THE EFFECT OF TETRACERA POTATORIA AND ITS CONSTITUENT BETULINIC ACID ON GASTRIC ACID SECRETION AND EXPERIMENTALLY-INDUCED GASTRIC ULCERATION


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Summary: This study was designed to examine possible antiulcerogenic activity of methanolic extract of Tetracera potatoria (MeTP) and betulinic acid isolated from it. Results showed that various doses of the extract (100, 200, 400 and 800 mg/kg body weight) significantly reduced experimentally induced gastric ulceration in pretreated animals in a dose-dependent fashion. Similarly, animals pretreated with 1 (0.2 and 2.0 mg/kg body weight) had significant reduction in mean ulcer indices recorded (p<0.05). The two doses of betulinic acid also significantly reduced basal gastric acid secretion in the animals (p<0.05). Although no significant changes were observed in gastric acid secretion with the low doses of 0.02 and 0.2 mg/kg body weight of the extract, there was however significant reduction in gastric acid secretion with animals treated with 2.0 mg/kg body weight extract (P<0.05). These results suggest that decreases in mean ulcer indices in response to the extract and betulinic acid appear to be dependent on the inhibition of gastric acid secretion.

Key Words: Betulinic acid; Indomethacin; Gastric ulceration; Tetracera potatoria, Dilleniaceae.

Introduction
Tetracera potatoria Azel belongs to the family Dilleniaceae (Burkill, 1985). It has various ethnomedical applications. The plant’s leaves or a portion of liane boiled in its own sap is used as a powerful diuretic, vermifugal, purgative and for stomach complaints. The sap is claimed to be used for the treatment of gastrointestinal sores(Burkill, 1985). The aqueous extract from the root is an active remedy for intestinal disorders in South Western, Nigeria (Adelaja, 1990).

Betulinic acid has been isolated from different plants across the world but not in Tetracera potatoria. Bringmann et al, 1997; Huang et al, 1995; Kamperdick et al, 1995; Sabira Begum et al, 1995; Zhu-Min et al, 1996; Siddique et al, 1997). The plants, Lantana camara and Betaia alnoides which are used for treating ulcers and wounds are known to possess betulinic acid as a major constituent(Kamperdick et al, 1995; Sabira-Begum et al, 1995). The compound has been studied and found to inhibit prostaglandin synthesis Carter, 1980). The very important role played by hydrochloric acid secreted by the parietal cell of the gastric mucosa in the aetiology of peptic ulcer is established (Card and Marks, 1960). A number of studies have in fact showed an increased gastric acid secretion in peptic ulcer patients (Njar et al, 1994; Njar et al, 1995).

In this paper, the isolation and identification of betulinic acid from the methanolic extract of Tetracera potatoria (MeTP) are described and the antiulcerogenic activities of betulinic acid and methanolic extract of T. potatoria in experimentally induced gastric ulceration in rats using indomethacin were examined. The effects of these drugs on gastric acid secretory response in rats were also determined.

Materials and Methods
General Procedures
Melting point: Kofler hot-stage apparatus; Weights: Metler 1118 balance; IR Spectra: JASCO 410 FTIR Spectrophotometer; EIMS: JEOL AX-505. One-dimensional [1H (399.945 MHz), 13C (100.577 MHz), APT] and two-dimensional (COSY, HETCOR, HMBC) spectra acquired on Varian G-300 NMR spectrometer. Accelerated Gradient Chromatography-silica gel 60 (Merck 40-63
µm); analytical TLC-TLC silica gel 60-F254, pre-coated alumina sheets (Merck) and visualized using UV (254 and 366 nm) and vanillin-sulfuric acid spray.

**Animals:** Adult male albino rats weighing between 180 and 200g of Wistar strain obtained from Pre-clinical animal house, College of Medicine, University of Ibadan, were used. They were fed with standard commercial rat feeds (Ladokun Feeds, Ibadan) and water was given *ad libitum.*

**Preparation of the Extract and Isolation of Betulinic Acid.**

The roots of *Tetracera potatoria* Afzel, were collected in Ago-Iwoye, a town in South West of Nigeria. The plant was authenticated by Professor Z. O. Gbile, Biological Sciences Department, Ogun State University (now Obafemi Awolowo University) Ago-Iwoye Nigeria and Mr. T. K. Odewo, Forestry Research Institute Nigeria (FRIN) Ibadan, Nigeria, where voucher specimens were deposited (FHI 105782).

Dried roots of *T. potatoria* (500g) were ground and soaked in Methanol at room temperature for 48h. The extract when filtered and concentrated in-vacuo yielded a dark-brown solid (18.9g). The methanolic extract (10g) was pre-absorbed on silica gel (30g) and fractionated on Accelerated Gradient Chromatography (AGC), a medium pressure liquid chromatographic method, using a gradient elution with hexane/ethylacetate mixtures. Fractionations were monitored with analytical TLC and similar fractions combined. The solid obtained from fractions which eluted with 20% (400ml) EtOAc in hexane was further purified by use of Vacuum Liquid Chromatography (CHCl₃, 100ml and CHCl₃ / MeOH, 9.8:0.2, 100ml) collected in 20ml portions. Combined fractions 3-8 afforded a colourless crystalline solid compound, betulinic acid (0.192g) mp 290-293 °C, [α]D₂⁰ +7.5° (c 0.38, pyridine) which recrystallised in MeOH. Betulinic acid weighing 70mg was acetylated using pyridine (1.5 ml) and acetic anhydride (3.0 ml) to give the compound, 2 (73mg) mp 299 °C, [α]D₂⁰ -19.5° (c 1.32, CHCl₃). Fourier Transform Infra-Red (2, KBr, cm⁻¹) 3060; 2940; 2870; 1725; 1690; 1660; 1460; 1385; and 1245. Electron Impact Mass Spectroscopy, m/z (%): 498(36)[M⁺]; 438(93); 428(28); 395(40); 248(47); 203(30); 189(100).

¹H-NMR (400 MHz, CDCl₃, δ ppm): 4.37(1Hs, H-30); 4.61(1Hs, H-30); 4.48(1Hdd, 7,10 Hz, H-3); 3.01(1Hdt, 4, 11 Hz, H-19); 2.04(3Hs, OAc); 1.9(3Hs, CH₃-20); 0.97(3Hs, CH₃-14); 0.92(3Hs, CH₃-10); 0.85(3Hs, CH₃-8); 0.84(3Hs, CH₃-4) and 0.83(3Hs, CH₃-4).

¹³C-NMR (400 MHz, CDCl₃, δ ppm): 181.59(C-28); 171.06(C-1); 150.37(C-20); 109.74(C-30); 80.95(C-3); 46.92(C-19); 30.54(C-22); 21.31(CH₃-OAc); 19.34(CH₂-29).Homo-Nuclear Correlation and Hetero-Nuclear Correlation data in Table 1. The structure of betulinic acid was elucidated by spectroscopic analysis, including Attached Proton Test spectrum of its acetylated product and comparison with authentic sample. The NMR data agree with those reported by earlier workers (Andre nick et al, 1995). Copies of the original spectra are obtainable from the author of correspondence.
Table 1: 1H-NMR, HOMCOR and HMBC Spectra of Compound 2.

<table>
<thead>
<tr>
<th>Protons</th>
<th>δ (mult.; JHz)</th>
<th>1H-H COSY</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃-1</td>
<td>0.83 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH₃-4</td>
<td>0.84 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH₃-8</td>
<td>0.85 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH₂-10</td>
<td>0.93 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH₂-14</td>
<td>0.97 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH₂-20</td>
<td>1.69 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OAc</td>
<td>2.04 (3Hs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H-19</td>
<td>3.01 (1Hdd; 4,11)</td>
<td>1.4,1,6,1.97.</td>
<td>30.54(C22)</td>
</tr>
<tr>
<td>H-3</td>
<td>4.48 (1Hdd; 7,10)</td>
<td>1.6</td>
<td>16.03(C23), 27.94(C1), 37.79(C2) and 171.06(C1').</td>
</tr>
<tr>
<td>H-30</td>
<td>4.61 (1Hs)</td>
<td>1.7</td>
<td>46.97(C19), 19.34(C29).</td>
</tr>
<tr>
<td>H-30</td>
<td>4.73 (1Hs)</td>
<td>1.7</td>
<td>46.97(C19), 19.34(C29).</td>
</tr>
</tbody>
</table>

Indomethacin-Induced Ulceration.

The method of indomethacin induced gastric ulceration adopted was that described in previous work (Njar et al., 1994; Njar et al., 1995). Feeding of the animals was terminated 24h before the commencement of the experiment. The animals were however allowed access to water and were then randomly divided into seven treatment groups. Crude methanolic extract of T. potatoria (100, 200, 400 and 800mg/Kg) was administered orally to four groups of rats; betulinic acid (1.0 and 2.0mg/Kg) to two other groups and the seventh group was given normal saline to serve as control. One hour after the administration of extract, betulinic acid and normal saline indomethacin at 40mg/Kg Body weight (Merck, Sharp & Dohme, Canada) was administered subcutaneously to all the animals in all the groups. After 4h, the animals were killed by cervical dislocation. Their stomachs were removed, opened along the lesser curvature, washed in normal saline to remove any debris.

Assessment of Gastric Ulceration by means of “Scoring Technique”

The scoring of gastric ulceration was done according to the method used by Elegbe (1978). Macroscopic examination of the stomach was carried out with a hand lens with x2 magnification.

Scoring system:

Ulcer Score 0 = Normal stomach
0.5 = Punctate haemorrhage or pin-point ulcers
1.0 = Two or more haemorrhagic ulcers
2.0 = Ulcers greater than 3mm in diameter

Gastric Acid Secretion.

In the gastric acid secretory study, gastric acid secretion was measured in male albino rats of Wistar strain weighing between 180-220g using the adapted method of Ghosh and Schild (1958) as modified by Lai (1964). The animals were fed on rat’s pellets and 24h before the start of the experiment, the feeds were removed while water was still given freely. Anaesthesia was induced in the animals with urethane at a dose of 0.6ml per 100 g body weight of a 25% w/v solution given intraperitoneally. Each animal was surgically prepared for gastric acid secretory assay. The stomach lumen was perfused at a rate of 1.0 ±0.1ml/min (Watson –Marlow HR flow inducer) with normal saline at 37°C. The effluent was collected at 10 minutes intervals for titratable acid against 0.01N Sodium hydroxide using phenolphthaline as indicator. Basal gastric acid secretion was taken in all the six groups of animals. However, in groups II to VI, gastric acid secretory responses to different doses of intravenous injection of MeTp (0.02, 0.20 and 2.00 mg/Kg) and betulinic acid (0.2 and 2.0 mg/Kg) were examined. Mean basal gastric acid secretion was calculated from mean of four consecutive aliquots from unstimulated animals before intravenous administration of the test drugs (MeTp and Betulinic acid). Results were expressed as the Mean ± SEM. Statistical significance was tested by using the students’ t-test.

Results

The effects of methanolic extract of T. potatoria and betulinic acid on indomethacin-induced gastric ulceration are shown in Table 2.
Table 2: The Effects of Methanolic Extract of *T.* potatoria and its Constituent, Betulinic Acid on Indomethacin Induced Gastric Ulceration in Rats

<table>
<thead>
<tr>
<th>Treatment (^a)</th>
<th>Dose (mgkg(^{-1}) BW)</th>
<th>Mean Ulcer index (^b)</th>
<th>Inhibition of ulceration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>-</td>
<td>7.5 ± 0.54</td>
<td>-</td>
</tr>
<tr>
<td>MeTp</td>
<td>100</td>
<td>4.3 ± 0.65(^c)</td>
<td>42.67</td>
</tr>
<tr>
<td>MeTp</td>
<td>200</td>
<td>1.3 ± 0.41(^c)</td>
<td>82.67</td>
</tr>
<tr>
<td>MeTp</td>
<td>400</td>
<td>0.6 ± 0.33(^c)</td>
<td>92.00</td>
</tr>
<tr>
<td>MeTp</td>
<td>800</td>
<td>0.5 ± 0.28(^c)</td>
<td>93.33</td>
</tr>
<tr>
<td>Betulinic Acid</td>
<td>1.0</td>
<td>0.7 ± 0.22(^c)</td>
<td>90.67</td>
</tr>
<tr>
<td>Betulinic Acid</td>
<td>2.0</td>
<td>0.3 ± 0.21(^c)</td>
<td>96.00</td>
</tr>
</tbody>
</table>

\(^a\) Five animals were used in each test; \(^b\) Values are Mean ± SEM. \(^c\) Significantly lowered when compared with control (P< 0.05).

In rats treated with MeTp (100, 200, 400 and 800 mg/kg), the mean ulcer index decreased progressively and significantly with increasing doses of extract when compared with the control animals (P<0.05). Betulinic acid (1.0 and 2.0 mg/Kg) also exhibited significant inhibitory effect on indomethacin - induced gastric ulcers (p<0.05).

Gastric acid secretions in rats that received intravenous injection of betulinic acid (0.2 and 2.0 mg/kg) post-basal secretion were 1.80 ±0.03 and 1.13 ±0.03 μeq/10min respectively (Table 3). These values were significantly lower compared with the value of 2.05 ±0.03μeq/10min seen in the control animals (p< 0.05). Unlike betulinic acid, MeTp showed no significant change in gastric acid secretion at 0.02 and 0.2 mg/kg compared to control (p>0.05). At higher dose of 2.0 mg/kg MeTp, significant decrease in gastric acid output was observed (p<0.05).

Table 3: Gastric Acid Secretory Responses in Rats to Graded Doses of Methanolic Extract of *T.* potatoria and Betulinic Acid.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mgkg(^{-1}) BW)</th>
<th>Gastric Acid secretion (μeq/10min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (Basal)</td>
<td>-</td>
<td>2.05 ± 0.09</td>
</tr>
<tr>
<td>II</td>
<td>MeTp</td>
<td>0.02</td>
<td>2.13 ± 0.09</td>
</tr>
<tr>
<td>III</td>
<td>MeTp</td>
<td>0.20</td>
<td>1.85 ± 0.07</td>
</tr>
<tr>
<td>IV</td>
<td>MeTp</td>
<td>2.00</td>
<td>1.50 ± 0.07(^c)</td>
</tr>
<tr>
<td>V</td>
<td>Betulinic Acid</td>
<td>0.20</td>
<td>1.80 ± 0.03(^c)</td>
</tr>
<tr>
<td>VI</td>
<td>Betulinic Acid</td>
<td>2.00</td>
<td>1.13 ± 0.03(^c)</td>
</tr>
</tbody>
</table>

\(^d\) Values are Mean ± SEM, n = 6
\(^c\) Significantly lowered when compared with control (P< 0.05).

BW : Body weight

Discussion
In the present study, significant protection against indomethacin-induced gastric ulceration was observed with *T.* potatoria extract (MeTp) and its compound, betulinic acid (Table 2). Both doses of betulinic acid used in this work showed significant gastric acid suppression when compared with the control animals (Table 3). At 0.02 and 0.2 mg/Kg dose levels, MeTp did not significantly affect basal gastric acid secretion in the animals. This may be due to low concentrations of the active principle (betulinic acid) in the extract at these dose levels.
Tetracera and potatoria gastric acid secretion

The results shown in Tables 2 and 3 clearly suggest that there is a good relationship between the dose-dependent reduction of gastric acid secretion of butulinic acid and its antiulcer effect. These findings support the concept that increased gastric acid secretion is a major factor responsible for incidence of peptic ulcer in human.

Though some workers have reported that several prostaglandins and prostaglandin analogues are potent antisecretory and antiulcer agents most especially prostaglandin of the A, E and F types. The antiulcer effect of butulinic acid may be similar to that of sodium carbonoxolone, a related triterpenoid. It should be mentioned that butulinic acid has recently been reported to inhibit prostaglandin synthesis in an in-vitro experiment (Carter, 1980). Sodium carbonoxolone, a triterpenoid has been found to be effective as an antiulcer agent because it protects the mucosa from acid effects by selectively inhibiting prostaglandin F2 (Aguwa and Okunji, 1986). Butulinic acid a related triterpenoid may likely act in similar manner as sodium carbonoxolone.

Following previous research findings on peptic ulceration, quite a good number of drugs have been employed clinically to relieve or heal peptic ulcers, among which are the four known antagonists (Cimetidine, Ranitidine, Nizatidine and Famotidine) of H2-receptor, which share prestructural configuration of a five membered ring system with a flexible side chain attached to polar uncharged group (Yamada, 1996). Interestingly, butulinic acid, the active compound in McTc shares the same five membered ring structure of the classical H2-receptor antagonists. This compound may therefore be acting as an antihistaminic agent. It is however well established that stimulation of gastric acid secretion is inhibited competitively by selective H2-receptor antagonists (Hirschowitz and Molina, 1983). Further work is in progress to elucidate the mechanism of action of butulinic acid as an antiulcer agent.

These results, however, validate the use of T. potatoria sap in the treatment of gastrointestinal sores. Also, there is high hope for a more dependable peptic ulcer therapeutic agent emanating from this plant in the nearest future.

References


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