NISEB Journal Vol. 19, No. 2. June, 2019 1595-6938/2019
Printed in Nigeria (2019) Society for Experimental Biology of Nigeria
http://www.ojs.klobexjournals.com/index.php/nisebj

# Evaluation of the Analgesic Efficacy of Paracetamol in the Presence of *Hibiscus sabdariffa* Calyx Aqueous Extract in Rats

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## Abstract
*The influence of various doses of the aqueous extract of Hibiscus sabdariffa calyx (AEHSC) on the efficacy of paracetamol when co-administered to rats experiencing formalin – induced pain has been examined. After appropriate treatments, each rat was monitored for pain sensation for a total period of 90 minutes. In the formalin only group of rats(group B; 250µl of 5% formalin in saline / kg bw) the pain lingered till the 90$^{th}$ minute. In the paracetamol (500 mg/ kg bw in dimethylsulphoxide orally by gavage) and formalin group (group C) the pain sensation abated by the 70$^{th}$ minute. At all the AEHSC treated groups (100,200,300,400, 500 and 600 mg/kg bw by gavage), groups D,E,F,G,H and I in which the extract was administered along with paracetamol and formalin, pain sensation ceased by the 70$^{th}$ minute as shown in group C rats. The most effective dose of the extract was 500 mg /kg bw in view of the steady drop in pain sensation till the 70$^{th}$ minute when it terminated. Evidently the analgesic efficacy of paracetamol was not lost in the presence of AEHSC.*

**Keywords**: Analgesic efficacy, Formalin, Paracetamol, *Hibiscus sabdariffa* calyx, Rats

## Introduction
Paracetamol is a commonly used over-the-counter analgesic and antipyretic drug (1). The conventional dose of paracetamol is 500 to 1000mg every 4 to 6 hours with a maximum daily dose of 4000mg for a 70kg adult human. The toxic action of this drug is attributed to its principal metabolite, N-acetyl-p-benzoquinoneimine (NAPQ 1) (2).
This drug is readily available; a factor that makes its misuse or overdose almost inevitable. Overdose can lead to kidney and liver injuries (3,4). It has been reported that in some individuals, paracetamol toxicity can result from normal usage and dose (5) although another school of thought believes that this is due to overuse or combinations of the drug with opioids (6). Results obtained from animal model studies reveal that non-therapeutic doses of paracetamol are toxic. This has been demonstrated using mice (7,8) and rats (9). In order to protect the kidney and liver from the side effects of paracetamol, even at therapeutic doses, the action of extracts of botanicals such *Hibiscus sabdariffa* L. calyx have been examined (7,8,10,).
It is conceivable that the toxic effects of paracetamol, even if it occurs at the therapeutic doses in some humans as has been suggested (5) can be diminished or completely eliminated by co-administration with *H. sabdariffa*calyx aqueous extract. Orji and Obi (7,8) hinged their study on this hypothesis and found that all indices of kidney and liver injuries were profoundly and significantly reduced in mice to which non-therapeutic dose of paracetamol and the extract were concurrently administered. However, since the purpose of paracetamol ingestion is for its analgesic and antipyretic actions, there is the concern whether these desirable actions of the drug will be lost when co-administered with aqueous extract of *H. sabdariffa*calyx (AEHSC). Hence, the aim of this study was to evaluate whether the analgesic potency of paracetamol will be lost when co-administered with AEHSC using rats as the animal model.

## Materials and Methods
### Materials
Animals: Forty-five male rats (Wistar strain: weight range 112-188) were obtained from Department of Biochemistry Animal Unit, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
Plant material: *Hibiscus sabdariffa* L. calyces were purchased from Oba Market, Benin City, Edo State, Nigeria.
Chemicals/Reagents: The reagents/chemicals used for this study were physiological sodium chloride solution (UNISAL, 0.9%, NaCl), formalin (Cil Chemicals, Northwood, U.K), dimethylsulphoxide (Loba Chemie Ltd, Mumbai, India) and paracetamol base powder (Tianjain-Bofa, China).

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**Methods**

***Animal treatment for acclimatization and experiment:***

The rats were kept in wood framed and iron meshed sides and base cages for one month to acclimatize before the commencement of the experiments. They were allowed unrestricted access to food (growers mash) and water. This allowed them to acclimatize to their new location within the animal house designated for the study. For the study, the rats were divided into nine experimental groups of 5 rats each.

**Preparation of pain-inducing agent and paracetamol solutions:**

Formalin (5% in saline, v/v) was prepared by adding saline to 5 ml formalin in 100 ml volumetric flask until the 100 ml mark was reached. Paracetamol base powder (3.146g) was dissolved in 6.5 ml dimethylsulphoxide/water mixture (2.25: 1 v/v) to obtain a 0.484 mg/µl paracetamol stock solution.

***Preparation of Hibiscus sabdariffa Linn calyx aqueous extract:***

Ten grams of pulverized dried calyces were weighed and suspended in 100ml distilled water, stirred and left in the refrigerator at 4$^{o}$C for 24 hours. Thereafter, the infusion was filtered through four layers of cheese cloth. In order to know what quantity and volume of the extract to administer to a given rat the solid content in 1ml of the filtrate was determined as described previously (11, 12) and the entire filtrate refrigerated at 4$^{o}$C and used within 48 hours.

***Administration of formalin, paracetamol and AEHSC and monitoring of pain response:***

Group A rats were administered saline by injection into the left hind paw (250 µl kg$^{-1}$ body weight), while group B rats were administered formalin via injection (250 µl kg$^{-1}$bw of 5% formalin in saline via the left hind paw). Group C rats were administered paracetamol dissolved in dimethylsulphoxide (DMSO) orally by gavage (500 mg kg$^{-1}$bw) followed by formalin injection in the manner described for group B rats. Groups D, E, F, G, H and I rats were treated with paracetamol (500 mg kg$^{-1}$bw) concurrently with AEHSC orally by gavage (100, 200, 300, 400, 500 and 600 mg kg$^{-1}$bw respectively) followed by formalin injection.

The response of the rats to saline or formalin induced pain was monitored using the method described by Gong *et al,* (13). Immediately after saline or formalin injection to each rat in the appropriate group, it was placed in a transparent observation chamber and the stopwatch started. Flinching of the left hind limb was monitored for the first 5 minutes and thereafter for every ten minutes (13) for a duration of 90 minutes.

**Statistical Analysis:**

Results are shown as means ±SEM. Data were analyzed using one way analysis of variance (ANOVA) and post hoc least square difference (LSD) test by SPSS software version 23 for window (SPSS, IBM Chicago, IL, USA) with $p \leq 0.05$ considered as statistically significant.

## Results

The pattern of sensitivity of the experimental rats to formalin-induced pain in terms of flinches per minute is presented in Table 1.Two peaks of pain sensation emerged for rats treated with formalin alone (group B), a 10$^{th}$ and 50$^{th}$ minute peaks. However, in this group the pain did not cease even at the 90$^{th}$ minute when relative to the 10$^{th}$ minute pain intensity, the pain had reduced by 93.22%. Rats exposed to paracetamol before formalin injection (group C ) also had two peaks of pain sensation, the 10$^{th}$ and 30$^{th}$ minute peaks. The second peak in this group appeared at the 30$^{th}$ and not at the 50$^{th}$ minute and by the 60$^{th}$ minute the pain had reduced by 98.31% relative to the 10$^{th}$ minute value.

The groups of rats treated with paracetamol and the extract (100 and 200 mg /kg bw) first before formalin injection also exhibited the 10$^{th}$ and 30$^{th}$ minutes two peak pain sensation points. Rats treated with paracetamol and higher doses of the extract before formalin injection, namely, the 300 to 600 mg /kg bw extract groups, had their first peak at the 5$^{th}$ minute and the second at the same 30$^{th}$ minute point except group H (the 500 mg /kg bw extract group) which had only one peak at the 5$^{th}$ minute.Relative to each group's highest pain sensation peak, there were 97.21, 95.87, 99.48, 99.58, 99.21 and 99.90% reduction in pain in groups D to I respectively, before complete cessation (Table 1).

Unlike the formalin only group of rats whose pain sensation lingered unabated even at the 90$^{th}$minute,all other groups, the paracetamol plus formalin group, groups D,E,F,G and H had the pain abolished at the 70$^{th}$ minute except group I rats which though evidenced pain termination at the 70$^{th}$ minute still showed tiny evidence of residual pain at the 80$^{th}$ and 90$^{th}$ minutes ( Table 1).

Vertical or within column comparisons of the pain sensation intensities in terms of flinches per minute are presented in Table 2. The 5$^{th}$ minute peak pain sensation of rats in groups D,E,F ,H and I were significantly ($p \leq 0.05$ ) greater than that of group B, formalin only group. Only groups E and I values were significantly greater than that of group C, the paracetamol and formalin group. In the 10$^{th}$ minute the number of flinches per minute of groups G,H and I rats were significantly *( $p \leq 0.05$* ) lower than that of group C rats. In the 50$^{th}$ to 60$^{th}$ minutes interval the values for groups C,E,F,G,H and I were significantly *( $p \leq 0.05$* ) lower than that of group B, the formalin only group of rats.

The horizontal or within row comparisons of the pain sensation intensities of rats injected with formalin , rats administered paracetamol and injected formalin and those administered extract and paracetamol and injected formalin are presented in Table 3. The 10$^{th}$ minute flinches per minute of the formalin only group, the paracetamol plus formalin, paracetamol plus 100, 200, 300 and 400 mg extract kg$^{-1}$bw plus formalin were significantly *($p \leq 0.05$)* greater than that of any other time point.

- **Table1: Effects of Paracetamol and Varying Doses of AEHSC on Formalin-Induced Pain Sensation**

| Group | Treatment | Pain Sensation (Flinches/min) Mean ± SEM(n=5) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $5^{th}$ min | $10^{th}$ min | $20^{th}$ min | $30^{th}$ min | $40^{th}$ min | $50^{th}$ min | $60^{th}$ min | $70^{th}$ min | $80^{th}$ min | $90^{th}$ min |
| A | Saline (250µl $kg^{-1}$bw) | NFO* | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO |
| B | Formalin (FM) (250µl $kg^{-1}$bw) | 0.16 ± 0.16 | 2.36 ± 0.71 | 0.11 ± 0.05 | 0.48 ± 0.20 | 0.29 ±0.06 | 0.59 ± 0.20 | 0.39 ± 0.11 | 0.30 ± 0.09 | 0.17 ± 0.10 | 0.04 ±0.03 (93.22%)*** |
| C | Paracetamol (PCM) (500 mg $kg^{-1}$ b w) FM (250µl $kg^{-1}$bw) | 0.64 ± 0.17 | 3.24 ± 1.51 | 0.81 ± 0.42 | 0.83 ± 0.38 | 0.60 ± 0.30 | 0.16 ± 0.09 | 0.04 ± 0.03 (98.31%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| D | PCM + AEHSC** (100mg $kg^{-1}$bw) + FM | 1.60 ± 0.35 | 2.12 ± 0.46 | 0.13 ±0.11 | 0.96 ±0.43 | 0.59 ± 0.29 | 0.26 ±0.26 | 0.05 ±0.05 (97.64%) | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ± 0.00 |
| E | PCM + AEHSC (200mg $kg^{-1}$bw) + FM | 2.24 ±0.75 | 2.42 ± 0.91 | 0.80 ±0.30 | 0.96 ± 0.44 | 0.33 ±0.18 | 1.10 ± 0.09 (95.87%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| F | PCM + AEHSC (300mg $kg^{-1}$bw) + FM | 1.94 ± 0.51 | 1.22 ±0.29 | 0.45 ±0.16 | 0.60 ±0.11 | 0.13 ±0.07 | 0.01 ±0.01 | 0.01 ±0.01 (99.48%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| G | PCM + AEHSC (400mg $kg^{-1}$bw) + FM | 0.96 ±0.43 | 0.72 ± 0.29 | 0.17 ±0.07 | 0.33 ±0.18 | 0.06 ±0.06 | 0.004 ±0.004 | 0.004 ±0.004 (99.58%) | 0.00=0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| H | PCM + AEHSC (500mg $kg^{-1}$bw) + FM | 1.52 ±0.55 | 0.74 ±0.05 | 0.34 ±0.18 | 0.22 ±0.18 | 0.15 ±0.14 | 0.012 ±0.012 (99.21%) | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| I | PCM + AEHSC (600mg $kg^{-1}$bw) + FM | 2.04 ±0.47 | 0.64 ±0.28 | 0.45 ±0.15 | 0.96 ±0.17 | 0.68 ±0.19 | 0.21 ±0.07 | 0.02 ±0.01 | 0.00 ±0.00 | 0.03± 0.03 | 0.002 ±0.002 (99.90%) |

- *NFO: No flinch observed
- **AEHSC = Aqueous extract of *Hibiscus sabdariffa* calyx
- *** Values in bracket represent percentage reduction in pain intensity relative to the group's peak pain intensity.

**Table 2: Vertical or Within Column Comparisons of Pain Sensation Intensities**

| Group | Treatment | Pain Sensation (Flinches/min) Mean ± SEM(n=5) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $5^{th}$ min | $10^{th}$ min | $20^{th}$ min | $30^{th}$ min | $40^{th}$ min | $50^{th}$ min | $60^{th}$ min | $70^{th}$ min | $80^{th}$ min | $90^{th}$ min |
| A | Saline (250µl $kg^{-1}$bw) | NFO* | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO |
| B | Formalin (FM) (250µl $kg^{-1}$bw) | 0.16 ± 0.16 | 2.36 ± 0.71 | 0.11 ± 0.05 | 0.48 ± 0.20 | 0.29 ±0.06 | 0.59 ± 0.20 | 0.39 ± 0.11 | 0.30 ± 0.09 | 0.17 ± 0.10 | 0.04 ±0.03 |
| C | Paracetamol (PCM) (500 mg $kg^{-1}$bw) FM (250µl $kg^{-1}$bw) | 0.64 ± 0.17 | 3.24 ± 1.51 | b 0.81 ± 0.42 | 0.83 ± 0.38 | 0.60 ± 0.30 | b 0.16 ± 0.09 | b 0.04 ± 0.03 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| D | PCM + AEHSC^ (100mg $kg^{-1}$bw) + FM | b** 1.60 ± 0.35 | 2.12 ± 0.46 | c 0.13 ±0.11 | 0.96 ±0.43 | 0.59 ± 0.29 | 0.26 ±0.26 | b 0.05 ±0.05 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ± 0.00 |
| E | PCM + AEHSC (200mg $kg^{-1}$bw) + FM | b, c 2.24 ±0.75 | 2.42 ± 0.91 | b, d 0.80 ±0.30 | 0.96 ± 0.44 | 0.33 ±0.18 | b 0.10 ± 0.09 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| F | PCM + AEHSC (300mg $kg^{-1}$bw) + FM | b 1.94 ± 0.51 | 1.22 ±0.29 | 0.45 ±0.16 | 0.60 ±0.11 | 0.13 ±0.07 | b 0.01 ±0.01 | b 0.01 ±0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| G | PCM + AEHSC (400mg $kg^{-1}$bw) + FM | 0.96 ±0.43 | c 0.72 ± 0.29 | c, e 0.17 ±0.07 | 0.33 ±0.18 | c, d 0.06 ±0.06 | b 0.004 ±0.004 | b 0.004 ±0.004 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| H | PCM + AEHSC (500mg $kg^{-1}$bw) + FM | b 1.52 ±0.55 | c 0.74 ±0.05 | 0.34 ±0.18 | 0.22 ±0.18 | 0.15 ±0.14 | b 0.012 ±0.012 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| I | PCM + AEHSC (600mg $kg^{-1}$bw) + FM | b, c 2.04 ±0.47 | c 0.64 ±0.28 | 0.45 ±0.15 | 0.96 ±0.17 | f, g 0.68 ±0.19 | b 0.21 ±0.07 | b 0.02 ±0.01 | 0.00 ±0.00 | b 0.03 ±0.03 | b 0.002 ±0.002 |

*NFO: No flinch observed

^AEHSC = Aqueous extract of *Hibiscus sabdariffa* calyx

**Values with lowercase letters a, b, c ecetera as superscripts within column are significantly different from the value of the group bearing the corresponding uppercase letter A, B, C, D, E, F, G, H or I ($p \leq 0.05$).

**Table 3: Horizontal or Within Row Comparisons of Pain Sensation Intensities**

| Group | Treatment | Pain Sensation (Flinches/min) Mean ± SEM(n=5) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5th min $A^{I}$** | 10th min $B^{I}$ | 20th min $C^{I}$ | 30th min $D^{I}$ | 40th min $E^{I}$ | 50th min $F^{I}$ | 60th min $G^{I}$ | 70th min $H^{I}$ | 80th min $I^{I}$ | 90th min $J^{I}$ |
| A | Saline (250µl $kg^{-1}$bw) | NFO* | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO | NFO |
| B | Formalin (FM*) (250µl $kg^{-1}$bw) | 0.16 ± 0.16 | $a^{I}$*** 2.36 ± 0.71 | $b^{1}$ 0.11 ± 0.05 | $b^{1}$ 0.48 ± 0.20 | $b^{I}$ 0.29 ±0.06 | $b^{I}$ 0.59 ± 0.20 | $b^{I}$ 0.39 ± 0.11 | $b^{I}$ 0.30 ± 0.09 | $b^{I}$ 0.17 ± 0.10 | $b^{I}$ 0.04 ±0.03 |
| C | Paracetamol (PCM) (500 mg $kg^{-1}$bw) FM (250µl $kg^{-1}$bw) | 0.64 ± 0.17 | $a^{I}$ 3.24 ± 1.51 | $b^{I}$ 0.81 ± 0.42 | $b^{I}$ 0.83 ± 0.38 | $b^{I}$ 0.60 ± 0.30 | $b^{I}$, 0.16 ± 0.09 | $b^{I}$ 0.04 ± 0.03 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| D | PCM + AEHSC^ (100mg $kg^{-1}$bw) + FM | 1.60 ± 0.35 | 2.12 ± 0.46 | $a^{I}$, $b^{I}$ 0.13 ±0.11 | $b^{I}$, $c^{I}$ 0.96 ±0.43 | $a^{I}$, $b^{I}$ 0.59 ± 0.29 | $a^{I}$, $b^{I}$ 0.26 ±0.26 | $a^{I}$, $b^{I}$ , $d^{I}$ 0.05 ±0.05 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ± 0.00 |
| E | PCM + AEHSC (200mg $kg^{-1}$bw) + FM | 2.24 ±0.75 | 2.42 ± 0.91 | $a^{I}$, $b^{I}$ 0.80 ±0.30 | $a^{I}$, $b^{I}$ 0.96 ± 0.44 | $a^{I}$, $b^{I}$ 0.33 ±0.18 | $a^{I}$, $b^{I}$ 1.10 ± 0.09 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| F | PCM + AEHSC (300mg $kg^{-1}$bw) + FM | 1.94 ± 0.51 | $a^{I}$ 1.22 ±0.29 | $a^{I}$, $b^{I}$ 0.45 ±0.16 | $a^{I}$, $b^{I}$ 0.60 ±0.11 | $a^{I}$, $b^{I}$ 0.13 ±0.07 | $a^{I}$, $b^{I}$ 0.01 ±0.01 | $a^{I}$, $b^{I}$ , $d^{I}$ 0.01 ±0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| G | PCM + AEHSC (400mg $kg^{-1}$bw) + FM | 0.96 ±0.43 | 0.72 ± 0.29 | $a^{I}$, $b^{I}$ 0.17 ±0.07 | 0.33 ±0.18 | $a^{I}$, $b^{I}$ 0.06 ±0.06 | $a^{I}$, $b^{I}$ 0.004 ±0.004 | $a^{I}$, $b^{I}$ 0.004 ±0.004 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| H | PCM + AEHSC (500mg $kg^{-1}$bw) + FM | 1.52 ±0.55 | 0.74 ±0.05 | $a^{I}$ 0.34 ±0.18 | $a^{I}$ 0.22 ±0.18 | $a^{I}$, $b^{I}$ 0.15 ±0.14 | $a^{I}$, $b^{I}$ 0.012 ±0.012 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| I | PCM + AEHSC (600mg $kg^{-1}$bw) + FM | 2.04 ±0.47 | $a^{I}$ 0.64 ±0.28 | $a^{I}$ 0.45 ±0.15 | $a^{I}$ 0.96 ±0.17 | $a^{I}$ 0.68 ±0.19 | $a^{I}$, $d^{I}$ 0.21 ±0.07 | $a^{I}$, $b^{I}$ , $d^{I}$, $e^{I}$ 0.02 ±0.01 | 0.00 ±0.00 | $a^{I}$, $b^{I}$ , $d^{I}$, $e^{I}$, 0.03 ±0.03 | $a^{I}$, $b^{I}$ , $d^{I}$, $e^{I}$ 0.002 ±0.002 |

*NFO: No flinch observed

^AEHSC = Aqueous extract of *Hibiscus sabdariffa* calyx

**Row of primed uppercase letters.

***Values with primed lowercase letters, $a^{I}$, $b^{I}$, $c^{I}$ et cetera as superscripts across a row are significantly different from the value of the group bearing the corresponding primed uppercase letter $A^{I}$, $B^{I}$, $C^{I}$, $D^{I}$, $E^{I}$, $F^{I}$, $G^{I}$, $H^{I}$, $I^{I}$ or $J^{I}$ ($p \leq 0.05$).

The 5th minute flinches per minute of the group administered paracetamol plus 300, 400, 500 and 600 mg extract $kg^{-1}$bw along with formalin were significantly ($p \leq 0.05$) greater than that of any other time point along the row. Among these groups the 10th and 30th minute values of some (Table 3 groups D and F ) are significantly ($p \leq 0.05$) greater than those of other time points.

## Discussion

Formalin has been used as a pain-inducing agent in various investigations aimed at evaluating pain in animal models (13,14,15) and found to possess this ability. This is in harmony with the findings presented in this report in which rats injected with formalin in saline betrayed evidence of pain by flinching the left hind limb bearing the paw through which the pain-inducing agent was injected. The flinching frequency peaked between the 5th and 10th minute post formalin injection (Table 1). Injection of saline, the vehicle in which the formalin was administered did not cause limb flinching behavior (Table 1, group A).

The pain response of the rats in the formalin only group, following its injection was apparently biphasic. An initial interval that occurred between the 5th and 20th minute and the second less intense pain interval from the 20th to the 90th minute. These apparently correspond to the phase I, (the acute phase) and the phase II (the tonic phase) described by Gong *et al*. (13). In what corresponds to phase II in this study, the intensity of pain rose and ebbed every ten minutes until the 50th minute. From the 50th minute, the pain intensity diminished gradually and uniformly till the 90th minute. The biphasic pattern was also alluded to by other previous workers (14,15). To these later authors, the initial phase is acute response, linked to C-fibre afferent nociceptors. The phase II is believed by others to have arisen from central sensitization of the spinal dorsal horn neurone (16). The initial phase, which in this study was somewhere between the 5th and 20th minute for the formalin only and the other groups, was apparently not short as reported by Gong *et al.* (13) while the phase II pattern and duration are generally in accord with their finding.

Prior administration of paracetamol to rats in group C, before formalin injection did not reduce the intensity of formalin-induced pain in these rats as the 30th and 40th minute flinch rates revealed. The same pattern of rise and fall in pain intensity observed in the formalin only group was also present in the formalin plus paracetamol treated group up to the 40th minute. Paracetamol in this study did not appear to have inhibited pain within the 5th and 40th minute intervals, the apparent phase I period in this study. Its pain inhibitory effect became manifest at the 50th minute. This appears to agree with the findings of earlier investigators that some drugs such as monocycline (17) and many analgesics which include non-steroidal anti-inflammatory drugs (NSAIDs) and N-methyl-D-aspartic acid (NMDA) antagonists, morphine and gabapentin do not inhibit phase I pain responses but inhibit those of phase II (15). If this is so with paracetamol, it would explain why it's pain relieving action appeared not in the 5th and 40th minute segment, which is the apparent phase I in this study, but much later in the segment that is the apparent phase II. This makes the non-response of the formalin treated rats to paracetamol pain "killing" action at the early period, not entirely surprising.

As can be seen from Table 1, starting from the 40th minute, the formalin and paracetamol group of rats had profoundly reduced formalin-induced pain due to paracetamol administration. The analgesic action of paracetamol was evident from the 40th minute to the 60th minute resulting in cessation of pain from the 70th to the 90th minute. The 30th to the 90th minute may therefore, truly represent the phase II pain period in this group of rats. Evidently, paracetamol reduces formalin-induced pain in phase II just like indomethacin (18), monocycline (17) and other NSAIDs (15).

In view of the fact that paracetamol affected the "second phase" pain sensation in this study, the effect of AEHSC on its analgesic efficacy was analyzed within the same pain sensation period in the rats, starting from the 50thminute (Tables 1,2,3). AEHSC when used at a dose of 300 -500 mg / kg bw and the effects examined from the 50th minute, did not appear to have impaired the analgesic action of paracetamol**.** This can be deduced by comparing the flinching pattern of the rats in group C (formalin plus paracetamol group) with the pattern exhibited by the rats in the 300-500 mg AEHSC $kg^{-1}$bw, paracetamol and formalin exposed groups. Paracetamol inhibits the ability of cells to produce prostaglandin, the molecule that triggers pain and inflammation (19). Currently, drug impairment of prostaglandin production is attributed to two possible mechanisms of action. One version is that, it does this by competing with arachidonic acid, the subtrate of prostaglandin synthase for its active site. A second version is that drugs inhibit either cyclooxygenase-2 (COX-2) or cyclooxygenase-3 (COX-3) via a metabolite produced bythe peroxidase function of the isoenzymes (6,20,21,22). The fact that paracetamol still retained its analgesic action in the presence of the extract is an indication that the extract does not interfere with the drug's ability to either inhibit prostaglandin synthase or the COX isoenzymes. The pain relieving potential of paracetamol is also attributed to the ability of one of its metabolites, N-arachidonoylphenolamine (23) to inhibit the re-uptake of endogenous cannabinoids by neurons. This makes the cannabinoids more available biologically to reduce pain. Also, the metabolite of paracetamol produced by the peroxidase function of COX-2 is believed to be capable of depleting cellular glutathione status which is a cofactor of a number of enzymes, such as prostaglandin E synthase (19). Again, if these modes of action of paracetamol were involved in its analgesic

effects in this study, it is highly unlikely that the extract did interfere with any of them otherwise the pain sensation would have prolonged in these groups to 90 or near 90 minutes.

## References


1. Obu HA, Chinawa J, Ubesie A , Eke CB, Ndu IK : Paracetamol use ( and/or misuse) in children in Enugu, South-East, Nigeria. BMC Pediatrics 12 (1): 103-107, 2012.
2. Hodgman MJ,Garrard AR: A review of acetaminophen poisoning. Critical Care Clinics 28: 499-516, 2012.
3. Larson AM, Polson J, Fontana RJ, Davern TJ: Acetaminophen-induced acute liver failure: results of a United States multicenter prospective study. Hepatology (Baltimore, MD), 42(6): 1364-1372, 2005.
4. Daly FF, Fountain JS, Murray L, Graudins A: Guidelines for the management of paracetamol poisoning in Australia and New Zealand-explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australian poisons information centers. The Medical Journal of Australia 188(5): 296-301, 2008.
5. Vuppalanchi R, Liangpunsakul S, Chalasani N: Etiology of new-onset jaundice: how often is it caused by idiosyncratic drug-induced liver injury in the United States? American Journal of Gastroenterology, 102(3):558-562, 2007.
6. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF: The modern pharmacology of paracetamol: therapeutic action, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology 21(3): 201-232, 2013.
7. Orji BO, Obi FO: Effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on paracetamolhepatoxicity in mice. Journal of Pharmaceutical, Chemical and Biological Sciences, 5(2): 108-117, 2017.
8. Orji BO, Obi FO: Subchronic and chronic studies of the effects of concurrent administration of paracetamol and aquesous extract of *Hibiscus sabdariffa* Linn calyx on paracetamolhepatoxicity in mice. Journal of Pharmcognosy and Phytochmemistry 7(1): 2311-3217, 2018.
9. Mohammed MS, Mohammed AN, Tamer AJ: Immunochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in Wistar rats. BMC Complementary Alternative Medicine 14(1):1-11, 2014.
10. Ali BH, Mousa HH, EL-Mougy S: The effects of water extract of anthocyanins of *Hibiscus sabdariffa* L. on paracetamol-induced hepatotoxicity in rats. Phytotherapy Research 17:56-59,2003.
11. Obi FO, Uneh E: pH dependent prevention of carbon tetrachloride-induced lipoperoxidation in rats by ethanol extract of *Hibiscus rosasinensis* petal. Biokemistri 13:42-50, 2003.
12. Obi FO, Fadairo EA: Influence of anthocyanin-free aqueous extract of *Hibiscus sabdariffa* Linn petal on cadmium toxicity in male rats. Nigeria Journal of Life Sciences 3(1): 78-88, 2013.
13. Gong N, Huang Q, Chen Y, Xu M, Ma S, Wang YX: Pain assessment using the rat and mouse formalin tests. BioProtocol 4(21): 1-7, 2014.
14. Sevostianova N, Zvartan E, Baspalov A, Danysz W: Effects of morphine on formalin-induced nociception in rats. European Journal of Pharmacology 402(1-3): 109-113, 2003.
15. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deraniank KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM: TRPA 1 mediates formalin-induced pain. Proceedings of National Academy of Sciences 104(33): 13525-1353, 2007.
16. Coderre TJ, Katz J, Vaccarino AL, Melzack R: Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 52(3):259-285, 1993.
17. Cho IK, Chung YM, Park CK, Park SH, Li HV, Kim D, Piao ZG, Choi SY, Lee SJ, Park K, Kim JS, Jung SG, Oh SB: Systemic administration of monocycline inhibits formalin-induced inflammatory pain in rats. Brain Research 1072(1): 208-214, 2006.
18. Detweiler DJ, Rohde DS, Basbaum AL: The development of opioid tolerance in the formalin test in the rat. Pain 63(2): 251-254, 1995.
19. Graham GG, Scott KF: Mechanism of action of paracetamol. American Journal of Therapeutics 12(1): 46-55, 2005.
20. Graham GG, Day RO, Milligan MK, Ziegler JB, Kettle AJ: Current concepts of the actions of paracetamol(acetaminophen) and NSAIDs. Inflammopharmacology 7(3): 255-263, 1999.
21. Graham GG, Scott KF: Mechanisms of action of paracetamol and related analgesics. Inflammopharmacology 11(4): 401-413, 2003.
22. Hinz B, Dormann H, Brune K: Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. FASEB Journal 22(2): 383-390, 2008.
23. Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S: Paracetamol: new vistas of an old drug. CNS Drug Review 12(3-4); 250-275, 2006.