

**ANTI-NOCICEPTIVE EFFECT OF ACTIVATED CHARCOAL OF *TERMINALIA CATAPPA* STEM BARK ON ACETIC ACID-INDUCED PAIN IN WISTAR ALBINO RATS****\*<sup>1</sup>Samuel I. Ojeaburu, <sup>1,2,3</sup>Olusanya Olasehinde and <sup>1</sup>Rosephine Enadeghe**<sup>1</sup>Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria<sup>2</sup>Laboratory of Protein and Peptide Pharmaceuticals and Proteomics Laboratory, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.<sup>3</sup>University of Chinese Academy of Sciences, Beijing 100049, China.\*Corresponding authors' email [samuel.ojeaburu@uniben.edu](mailto:samuel.ojeaburu@uniben.edu) Phone: +2348035844852**ABSTRACT**

There are numerous medicinal plants with analgesic potential. Activated charcoal is often used in traditional medicine to treat poisons, pains, and hangovers. The quest for effective and safe analgesics has been an ongoing challenge in modern medicine, as many conventional analgesics are associated with adverse effects, such as gastrointestinal complications. This study evaluated the anti-nociceptive effect of pulverized activated charcoal gotten from *Terminalia catappa*. Twelve rats Male and female Albino rats weighing between 150-180 g (Average weight is 150 g) were divided into four groups of three rats each. Group 1 received distilled water while groups 2-4 received 0.5 ml/kg body weight of 0.8% of acetic acid intraperitoneally, 30 mins after treatment with extract (200 mg/kg body weight of activated charcoal) and standard drugs (300 mg/kg body weight of Aspirin). The results revealed a significant decrease ( $p \leq 0.05$ ) in the number of writhing in group 3 and 4 treated with 200 mg/kg body weight activated charcoal ( $4.21 \pm 0.11$ ) and 300 mg/kg body weight Aspirin ( $4.00 \pm 0.21$ ) when compared with group 2 ( $11.31 \pm 0.12$ ) not treated acetic acid pain induced. Antioxidant parameters evaluated revealed a significant increase ( $p \leq 0.05$ ) in sodium dismutase (SOD), catalase (CAT), glutathione reductase (GSH), glutathione peroxidase (GPX) and total protein (TP) concentrations when compared to control group 1, and in group 2 untreated acetic acid pain induced rats. A significant decrease ( $p \leq 0.05$ ) in Malondialdehyde (MDA) levels in group 3 and 4 when compared to group 2 not treated acetic acid pain induced.

**Keywords:** Anti-nociceptive, Poisons, Pain, Writhing, Hangovers, Inflammation**INTRODUCTION**

Pain, a complex and multifaceted phenomenon, is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Raja *et al.*, 2020). It is a primary clinical concern and a leading cause of disability worldwide, affecting millions of individuals and imposing substantial economic and social burdens (Sivakumar and Deepa, 2020). The quest for effective and safe analgesics (pain-relieving agents) has been an ongoing challenge in modern medicine, as many conventional analgesics are associated with adverse effects such as gastrointestinal complications, cardiovascular risks, and potential drug dependence (Ma *et al.*, 2023). Reports indicate that *Terminalia catappa* possesses diverse bioactive compounds, such as tannins, flavonoids, saponins, and triterpenoids (Mwangi *et al.*, 2024).

Several studies have investigated the potential medicinal properties of different parts of *Terminalia catappa*. For example, the methanolic extract of *Terminalia catappa* leaves has been shown to effectively reduce inflammation and ease pain in animal models (Mwangi *et al.*, 2024). On the other hand, herbalists often transform the plant's stem bark to achieve analgesic effects. Its highly porous structure and large surface area allow it to adsorb and bind to various molecules, including toxins, allergens, and inflammatory mediators (Lawtae and Tangsatitkulchai, 2021). When administered to treat acute poisoning, activated charcoal binds and removes ingested toxins from the gastrointestinal tract, preventing their absorption into the systemic circulation (Bonilla-Velez and Marin-Cuero, 2017). The acetic acid-induced writhing test is a well-established and widely used experimental model for evaluating the analgesic potential of various substances (Daniel Le Bars *et al.*, 2001). In this model, the intraperitoneal

(abdominal cavity) injection of acetic acid in rodents, such as mice or rats, induces visceral pain, leading to characteristic writhing movements. We record the number of writhing movements and use them to indicate the animals' pain intensity (Gawade, 2012). Over-the-counter pain relievers like Paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs), like Ibuprofen have been used over the years as anti-nociceptive agents with their attendant side effects (Romano *et al.*, 2012). Considering the frequent indication of the activated charcoal of *Terminalia catappa* stem bark for the treatment of inflammation of several aetiologies in Nigerian folk medicine, this study therefore evaluated the anti-nociceptive effect of pulverized activated charcoal derived from *Terminalia catappa*.

**MATERIALS AND METHODS****Preparation of charcoal**

Hard bark of *Terminalia catappa* was gotten fresh from the tree, chopped into pieces, dried for 30 days and subjected to a Thermofitcher muffle furnace at 1450 °C in the absence of oxygen for about an hour to allow all the stem bark to turn activated charcoal. Charcoal was pulverized to smooth powder and then subjected to analysis.

**Pain induction/Analgesic evaluation**

The induction of pain and analgesic activity of activated charcoal was evaluated using the acetic acid-induced writhing test. The rats were divided into four groups labelled groups 1-4; each group consisting of three rats. Groups 1 and 2 received distilled water as a normal and negative control; groups 2 and 4 received 200 mg/kg body weight activated charcoal and 300 mg of aspirin, respectively. Thirty minutes after, intraperitoneal injections of 0.6% acetic acid in 100 ml saline

water into the abdominal region of all the rats in groups 2-4 after 30 minutes of treatment. The percentage inhibition of activated charcoal against acetic acid was calculated using the following formula;

$$\% \text{ inhibition} = \frac{N_C - N_T}{N_C} \times 100$$

where

$N_C$

= Number of viable cells in the control tube, and  $N_T$

= Number of viable cells in the testing tube

#### Blood collection and sample preparation

Blood samples were obtained by cardiac puncture and stored in heparin bottles. The samples were then subjected to centrifugation at a temperature of 3 degrees Celsius for a duration of 15 minutes. This centrifugation process was performed to separate the serum, which was subsequently used for the examination of various biochemical parameters, including MDA levels and spectrophotometric measurements of SOD, GSH, CAT, TP, and GPX.

#### Determination of SOD activity

The measurement of SOD activity was conducted using the method given by Popov and Lewin of Popov and Lewin (Popov and Lewin, 1999). Xanthine oxidase was employed as the substrate in this approach. The blank and sample tubes were measured using distilled water as a reference at a wavelength of 560 nm. The results were quantified in units per litre (U/L).

#### Determination of CAT activity

The activity of the CAT enzyme was assessed using Aebi's method, as described in Aebi (1984) publication. The test measures the rate at which  $H_2O_2$  breaks down at a wavelength of 240 nm. The results were quantified and reported in units per litre (U/L).

#### Determination of GSH level

The glutathione level was determined using the method established by Beutler *et al.* (1963). 200  $\mu$ l of serum was

supplemented with 800  $\mu$ l of phosphate buffer. The initial absorbance (OD1) at a wavelength of 412 nm was measured. Subsequently, 100  $\mu$ l of Ellman's reagent was introduced into the identical tube, and the second absorbance (OD2) was documented.

#### Determination of GPX activity (U/mg)

Glutathione peroxidase activity was measured using the Beutler *et al.* (1963) technique. 200  $\mu$ l of serum was supplemented with 800  $\mu$ l of phosphate buffer. The initial absorbance (OD1) at a wavelength of 412 nm was measured. Subsequently, 100  $\mu$ l of Ellman's reagent was introduced into the identical tube, and the second absorbance (OD2) was documented.

#### Determination of MDA level

The MDA level was assessed using the methodology described by Gutteridge (1981). The absorbance measurements were taken using a UV/Vis spectrophotometer at a wavelength of 532 nm.

#### Determination of total protein

The activity of the CAT enzyme was assessed using Aebi's method, as described in Aebi (1984) publication. The test operates on the basis of measuring the rate at which  $H_2O_2$  breaks down at a wavelength of 240 nm. The results were quantified in units per litre (U/L).

#### Statistical Analysis

Mean and standard deviation were used in the data's descriptive statistics. In paired group comparisons regarding continuous variables, the T-test was utilized where standard deviation was achieved, and Mann-Whitney U statistics was used where it was not. In addition, ROC curve analysis was performed to evaluate their performance in differentiating the patient group from the control group. The statistical significance level was  $p < 0.05$  in the calculations and SPSS (version: 13) package.

## RESULTS AND DISCUSSION

**Table 1: Effect of Activated charcoal on abdominal writhing**

Groups	Treatment	Number of writhing	Temperature( $^{\circ}$ C)
Group 1	Control (water only)	0.00 $\pm$ 0.00	32
Group 2	Control(2 ml 0.8% Acetic acid no treatment)	11.01 $\pm$ 0.1	39
Group 3	200 mg activated charcoal +2 ml 0.8% acetic acid	4.21 $\pm$ 0.5*	34
Group 4	300 mg Aspirin + 2 ml 0.8% Acetic	4.00 $\pm$ 0.1*	34

Animals in groups 3 and 4, which were given 200 mg of activated charcoal and 300 mg of Aspirin, respectively, and then exposed to acetic acid thirty minutes later, exhibited a notable reduction in the frequency of writhing episodes compared to animals in group 2, which were not treated but

triggered with pain. This reduction was statistically significant ( $p < 0.05$ ). Values are multiple and are represented as the average  $\pm$  standard error of the mean. Values marked with an asterisk (\*) exhibit a significant difference when compared to group 1 ( $p < 0.05$ ).

**Table 2: Effect of Activated charcoal on some antioxidant parameters**

Parameters	Groups			
	Group 1	Group 2	Group 3	Group 4
GPX (U/mg protein)	23.99 $\pm$ 0.690	10.38 $\pm$ 1.359*	18.63 $\pm$ 2.339*	20.14 $\pm$ 1.736
TP (g/L)	75.45 $\pm$ 2.405	78.65 $\pm$ 3.814	80.47 $\pm$ 8.141	86.59 $\pm$ 7.800
MDA (mole/mg protein) *10 $^{\wedge}$ 3	0.16 $\pm$ 0.0001	0.198 $\pm$ 0.0001*	0.156 $\pm$ 0.0009*	0.130 $\pm$ 0.0005
GSH (mol/L)	0.085 $\pm$ 0.0014	0.037 $\pm$ 0.003*	0.062 $\pm$ 0.0094*	0.092 $\pm$ 0.0057
SOD (U/mg protein)*10 $^{\wedge}$ 3	11.63 $\pm$ 0.033	5.05 $\pm$ 0.659*	9.29 $\pm$ 0.962*	10.99 $\pm$ 0.460
Catalase (U/mg protein)*10 $^{\wedge}$ 3	3.06 $\pm$ 0.089	1.21 $\pm$ 0.205*	2.48 $\pm$ 0.237*	2.89 $\pm$ 0.122

Values are oxidative stress indices and are expressed as mean  $\pm$  SEM. Values with asterisk (\*) are significantly different when compared with group 1 ( $p < 0.05$ ).

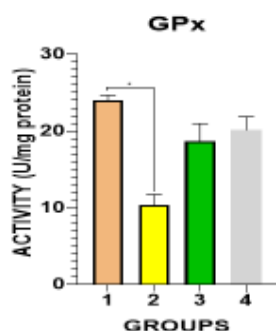


Figure 1: Rats exposed to 200 mg activated charcoal and 300 mg Aspirin (group 3 and 4) and later induced with acetic acid pain showed significant ( $p < 0.05$ ) increase in GPx activity when compared to group 2 exposed to distilled water and later induced with pain (acetic acid). Each column represents the mean  $\pm$  SEM. Values of GPx level.

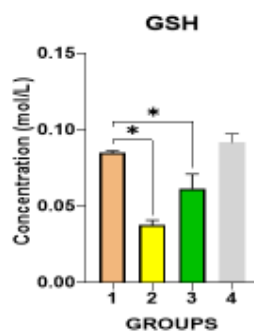


Figure 2: Rats exposed to 200mg activated charcoal and 300 mg Aspirin (group 3 and 4) and later induced with acetic acid pain showed significant ( $p < 0.05$ ) increase in GSH activity when compared to group 2 exposed to distilled water and later induced with pain (acetic acid). Each column represents the  $\pm$  SEM. Values of GSH level.

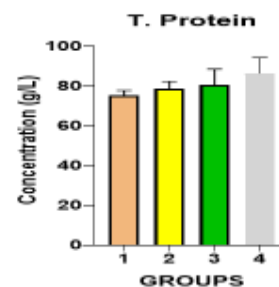


Figure 3: Rats exposed to distilled water, 200 mg activated charcoal and 300 mg Aspirin (group 2, 3 and 4) and later induced with acetic acid pain model showed an increase that was not significant ( $p > 0.05$ ) when compared to control group.

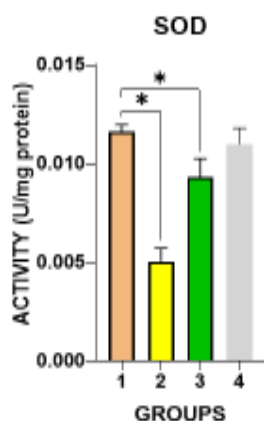


Figure 4: Rats exposed to 200 mg activated charcoal and 300 mg Aspirin (group 3 and 4) and later induced with acetic acid pain showed significant ( $p < 0.05$ ) increase in SOD activity when compared to group 2 exposed to distilled water and later induced with pain (acetic acid). Each column represents the mean  $\pm$  SEM. Values of SOD level.

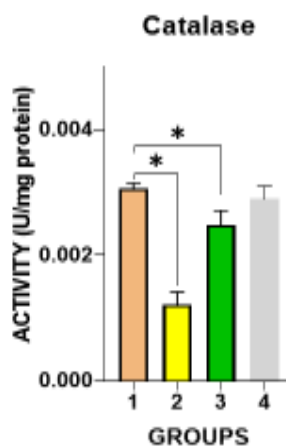


Figure 5: Rats exposed to 200 mg activated charcoal and 300 mg Aspirin (group 3 and 4) and later induced with acetic acid pain showed significant ( $p < 0.05$ ) increase in CAT activity when compared to group 2 exposed to distilled water and later induced with pain (acetic acid). Each column represents the mean  $\pm$  SEM. Values of CAT level.

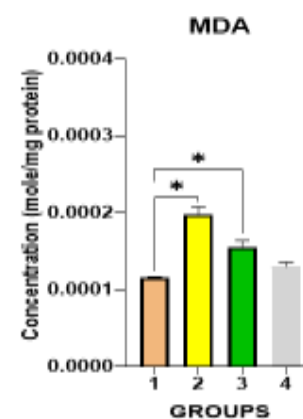


Figure 3: Rats exposed to distilled water group 2 and later induced with acetic acid pain model showed an increase that was not significant ( $p > 0.05$ ) when compared to group 1, 3 and 4.

## Discussion

Medicinal plants have long been investigated for their pain-alleviating properties, with ongoing research aimed at substantiating their therapeutic claims. The efficacy of these plants can vary depending on factors such as specific conditions, dosage, and route of administration. The data presented in Table 1 underscore the potential therapeutic benefits of activated charcoal (AC) and aspirin in acetic acid-induced pain models in animals. Rats administered with 200 mg of activated charcoal and 300 mg of aspirin (Groups 3 and 4) exhibited a significant ( $p < 0.05$ ) reduction in writhing episodes (4.21 and 4.00, respectively) compared to untreated animals induced with pain (Group 2, 11.00). Writhing movements, indicative of pain intensity and analgesic effect of AC, were recorded following induction with acetic acid into the peritoneal cavity. This injection typically stimulates the release of inflammatory mediators such as prostaglandins

(PGE<sub>2</sub>), bradykinin, histamine, serotonin, and reactive oxygen species from the peritoneal lining and visceral tissues. Reactive oxygen species (ROS) are byproducts of normal cellular metabolism, particularly in the mitochondria during ATP production. Medicinal plants exert analgesic effects through various biochemical mechanisms, modulating the endogenous opioid system (like endorphins, enkephalins, and dynorphins), which is crucial for pain regulation. The control group (Group 1) treated with water alone exhibited no writhing in agreement with Smith *et al.*, 2022. The untreated group (Group 2) induced with acetic acid showed a significant number (increase) of writhing episodes ( $11.01 \pm 0.1$ ), which is consistent with the well-established acetic acid-induced abdominal writhing model for evaluating analgesic activity (Jones and Brown, 2019). Interestingly, animals treated with 200 mg of activated charcoal (Group 3) or 300 mg of aspirin (Group 4) before the induction of acetic

acid pain demonstrated a substantial reduction in the number of writhing episodes ( $4.21 \pm 0.5$  and  $4.00 \pm 0.1$ , respectively) compared to the untreated group (Group 2) (Smith *et al.*, 2022). This significant decrease in writhing episodes suggests that activated charcoal and aspirin possess analgesic properties and can effectively alleviate acetic acid-induced visceral pain in this animal model. The acetic acid-induced writhing model is a widely accepted method for evaluating the analgesic potential of various compounds (Collier *et al.*, 1968). The writhing response is mediated by releasing various inflammatory mediators, such as prostaglandins, histamine, and serotonin (D. Le Bars *et al.*, 2001). The observed reduction in writhing episodes with activated charcoal and aspirin treatment could be attributed to their ability to modulate these inflammatory pathways and associated pain perception (Silva-Correa *et al.*, 2021).

Aspirin, a nonsteroidal anti-inflammatory drug (NSAID), is well-known for its analgesic and anti-inflammatory properties, which are primarily mediated through the inhibition of cyclooxygenase (COX) enzymes and subsequent reduction in prostaglandin synthesis (Vane and Botting, 1998, 2003). The analgesic effect of aspirin observed in this study is consistent with its established mechanism of action and previous reports (Cadavid, 2017).

However, the analgesic effect of activated charcoal is not as well-documented, and the underlying mechanisms are not fully understood. Activated charcoal is primarily known for its ability to adsorb and remove toxins, drugs, and other substances from the gastrointestinal tract (Juurlink, 2016). It is possible that the observed analgesic effect of activated charcoal in this study could be due to its adsorptive properties, wherein it may adsorb specific inflammatory mediators or pain-inducing substances from the gastrointestinal tract, thereby reducing the perception of visceral pain (Hassen and Abdulkadir, 2022). Additionally, activated charcoal has been reported to possess antioxidant and anti-inflammatory properties, which could contribute to its analgesic effect (Zhang *et al.*, 2022). AC may contain phytochemicals that have OH and C=O groups. These compounds with OH groups and oxygen atoms C=O can donate hydrogen atoms to free radicals, neutralize their scavenging effect and chelate metals (Enadeghe and Omeregje, 2023). The presence of alternating single and multiple bonds in phytochemicals in AC may provide resonance stability to the molecule, thus allowing easy interaction with reactive oxygen species (ROS) (Enadeghe and Omeregje, 2023). The antioxidant potential of activated charcoal may alleviate pain by excessively reducing oxidative stress. Activated charcoal may reduce pain by inhibiting the inflammatory mediators and enzymes including like prostaglandins (PGE<sub>2</sub>), cyclooxygenase (COX) thus reducing the synthesis of prostaglandins a mechanism similar to aspirin.

Data in Table 2 show the effect of activated charcoal and aspirin on various antioxidant parameters, and the data suggests a potential role of these compounds in modulating oxidative stress and enhancing antioxidant defence mechanisms. Excessive ROS can damage cellular components, leading to oxidative stress. Antioxidant enzymes protect cells from oxidative damage by neutralizing or mopping ROS.

The control group (Group 1) exhibited higher levels of antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase compared to the untreated group (Group 2). This result agrees with Gusti *et al.* (2021), who reported the same increase. This observation is consistent with the expected physiological levels of these enzymes, which play crucial roles in scavenging reactive

oxygen species (ROS) and maintaining the redox balance within healthy cells (Zandi and Schnug, 2022).

Conversely, the untreated group (Group 2) showed significant reduction levels of these antioxidant enzymes, accompanied by an elevated level of malondialdehyde (MDA), a marker of oxidative stress (Cordiano *et al.*, 2023). This finding suggests that the experimental condition or treatment protocol used in this study induced oxidative stress, leading to a depletion of antioxidant defences and increased lipid peroxidation, as evidenced by the higher MDA levels.

Interestingly, treatment with activated charcoal (Group 3) and aspirin (Group 4) significantly improved the levels of GPX, SOD, and catalase and reduced MDA levels compared to the untreated group (Group 2). This result agrees with Cordiano *et al.* (2023), who reported the same. SOD converts superoxide radicals into hydrogen peroxide and oxygen. Observation indicates that activated charcoal and aspirin possess antioxidant properties and can potentially alleviate oxidative stress by enhancing the activity of antioxidant enzymes and reducing lipid peroxidation.

The antioxidant properties of aspirin have been extensively studied. They are attributed to their ability to scavenge free radicals, inhibit the production of ROS, and modulate the activity of antioxidant enzymes (Falco *et al.*, 2023). Aspirin has also been shown to upregulate the expression of various antioxidant enzymes, including SOD, catalase, and glutathione-related enzymes, contributing to its overall antioxidant effects (Jian *et al.*, 2016).

On the other hand, the antioxidant potential of activated charcoal may be its ability to adsorb and remove free radicals, reactive oxygen species, and other pro-oxidant molecules, thereby alleviating oxidative stress (Chaudhary *et al.*, 2023). Additionally, activated charcoal has been reported to possess intrinsic antioxidant properties due to its porous structure and the presence of functional groups that can scavenge free radicals (Enadeghe and Omeregje, 2023)

## CONCLUSION

The antioxidant and anti-inflammatory properties of activated charcoal and aspirin may synergistically contribute to their observed analgesic effects. Oxidative stress and inflammation are intricately linked processes, and the ability of these compounds to mitigate oxidative stress may potentially modulate inflammatory pathways and influence pain perception (Sidhic *et al.*, 2023). In conclusion, the findings of this study suggest that activated charcoal exerts analgesic effects through multiple mechanisms, including adsorption, anti-inflammatory actions, antioxidant properties, and modulation of neurotransmitter activity. These attributes highlight activated charcoal as a promising and cost-effective candidate for analgesic therapy."

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