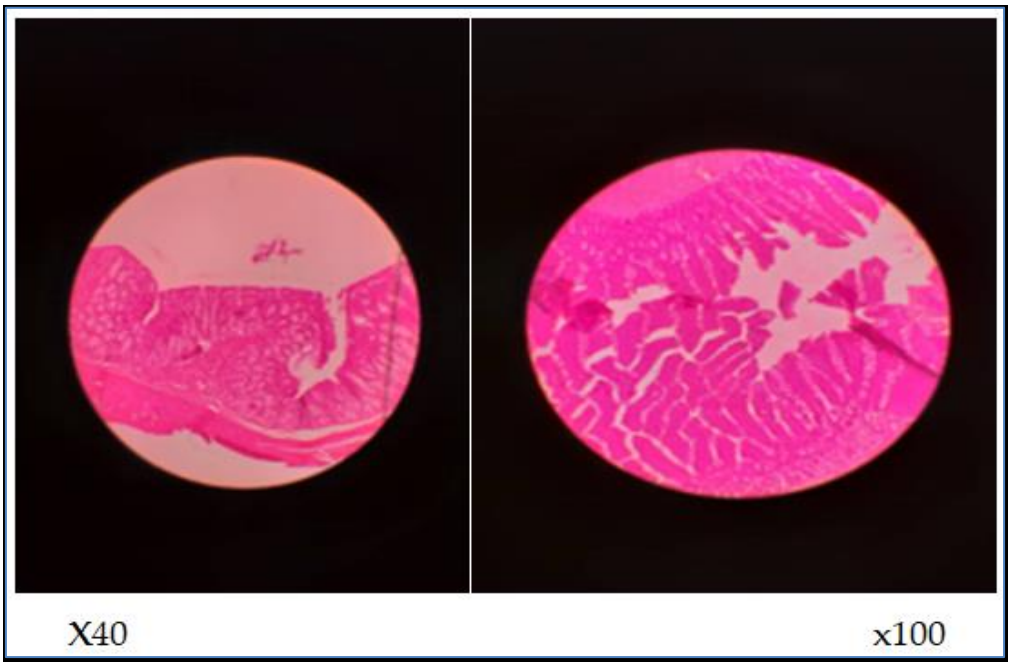

Figure 6: The histologic sections of the colon show tubular glands and crypts lined by uniform Goblets cells.

Group 4
Colon

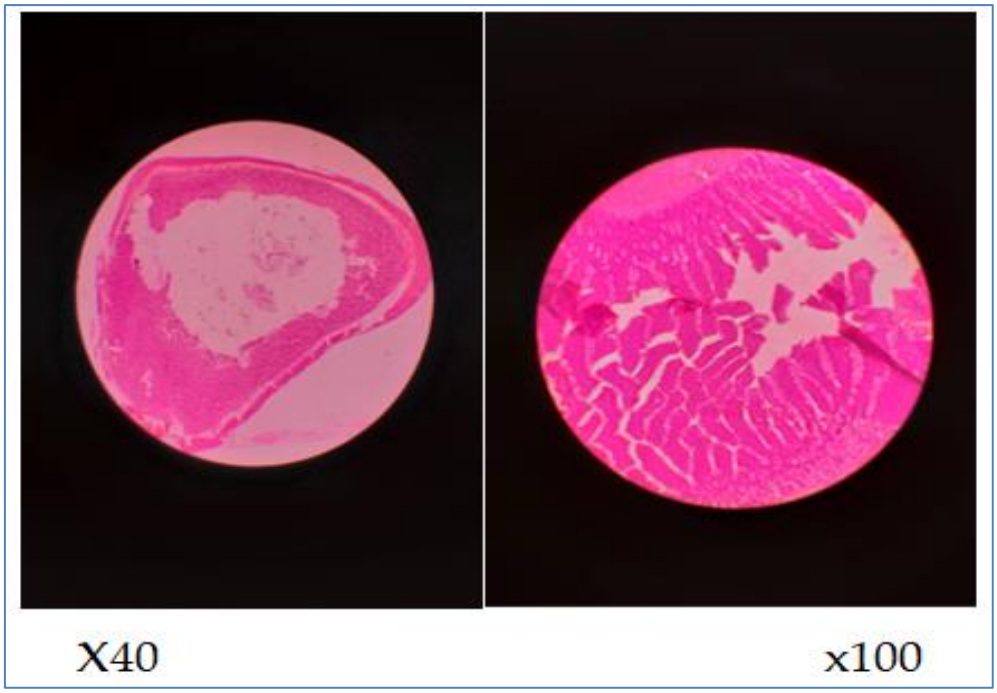

Figure 7: The histologic sections of the colon show tubular glands and crypts lined by uniform Goblets cells.

Group 1
Treated with DMH (30 mg/Kg)
Small intestine

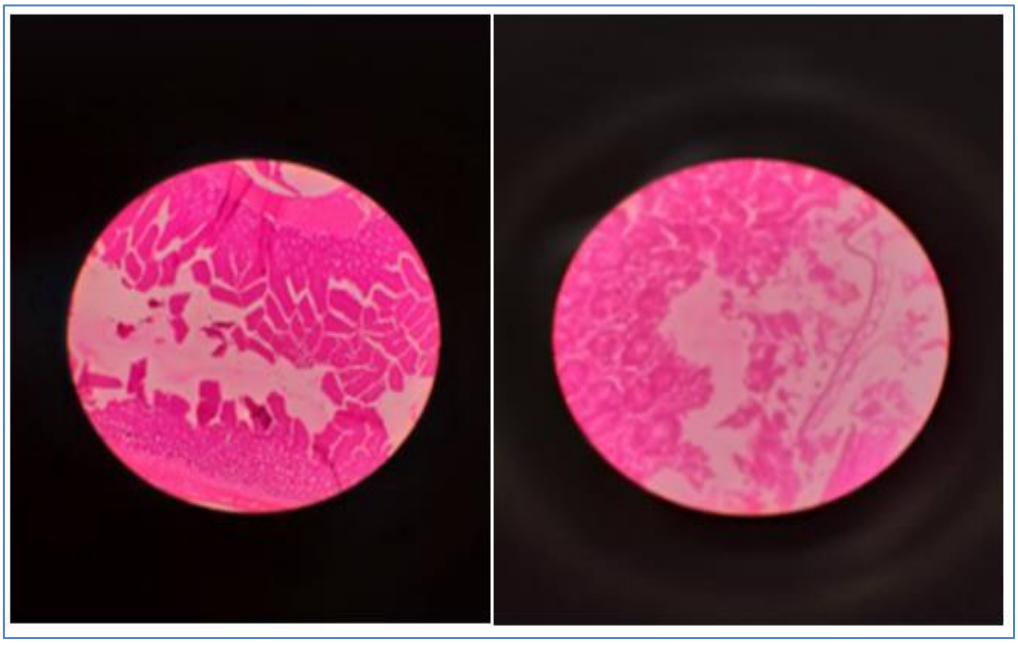

Figure 8: The histologic sections of the jejunal region small intestine show tall villus glands lined by normal absorptive columnar epithelial cells and a few Goblet cells. There are abundant fragments of cellulose material seen within the intestinal lumen.

Group 2
Small Intestine

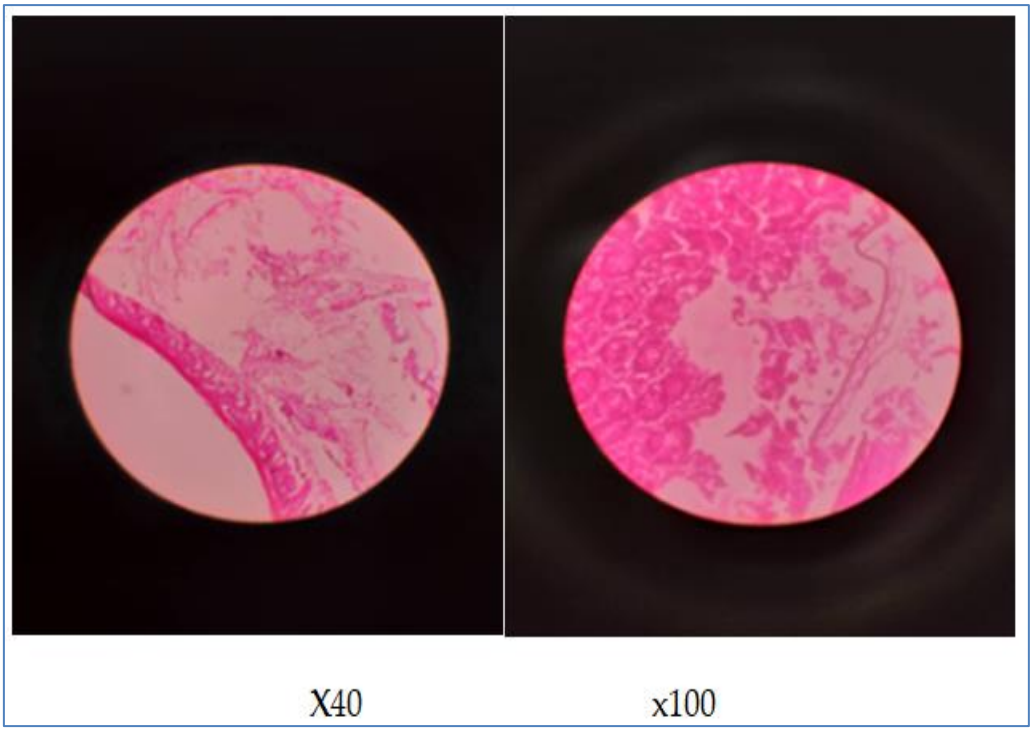

Figure 9: The histologic sections of the small intestine show tall villus glands lined by normal absorptive columnar epithelial cells. There are abundant fragments of cellulose material seen within the intestinal lumen.

Group 3
Small intestine

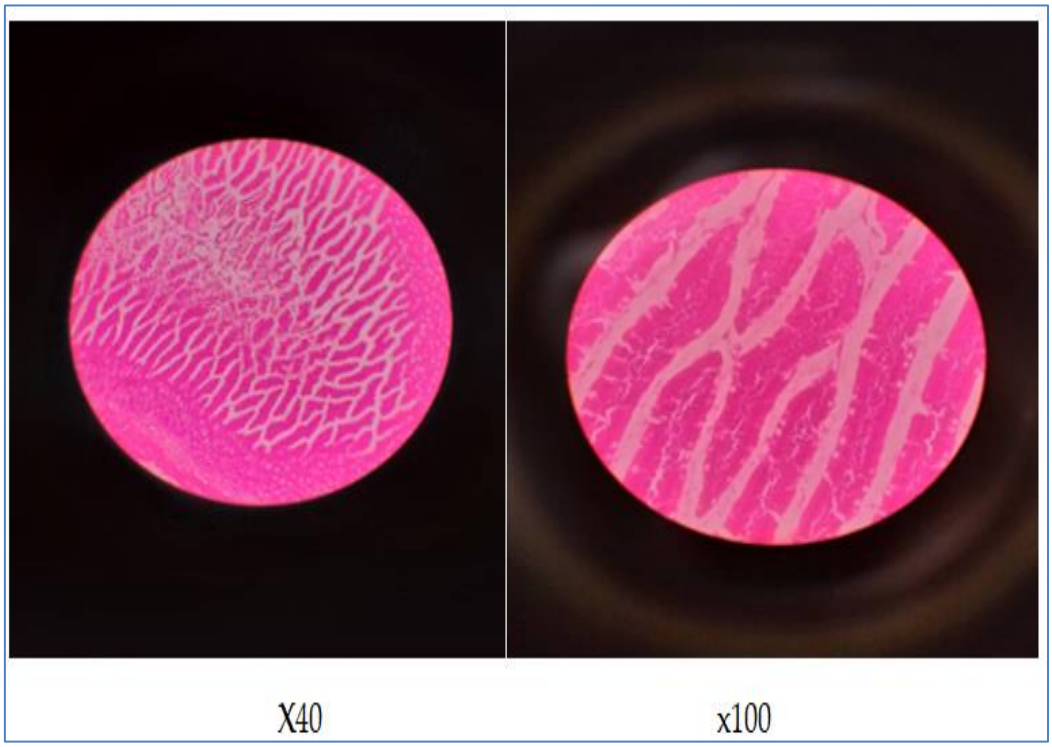

Figure 10: The histologic sections of the jejunal region small intestine show tall villus glands lined by normal absorptive columnar epithelial cells and a few Goblet cells.

Group 4

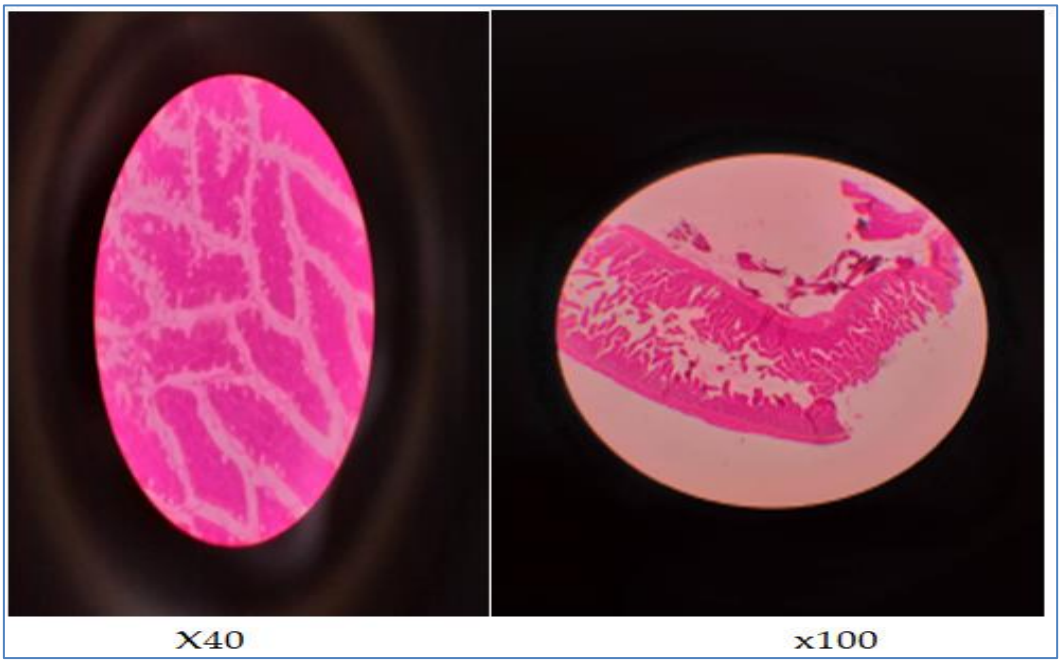

Figure 11: The histologic sections of the jejunal region small intestine show tall villus glands lined by normal absorptive columnar epithelial cells and a few Goblet cells.

Group 1
Treated with DMH (30 mg/Kg)
10 mg plant extract/Kg/5ml CMC
Liver

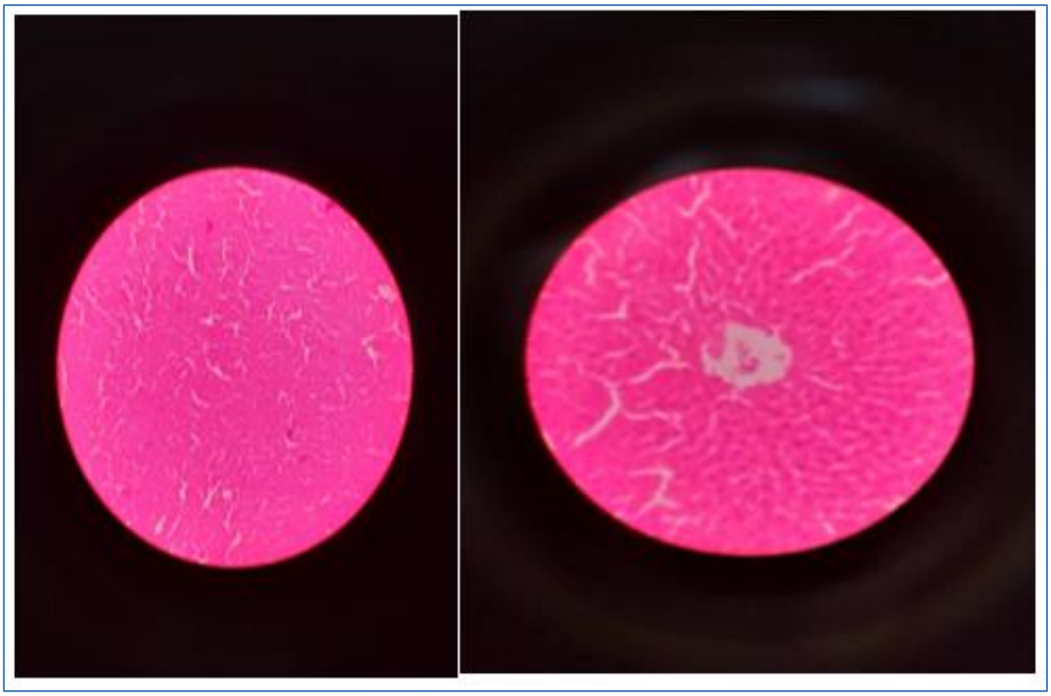

Figure 12: Histologic sections of the liver show trabeculae of mature hepatocytes with intervening portal triads and portal veins.

Group 2
Liver

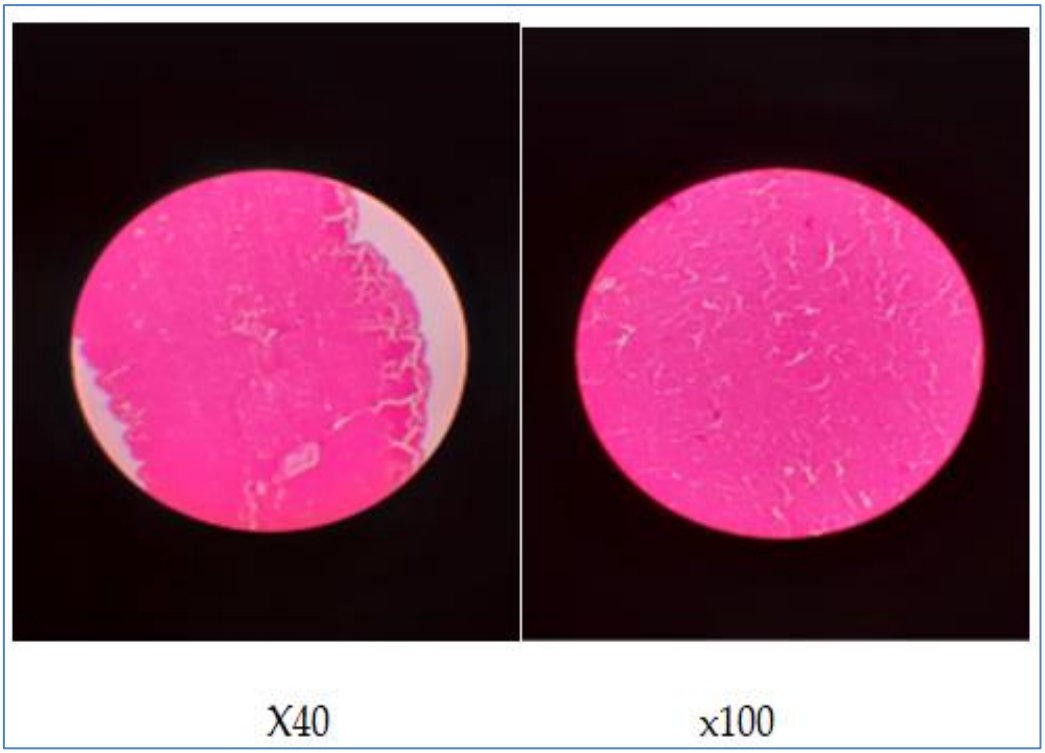

Figure 13: Histologic sections of the liver show trabeculae of mature hepatocytes with intervening portal triads and portal veins.

Group 3
Liver

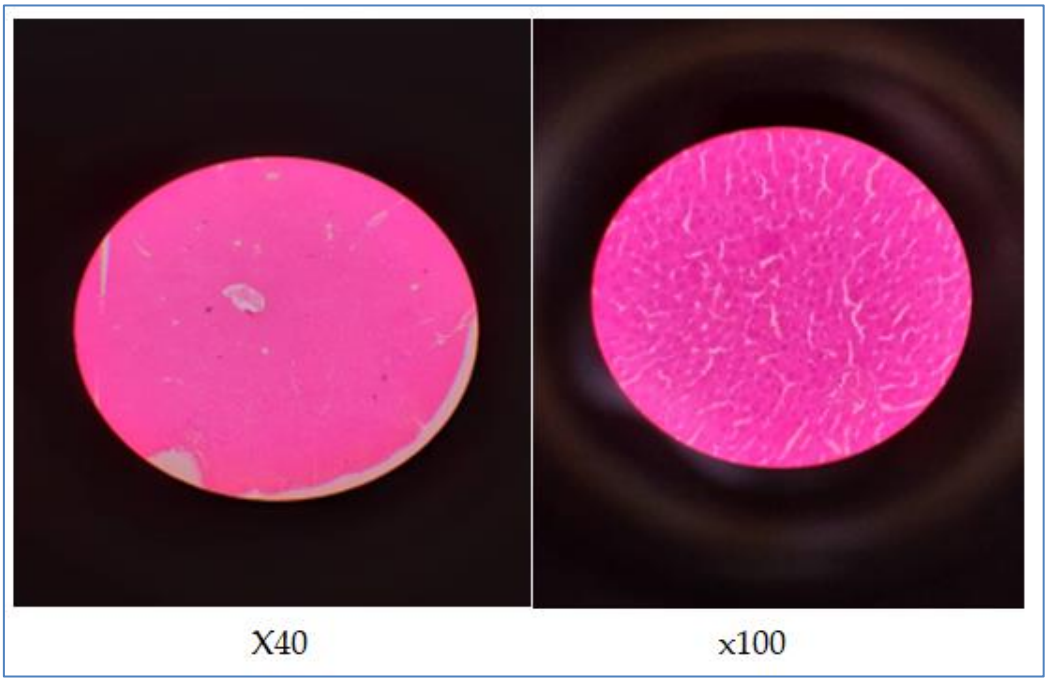

Figure 14: Histologic sections of the liver show trabeculae of mature hepatocytes with intervening portal triads and portal veins.

Group 4
Liver

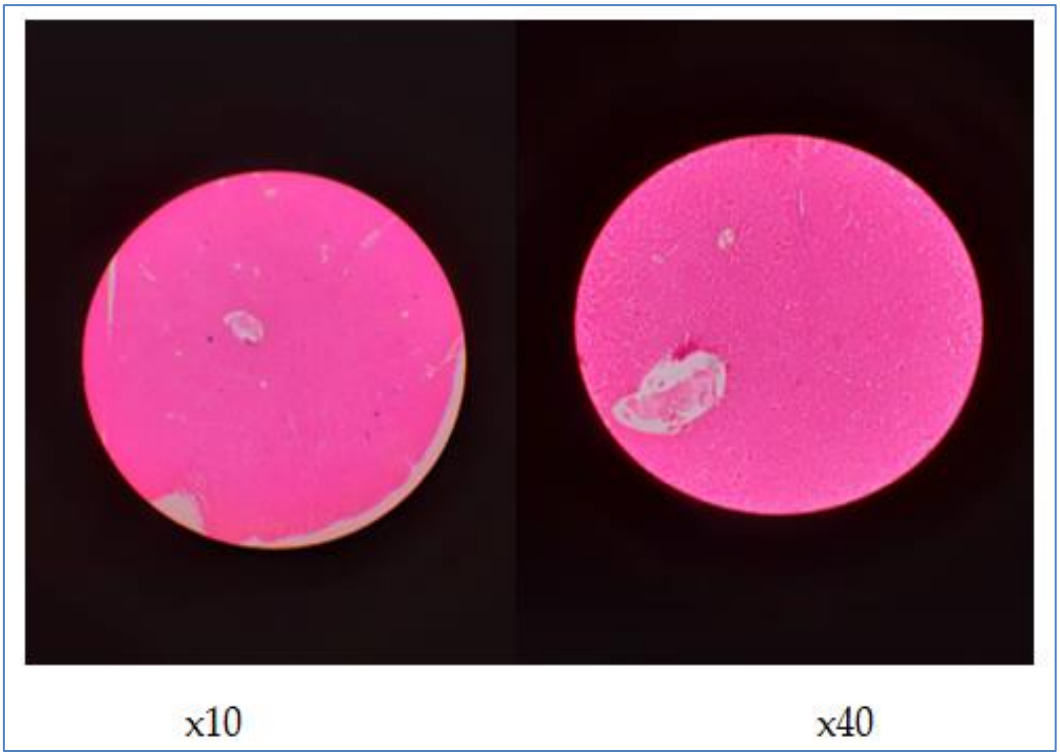

Figure 15: Histologic sections of the liver show trabeculae of mature hepatocytes with intervening portal triads and portal veins.

Tetrapleura tetraptera is a medicinal plant used in traditional folk medicine for treating various ailments such as pain, fever, inflammation, ulcers, wound, and leprosy. This plant has been reported to have a wide variety of uses which include the antimicrobial (Ekwenye & Okorie, 2010), antidiabetic, antihypertensive, anti-Alzhemic, anticonvulsant and anticancer activity Fields (Akah & Nwambie, 1993).

The lethal dose (LD_{50}) value of ethanol fruit of Tetrapleura tetraptera was found to be 1000mg/kg. This suggests that a concentration of the dry ethanolic extracts within the range of 50-1000mg/kg is not toxic to rats. This study, therefore, suggests that the dry fruit of *Tetrapleura tetraptera* at this dose is safe. The study of (Okokon *et al.,* 2007) supports our findings where the LD50 of the ethanolic extracts of *Tetrapleura tetraptera* used proved to be toxic in higher concentrations with a range of 1000-5000mg/kg and the LD50 of the extract in mice was calculated to be 3240.37mg/kg.

A class of phase II detoxification enzymes known as glutathione-S-transferases (GSTs) catalyze the conjugation of glutathione (GSH) to a wide range of endogenous and foreign electrophilic chemicals. It is possible that GSTs play two separate functions in the development of drug resistance via direct detoxification and functioning as an inhibitor of the MAP kinase pathway (Townsend & Tew, 2003). GSTs have been linked to the development of resistance toward chemotherapeutic drugs. In this study, rats treated with high-dose (200mg/kg) and low-dose (100mg/kg) extracts of *Tetrapleura tetraptera* fruits were observed to have shown (Fig 1) significantly increased activities ($p<0.05$) of GST in the serum when compared to negative control. This is in agreement with the result of (Erukainure *et al.,* 2011). Their result showed a significant increase in the antioxidant enzyme activity and the GST level of rats treated with crude extract of ethanolic fruits of *Tetrapleura tetraptera*. This suggests that both doses of ethanolic extracts of *Tetrapleura tetraptera* fruits used in this study may have antioxidant potential. This implies that *Tetrapleura tetraptera* may be associated with decreased oxidative stress and free radicals.

Catalases are ubiquitously present in aerobic organisms, including almost all mammalian tissues, in which they show the highest enzyme activity in the liver and erythrocytes. In erythrocytes, catalase is the first line of defence against H_2O_2 (Lukas A. Mueller *et al.,* 1997). There is evidence that moderate oxidative stress induces catalase expression in vascular cells and, thereby, could be beneficial in the prevention of further oxidative stress (Meilhac *et al.,* 2000). Superoxide anions can also inhibit catalase. In this study (as shown in Fig 2), it was observed that there was a significant increase ($p<0.05$) in the level of catalase in the high-dose ethanolic extract compared to the group administered with low-

dose extract of ethanolic *Tetrapleura tetraptera*. A significant increase was observed in both doses compared to the negative control, this may be as a result of free radicals scavenging activities of the extracts. This is similar to the findings of Narasimhacharya & Visavadiya, 2005) which suggest an increase in the level of catalase when experimenting with the hypolipidemic and antioxidant activities of *Asparagus racemous* in hypercholesteremic rats.

Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. SODs form the front line of defence against reactive oxygen species (ROS)-mediated injury (Kangralkar *et al.*, 2010). These proteins catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide and O_2^- level which damages the cells at excessive concentrations (Yasui & Baba, 2006). In this study (as shown in Fig 3), it was observed that there was a significant increase ($p>0.05$) in the level of superoxide dismutase (SOD) in high-dose ethanolic extract of *Tetrapleura tetraptera* fruits compared to the group administered with low-dose extract and an increase in both doses compared to the negative control which could be as a result of oxidative stress. This is similar to the study of (Badami *et al.*, 2003), which suggests a significant increase in the level of SOD when *Caesalpinia sappan* Heartwood has experimented on rats.

Histological examination is a standard for evaluating treatment related to pathological changes in tissues and organs of tested animals. In this study, the DMH administered to the rats (as shown in Fig 4- Fig 15) had no effect on the morphology of the liver, colon, and small intestine and cancer was not induced. This is because the potency of the dosage given to the rats was not enough to induce cancer but the DMH caused oxidative stress on the rat.

CONCLUSION

The result of the research of this study confirms the antioxidant potential of the alcoholic extract of *Tetrapleura tetraptera* fruits. The toxicity study from the LD_{50} analysis also confirms the safety and non-toxicity of the *Tetrapleura tetraptera alcoholic* extract on the rats. From the results from the antioxidant enzymes analysis it could be concluded that *Tetrapleura tetraptera fruits have* a potent antioxidant activity and this is due to their ability to scavenge free radicals, safe within the tested concentration also, the DMH used did not induce cancer but caused oxidative stress on the rat, which may be due to the low potency of the dosage used. Therefore, naturally occurring molecules in the fruits of *Tetrapleura tetraptera* can have a possible therapeutic application against oxidative damage-induced diseases.

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