Science World Journal Vol 5 (No 4) 2010
www.scienceworldjournal.org
ISSN 1597-6343

ANTI-GASTRIC ULCER AND ANTIINFLAMMATORY PROPERTIES OF BETULINIC ACID IN MALE ALBINO RATS

*ONWUCHEKWA, C.[1, 2] & OLUWOLE, F. S.[2]

[1]Department of Physiology, College of Health Sciences,
Usman Danfodio University, Sokoto Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences,
College of Medicine, University of Ibadan, Ibadan Nigeria.
*chinedukwa@yahoo.com

INTRODUCTION

Natural products have been used for combating human diseases since they exhibit a wide range of biological properties that can be exploited for medical application (Newman *et al.*, 2003). Naturally occurring substances play an increasing role in drug discovery and development. Natural products of plant origin are a major part of traditional medical system in developing countries and also in herbal remedies in the western countries. Hundreds of published reports have described the occurrence of lupane-type triterpene betulinic acid across a multitude of taxonomically diverse genera. Betulinic acid (BA) has been found to possess the following properties: anti-retroviral (2 Mayaux *et al.*, 1994), Evers *et al.*, 1996), anti-tumor (Pisha *et al.*, 1995; Schmidt *et al.*, 2007), and anti-bacteria (Pisha *et al.*, 1995, Setzer *et al.*, 2000).

Peptic ulcer is associated with inflammation of the stomach mucosal. Research on drugs for the treatment of both peptic ulcer and inflammation conditions are ongoing. Though there are reports of antiulcer and anti-inflammatory effect of a Betulin derivative (Karachurina *et al.*, 2002), there is insufficient information of the above properties on the potentially selective HIV inhibitor, Betulinic acid (BA). The aim of this study was to evaluate the effect of BA with respect to indomethacin-induced peptic ulcer and anti-inflammatory properties in experimental albino rats.

Experimental Design: Seventy-two (72) adult male albino rats of Wistar strains with average weight range 220-250 grams were used for the study. The rats were obtained from the Animal Center, University of Ibadan, Ibadan, Nigeria and kept in a well ventilated environment, with free access to food and water. Thirty-two rats were used for indomethacin-induced peptic ulcer Study. These were further divided into four groups. Group I was pretreated orally with Dimethyl sulfoxide (DMSO) in normal saline for seven days and serve as the control. Groups II, III, and IV were pretreated orally with BA (0.5 mg/kg, 1.5 mg/kg, and 3.0 mg/kg respectively) dissolved in DMSO for seven days. Forty rats were used in the anti-inflammatory study divided into four groups and pretreated as above with an additional Group V, that was administered with aspirin orally. All the rats were fed orally using gastric tube. The ethical guides for the handlings of laboratory animals were followed in the study.

Drugs: Indomethacin (Merck, Sharp & Dohme, Canada) and aspirin.

Effect of Betulinic Acid (BA) on indomethacin induced gastric ulceration: A total of thirty-two rats were used for this study. They were divided into four groups of eight rats and treated orally daily for 7 days. Group 1 was the control treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, and 3.0 mg/kg orally respectively.

The method of indomethacin induced gastric ulceration adopted was that described in previous works (Elegbe, 1978; Oluwole *et al.*, 2008). BA was administered orally as stated above. One hour after the administration of BA, indomethacin at 40mg/kg was administered subcutaneously to all the animals in all the groups. After 4 hours, the animals were sacrificed by cervical dislocation. Their stomachs were removed, opened along the greater curvature, and washed with in normal saline to remove any debris. Macroscopic examinations of the washed stomachs were carried out with a hand lens at X2 magnification. The gastric ulceration was assessed using the "Scoring Technique" of Elegbe (1978) where 0 = Normal Stomach; 0.5 = Punctuate haemorrhage or pin point ulcers

1.0 = Two or more haemorrhagic ulcers
2.0 = Ulcers greater than 3mm in diameter

Effect of Betulinic Acid on Carrageenin-induced oedema: A total of fourty rats were used for this study. They were divided into five groups of eight rats. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3, and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, and 3.0 mg/kg orally respectively. Group 5 was administered 2mg/kg Aspirin orally.

Oedema was induced in the rats by injection of carrageenin (0.1 ml, 1% w/v in normal saline) into the subplantar tissue of the right hind paw (Winter *et al.*, 1962). One hour later the paw circumference was measured using microscrew gauge and at half hourly intervals for 4 hours. The paw swelling was calculated as difference between the two readings. Percentage oedema inhibition was also calculated.

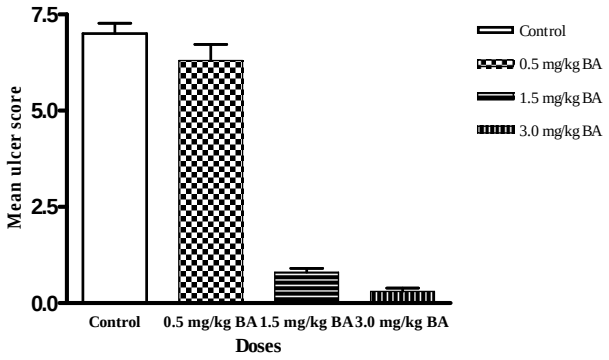

FIG. 1. THE EFFECT OF BETULINIC ACID (BA) ON INDOMETHACIN INDUCED GASTRIC ULCERATION

RESULTS

The results showed a significant difference ($p < 0.05$) in the 1.5 mg/kg BA treated rats (0.8 ± 0.10) and 3.0 mg/kg BA treated rats (0.3 ± 0.09) compared to the control rats (7.0 ± 0.27). There was no significant difference ($p > 0.05$) between the 0.5 mg/kg BA treated rats (6.3 ± 0.42) and the control rats (7.0 ± 0.27). In control rats, there was a progressive increase in paw circumference after

injection of carrageenin. Acute inflammation was observed at about the 2nd and 2.5th hour of administration of carrageenin. The BA and aspirin produced a significant decrease and dose dependent inhibition of the paw oedema formation compared to the control animals ($p < 0.05$). So also was the percentage change in oedema inhibition.

TABLE 1. THE EFFECT OF BETULINIC ACID ON CARRAGEENIN INDUCED PAW OEDEMA

Drug Dose (mg/kg)	Paw Circumference (mm)							
	0.5hr	1 hr	1.5 hrs	2 hrs	2.5 hrs	3 hrs	3.5 hrs	4 hrs
Normal Saline	6.8 ± 1.2	7.6± 0.88	8.6 ± 0.46	9.4 ± 0.22	9.7 ± 0.23	8.9 ± 0.21	8.8 ± 0.16	8.3 ± 0.22
BA 0.5	6.5 ±0.24 (4.41)	6.7 ± 0.19 (11.84)	7.5 ± 0.45 (12.79)	8.0 ± 0.19 (14.89)	8.2 ± 0.42 (15.46)	7.4 ± 0.29 (16.85)	7.4 ± 0.20 (15.91)	6.9±0.33 (16.87)
BA 1.5	7.0 ± 0.24 (2.94)	6.8 ± 0.16 (10.53)	7.7 ± 0.43 (10.47)	8.2 ± 0.21 (12.77)	8.4 ± 0.20 (13.40)	7.5 ±0.25 (15.73)	7.2± 0.12 (18.18)	6.7 ± 0.16 (19.28)
BA 3.0	6.5 ± 0.16 (4.41)	7.2 ± 0.41 (5.26)	7.5 ± 0.41 (12.79)	8.1 ± 0.21 (13.83)	8.3 ± 0.18 (14.43)	7.6 ± 0.19 (14.60)	7.1 ± 0.18 (19.32)	6.6 ± 0.18 (20.48)
Aspirin 2.0	5.2±0.12 (23.50)	5.4±0.11 (29.00)	5.9±0.14 (31.40)	6.5±0.12 (30.50)	6.8±0.11 (30.00)	6.9±0.13 (22.47)	6.5±0.10 (26.70)	6.1±0.10 (26.50)

The BA caused significant decrease in ulcer scores, thus suggesting an anti-gastric ulcer activity. One of the most studied of the triterpenoids is glycyrhetinic acid and its derivatives such as carbenoxolone, that can prevent or heal gastric ulcers (Doll *et al.*, 1962). Betulinic acid, a member of lupanes exhibiting one five membered ring may be acting in the same way as the glycyrhetinic acid. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system (Choi *et al.*, 1999; Santra *et al.*, 2000,). In addition, involvement of oxygen derived free radicals such as the superoxide anion, hydrogen peroxide, and hydroxyl radical are well established in the pathogenesis of ischaemic injury of gastrointestinal mucosa and in other models of mucosal damage induced by non-steroidal anti-inflammatory drugs (Joseph *et al.*, 1999)., ethanol (Simith *et al.*, 1996), feeding restriction and stress (Yelken *et al.,* 1999) This anti-ulcer may be due to the antioxidant effect of Betulinic acid. Levels of MDA are thought to reflect free radical mediated cell membrane damage (Demir *et al.*, 2003) and the ability of Betulinic acid to decrease malondialdehyde (MDA) may account for this effect. Thus reduction of MDA concentration may be due to the ability of Betulinic acid to increase anti-oxidant activity and these do lead to a reduction in lipid peroxidation of the gastric mucosa (Dela Lastra & Motiva, 1999).

Although the results correlate previous findings that revealed BA having anti- inflammatory effect, its effect is not as that of aspirin (a known non-steroidal anti-inflammatory drug) known to act by inhibiting prostaglandin synthesis. It had earlier been reported that BA inhibits prostaglandin synthesis in-vitro (Sotomatsu *et al.*, 1959), therefore, BA may be acting in the same way as aspirin. This is also reflected in the percentage inhibition of the paw oedema. The lupanes, and specifically betulinic acid, have been reported in various literatures to have anti-inflammatory property. The anti-inflammatory activity of betulinic acid is, at least in part, due to its capacity to inhibit enzymes involved in leukotriene biosynthesis, including 5-lipoxygenase (Sotomatsu *et al.*, 1959; Inoue *et al.*, 1986). The observed anti-inflammatory action of BA may be related to the inhibition of the non-neurogenic pathways and also be due to interaction with glucocorticoid receptors (Inoue *et al.*, 1986; Recio *et al.*, 1995; Mukherjee *et al.*, 1997; Huguet *et al.*, 2000). BA has been shown to interfere with NF-κB activation and NF- κB- regulated gene expression triggered by carcinogens and inflammatory stimuli (Takada & Aggarwal, 2003). This may provide a basis for the ability of Betulinic acid to suppress inflammation.

Our study thus shows that Betulinic acid has anti-gastric ulcer and anti-inflammatory properties that may be useful in peptic ulcer therapy but more studies are needed in this area.

REFERENCES

Choi M. A., Kim, B. S., Yu, R. (1999). Serum antioxidative vitamin levels and lipid peroxidation in gastric carcinoma patients. *Cancer Letters* 136: 89-93.

Dela Lastra, A. C. & Motilva, M. J. (1999). Protective effects of melatonin on indomethacin-induced gastric injury in rats. *Journal of Pineal Research* 26:101-107.

Doll, R., Hill, I. D., Hutton, C.& Underwood, D. J. (1962). Clinical trials of a Triterpenoid Liquorice compound in gastric and duodenal ulcer. *Lancet* 11: 793

Elegbe, R. A. (1978). Comparative studies on starvation and indomethacin induced gastric ulceration in albino rats. *Biochemical and Experimental Biology* 14(2): 159-166.

Evers, M., Poujade, C. & Solers, F. (1996). Betulinic Acid Derivatives: A new class of Human Immunodeficiency virus Type 1 specific inhibitors with a new mode of Action. *Journal of Medicinal Chemistry* 39:1056-1068.

Huguet, A. I., Recio, M. C., Manez, S., Giner, R. M., Rios, J. L. (2000). Effect of triterpenoids on the inflammation induced by protein kinase Cactivators, neuronally acting irritants and other agents. *European Journal of Pharmacology* 410:69–81.

Inoue, H., Mori, T., Shibata, S., Koshibara, Y., Murota, S. (1986). Inhibitory effect of glycyrrhetinic acid derivatives on lipoxygenase and prostaglandin synthetase. *Chemical Pharmacology* (Tokyo) 34 (2): 897-901

Joseph, R. M., Varela, V., Kanji, V. K. (1999). Protective effects of zinc in indomethacin-induced gastric mucosal injury: evidence for a dual mechanism involving lipid peroxidation and nitric oxide. *Alimentary Pharmacology and Therapeutics* 13:203-208.

Karachurina, L. T., Sapozhnikova, T. A., Zarudii, F. S., Flekhter, O. B. & Galin, F. Z. (2002). Antiinflammatory and Antiulcer Properties of Betulin Bis-Hemiphthalate. *Pharmaceutical Chemistry Journal*. Vol. 36, No. 8 pp. 488-491(4)

Mayaux, J. F., Bousseau, A., Panwels, R., De Clerq, E. & Pecq, J. B. (1994). Triterpene derivatives that blocks the entry of Human Immunodeficiency Virus Type I into Cells. *Proceedings of National Academy of Science, USA* 91:3564 -3568.

Mukherjee, P. K., Saha, K., Das, J., Pal, M. & Saha, B. P. (1997). Studies on the anti-inflammatory activity of rhizomes of Nelumbo nucifera. *Planta Medicus* 63:367–369.

Newman, D. J., Cragg, G. M., Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products 66*, 1022-1037.

Oluwole, F. S., Ayo, J. A., Omolaso, B. O., Emikpe, B. O. & Adesanwo, J. K. (2008). Methanolic extract of *Tetracera Potatoria,* an anti-ulcer agent increases gastric mucus secretion and endogenous antioxidants. *Nigeria Journal of Physiological Scence* Vol 23: 1-2. Pg 79-83.

Pisha, E., Chai, H., Lee, I. S., Chagwedera, T. E., Farnsworth, N. R., Cordell, A. C., Beecher, C. W. W., Fong, H. H. S., Kinghorn, A. D., Brown, D. M., Wani, M. C., Wall, M. E., Hieken, T. J., Das Gupta, T. K., Pezzuto, J. M. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Natural Medicine*1:1046–1051.

Recio, M. C., Giner, R. M., Manez, S., Gueho, J., Julien, H.R., Hostettmann, K., Rios, J. L. (1995). Investigations on the steroidal anti-inflammatory activity of triterpenoids from Diospyros leucomelas. *Planta Medicus* 61:9–12.

Santra, A., Chowdhury, A. & Chaudhuri, S. l. (2000). Oxidative stress in gastric mucosa in helicobacter pylori infection. *Indian Journal Gastroenterology*; 19: 21-3.

Schmidt, M. L., Kuzmanoff, K. L., Ling-Indeck, L. & Pezzuto, J. M. (2007). Betulinic Acid Induces Apoptosis in Human Neuroblastoma Cell Lines. *European Journal of Cancer* 33(12).

Setzer, W. N., Setzer, M. C., Bates, R. B. & Jackes, B. R. (2000). Biologically Active Triterpenoids of Syncarpia Glomulifera Bark Extract from Paluma, North Queensland, Australia. *Planta Medicus* 66:176–177.

Simith, G. S., Mercer, D. W., Cross, J. M. (1996). Gastric injury induced by ethanol and ischemia-reperfusion in the rat. Differing roles for lipid peroxidation and oxygen radicals. *Digestive Disorder Science*; 41: 1157-64.

Sotomatsu, S., Takalehi, Y., Hiroi, J., Namikata, A. & Okano, N. (1959). *Skin and Urology* 21: 138

Demir S, Yilmaz, M., Köseoğlu, M., Akalin, N., Aslan, D., Aydin A: (2003). Role of free radicals in peptic ulcer and gastritis. *Turkish Journal of Gastroenterology* 14 (1): 39-43

Takada, Y., Aggarwal, B. B. (2003). Betulinic acid suppresses carcinogen-induced NF-kappa B activation through inhibition of I kappa B alpha kinase and p65 phosphorylation: abrogation of cyclooxygenase-2 and matrix metalloprotease-9. *Journal of Immunology, 171*, 3278-3286.

Winter, C., Risley, E. & Nuss, O. (1962). Carrageenin-induced inflammation in the hind limb of sthe rats. *Federal Proceedings* 46: 118-126.

Yelken, B., Dorman, T., Erkasap, S. (1999). Clonidine pretreatment inhibits stress-induced gastric ulcer in rats. *Anesthetize and Analgesic*; 89: 159-62.