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*Full Length Research Paper*

# Microbiological and toxicological studies on cellulose generated from agricultural wastes

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**A pharmaceutical excipient is required to meet certain minimum standards for use in the manufacture of dosage forms. In this study, two of such requirements, microbiological and toxicological suitability, was investigated in respect of cellulose powder derived from an agricultural waste, maize cob. Microbial count data were obtained by inoculating a suspension of the cellulose into various types of agar. We also studied some of the possible toxicological effects of sub-acute ingestion of the cellulose in 2% tragacanth mucilage on adult male Wistar rats given 1.6 g/kg per day for 14 days. Weight changes, locomotor activity, some haematological parameters and the presence of gastrointestinal lesions were evaluated. Microbiological results indicate a 'no growth' status for yeast, fungi as well as for coliform and pseudomonas bacteria. The mesophilic bacterium, *Bacillus subtilis* returned a count of 100 cfu/g. Toxicological results show that animal weight was significantly ($P < 0.05$) reduced on the 14th day compared to weights on the 1st and 3rd days. Locomotor activity increased in a similar pattern being significantly higher ($P < 0.05$) on day 14 than on days 1 and 3. Platelet counts, white blood cell counts, and packed cell volume were not affected. There were no visible gastrointestinal (GI) lesions or morbidity and mortality in the animals. We conclude that the cellulose satisfied the British Pharmacopoeia requirement for pharmaceutical grade starch that it should be free from the coliform bacterium, *Escherichia coli*. Furthermore, the results obtained showed that the cellulose neither exerted adverse effects on the haematological status of the animals nor is it associated with any other significant toxicological event.**

**Key words**: maize cob, microbiological status, cellulose, toxicity, sub-acute ingestion, rats.

## INTRODUCTION

Non-starch polysaccharides such as cellulose have been used variously as pharmaceutical excipients and as dietary fibres. For example, while cellulose powder and microcrystalline cellulose of various grades are used as binders, fillers, disintegrants and lubricants in tableting (Uesu et al., 2000; Okhamafe and Azubuke, 1994; Okhamafe et al., 1995; Iwuagwu et al., 1996), other cellulosic substances are used as suspending agents in formulations, as bulk-forming laxatives to suppress hunger and in the management of colostomies (Smith et al., 2003; Pasman et al., 1997). According to Piggott et al. (2003), cellulose degradation by *Ruminococcus flavefaciens,* a cellulolytic ruminal bacterium occurred whereby it utilises cellobiose or cellulose as substrates for growth. Cellulose-based materials such as cotton, linen, paper and wood are particularly susceptible to direct attack by microorganisms. In any case, the ability to exist on almost any material characterizes microorganisms as primary agents of deterioration.

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Furthermore, microbial contamination of a pharmaceutical product may have its origin in the raw materials, equipment and packaging. It may also result from lack of good manufacturing practice (GMP) as well as its method of use. Since cellulose is a raw material of natural origin usually supplied for the manufacture of non-sterile pharmaceuticals, it is, therefore, important that it does not unduly support the growth of microorganisms as this may have pathologic consequences and/or undermine the aesthetic value of the preparations.

Over the years, we have steadily investigated in our laboratories the physicochemical, tablet and pharmacopoeial characteristics of powdered celluloses derived from agricultural wastes such as maize cob, groundnut shell, rice husk and sugarcane fibres, also called bagasse (Okhamafe and Azubuike, 1994; Okhamafe et al., 1995; Okhamafe et al., 1991; Okhamafe et al., 1992; Okor et al., 1992). Some of these wastes have proved to be low-cost sources of potential pharmaceutical grade cellulose. Although the British Pharmacopoeia does not prescribe microbial standards which powder cellulose must meet, it does, however, specify that *Escherichia coli* must be absent from pharmaceutical grade starch. This requirement could be extended to a non-sterile excipient such as cellulose. This study, in part, therefore examines the microbial suitability of cellulose derived from the agricultural waste, maize cob, as a pharmaceutical excipient.

Furthermore, whether they are used as pharmaceutical excipients or dietary fibres, cellulose and its derivatives are ingested, and this has occasioned the need to set acceptable daily intakes (ADI) for some of them in order to prevent possible incidences of adverse effects. ADIs are widely used to describe the “safe” levels of intake and are usually derived from chronic animal toxicity studies (Herrman and Younes, 1999). The no- observed effect level is then related to humans. Although there are no set toxicological tests that have to be performed on every chemical intended for commerce, sub-acute toxicity tests using high doses are often designed to characterise likely toxic effects (Klaassen and Eaton, 1991). Since the source of the cellulose, its subsequent modification and duration of ingestions are variables that may affect its toxicological profile, we undertook to examine the effect of ingestion of high levels of the cellulose on adult male rats.

## MATERIALS AND METHODS

### Microbiological assessment

0.1 g of cellulose was suspended in 10 ml of nutrient broth from which $10^{-1}$, $10^{-2}$ , and $10^{-3}$ dilutions in sterile distilled water were made. 0.1 ml each of the dilutions was inoculated on plates containing Saboraud agar in duplicates. The plates were incubated at an ambient temperature of 30°C for 72 h. In a similar manner, 0.1 ml each of the cellulose suspension was inoculated on plates of McConkey agar, nutrient agar, and 7% salt agar in duplicates. These were incubated at 37°C for 48 h and observed for colony formation.

### Toxicological experiments

**Animals:** Adult male Wistar rats weighing between 200 and 250 g were bred locally in the Department of Pharmacology and Toxicology Animal House, University of Benin. The animals were kept 5 per cage and fed standard rat chow (Livestock Feeds Plc, Benin City, Nigeria), and had free access to tap water with exposure to a 12 h light -dark cycle. Environmental temperature ranged from 23 (night) to 28°C (daytime). The rats were handled according to standard protocols for the use of animals for toxicological experiments.

**Preparation and administration of cellulose:** Cellulose from maize cob was suspended in 2% tragacanth mucilage and administered orally using gastric feeding needles (CU.FNC-16-3). Ten rats were given 1.6 g/kg body weight per day for 14 consecutive days, a 4-fold increase on the level of microcrystalline cellulose reportedly tolerated in humans (Martindale, 1982). The control group was administered 0.5 ml of the suspending agent daily for 14 consecutive days.

**Assessment of toxicities:** Animal body weights and locomotor activity were monitored on days 1, 3, 7, and 14 of daily cellulose administration. On each day the animals were also carefully observed for general signs of toxicity, lethargy, morbidity, mortality and food consumption. Animal locomotion was recorded for 20 min on each of the monitoring days by use of a sensitive electronic locomotor meter (40fc, Motron Products, Sweden) as previously described (Ozolua et al., 1996). On day 14, the animals were anaesthetised with pentobarbitone sodium (40 mg/kg, intraperitonial) and 5 ml blood samples were collected via carotid artery cannulation into bottles containing 0.5 ml of 3.8% sodium citrate and gently mixed. Platelet count (PC) and white blood count (WBC) were determined microscopically using the method of Dacie and Lewis (1991). Packed cell volume (PCV) was measured by filling microhaematocrit tubes with blood samples, centrifuging at 150 g for 5 min and reading values with a haematocrit reader. After each bleeding, the stomach and intestines were dissected longitudinally, washed with 10% formaldehyde saline and examined for lesions using a magnifying glass attached to a dissecting fluorescent lamp (Thousand and One Lamps, England).

**Drug and chemicals:** Cellulose powder was obtained from de-grained maize cobs as described in a previous work. All media used in microbiological experiments were obtained from Oxoid (Basingstoke, UK) and were prepared according to standard procedures. Pentobarbitone sodium (Sigma) and sodium citrate (BDH) were dissolved in water. Tragacanth (Halewood Chemicals Ltd, UK) mucilage was made by dispersing the powder in hot water. Other chemicals were used of analytical or reagent grade.

**Statistical analysis:** The data are presented as the mean ± standard error of the mean (s.e.m) or for animal weights, as SD (standard deviation) and *n* represents the number of rats used per group. Data were compared using Student’s t-test (GraphPad Prism Software, UK). $P < 0.05$ was regarded as significant.

## RESULTS

The microbial counts are shown in Table 1. The mesophilic bacteria observed were mainly *Bacillus*

**Table 1.** Microbial counts of cellulose in various media.

| Medium | No of Colonies* |
|---|---|
| Nutrient agar (bacterial mesophilic count) | 100 cfu/gm |
| 7% Salt agar (bacterial pseudomonads counts) | No growth |
| McConkey agar (bacterial coliform counts) | No growth |
| Saboraud agar | No growth |
| (Fungi counts) | No growth |
| (Yeast counts) | No growth |

Only *B. subtilis* grew on nutrient agar plates.
*****Results of duplicate experiments expressed in colony forming units (cfu).

**Table 2.** Effect of 14-day cellulose administration (1.6 g/kg/day) on animal weights.

| Period | Control Weight (g) | Cellulose Weight (g) |
|---|---|---|
| Day 1 | 220.2 ± 4.6 | 217.3 ± 5.9 |
| Day 3 | 218.9 ± 4.9 | 213.5 ± 4.5 |
| Day 7 | 221.7 ± 5.1 | 205.5 ± 5.2 |
| Day 14 | 228.6 ± 6.3 | 193.5 ± 5.3***** |

Mean weights are significantly lower on the $14^{th}$ day compared the $1^{st}$ and $3^{rd}$ days (*****, $P < 0.05$). Weight increase in the control group is not significant. $n = 10$ per group.

**Table 3.** Effect of 14-day cellulose administration (1.6 g/kg/day) on animal's locomotor activity.

| Period | Control Score/20 min | Cellulose Score /20 min |
|---|---|---|
| Day 1 | 701. 4 ± 67.2 | 684.7 ± 62.1 |
| Day 3 | 822.6 ± 79.7 | 739.5 ± 62.7 |
| Day 7 | 696.2 ± 55.3 | 945.3 ± 34.3 |
| Day 14 | 738.1 ± 61.7 | 1199 ± 123.9***** |

Activity increased significantly (*****, $P < 0.05$) on the 14 day from values on the $1^{st}$ and $3^{rd}$ days. There was no change in activity in the control group. $n = 10$ per group.

**Table 4.** Effect of daily cellulose administration (1.6g/kg/day for 14 days) on some haematological indices.

| Index | Control | | Cellulose | |
|---|---|---|---|---|
| | Day 1 | Day 14 | Day 1 | Day 14 |
| PCV (%) | 43.7 ± 3.1 | 40.0 ± 1.6 | 42.6 ± 2.2 | 39.3 ± 3.3 |
| PC (x $10^3$/ml) | 347 ± 44 | 421 ± 98 | 389 ± 57 | 528 ± 101 |
| WBC (x $10^3$/ml) | 3144 ± 553 | 3436 ± 829 | 3018 ± 784 | 3121 ± 862 |

Neither cellulose nor tragacanth (suspending agent) influenced these indices. $n = 10$ per group.

*substilis* on nutrient agar plates. There was no growth on McConkey agar, 7% salt agar, and saboraud agar. This is indicative of the absence of *Enterobactereacea,* such as *E. coli*, *Klebsiella species* , *Staphylococci*, and *yeasts* (fungi), respectively. Table 2 shows that cellulose administration significantly reduced animal body weight by the $14^{th}$ day. This weight loss was significant between the $1^{st}$ and $14^{th}$ day and between the $3^{rd}$ and $14^{th}$ day. Thus, weight loss was manifest from the 11th day of cellulose feeding. Tragacanth did not have any effect on the weight of the animals as seen in the control group.

Daily administration of cellulose increased the locomotor activity of the rats (Table 3). Like weight reduction, increase in activity is only significant between days 1 and 14; and between days 3 and 14. The results for the control group indicate that 2% tragacanth did not have any influence on the animals' locomotion. Table 4 shows the platelet counts (PC), white blood cell counts (WBC) and packed cell volume (PCV) after daily feeding with cellulose. In both the control and cellulose-fedrats, there were no inter- and intra-group variations for these indices.

Gross examination of the intestines and stomachs of the rats did not reveal any ulceration or other notable gastrointestinal lesions in both the control and cellulose-fed rats. Generally, behavioural changes were observed as increase in locomotor activity. No death was recorded within the period of exposure but animals on cellulose were observed to have manifested insignificantly reduced feed consumption.

## DISCUSSION

The absence of *Enterobactereacea,* such as *E. coli*, *Klebsiella species* , Staphylococci, and *yeasts* (fungi) is suggestive of high microbial excipient quality. Storage of powder excipients under dry conditions is usually unlikely to result in spoilage due to growth of microorganisms in the final products (Bos et al., 1989). Conversely, storage of tablets under tropical conditions of high temperature and relative humidity precipitates a dramatic loss of microbial integrity within a relatively short period of time (Bos et al., 1989). However, the findings of this study do not preclude the possibility that microbial integrity of tablets containing cellulose will be sustained when stored in a humid environment. Evidently, the high microbio-logical quality of this cellulose makes it suitable for pharmaceutical products manufacture.

The weight reduction in rats observed in this brand of cellulose could have been due to the combined effect of creating a sense of fullness and decreased intestinal transit time although these two parameters were not measured in this study. It seems likely that these two effects could have caused decreases in feed consumption and absorption, respectively. It is well known that some swellable hydrophilic polymers are used as appetite suppressants and laxatives. Data from *in vitro* studies earlier carried out (Okhamafe et al., 1991), show that this cellulose powder swells up to four times its original volume in the presence of water. In this regard, methylcellulose, for example, has been used as a laxative and a dietary supplement for weight reduction (Smith et al., 2003; Pasman et al., 1997).

Increase in locomotor activity became significant within 14 days. The activity instrument scores such traits as head and forepaw movements, and motility. Such increased activity could have been due to the weight reduction seen, i.e., the lower the weight of the animal (due to cellulose consumption), the more mobile the animal. Reduced locomotor activity is often an index of intoxication (Omogbai et al., 1999).

Studies have shown that diets containing large amounts of non-absorbable polysaccharides such as cellulose could decrease the absorption of calcium, magnesium, zinc and phosphorus (Reinhold et al., 1991; Ismail-Beigi et al., 1977). The present study has shown that cellulose feeding did not have any adverse effect on platelet counts, white blood cell counts and packed cell volume. A study on healthy male volunteers has shown that methylcellulose does not have any adverse haematological effects even at daily doses ten times higher than the ADI (Eastwood e al., 1990).

Visual macroscopic examinations of the gastrointestinal tract may sometimes reveal the presence of lesions. The present study neither revealed any ulceration, perforation nor other gastrointestinal lesions. However, stimulation of colonic epithelial cell proliferation in Sprague-Dawley rats through the production of short chain fatty acids has been reported for some dietary fibres including cellulose (Whitely et al., 1996), these at doses many times higher than may be consumed from intake as a pharmaceutical excipient. With more evidence that dietary fibre including cellulose reduce the incidence of colonic cancers (Hu et al., 2002), there is likelihood of increased human consumption.

Tragacanth, the suspending agent for cellulose in this study is itself composed of water-soluble and insoluble polysaccharides. Our present finding is that it has no effect on control rats and this agrees with the finding in humans where daily intake of as much as 9.9 g tragacanth for 21 days did not produce any change in haematological indices and plasma biochemistry (Eastwood et al., 1984).

The ADI has been defined as an estimate of the amount of a food additive, expressed on body weight, basis that can be ingested daily over a life time without appreciable health risks (Larsen and Richold, 1999). However, cellulose in the form and at the level studied is hardly consumed on daily basis and as a pharmaceutical material is usually ingested in very minute quantities. While the ADI for methylcellulose is 25 mg/kg, that of cellulose is not known.

In conclusion, our data indicate that our cellulose extracted from maize cob is free from pathogenic bacteria and fungi. The data also show that at sub-acute daily intake of 1.6 g/kg, this cellulose causes a decrease in body weight with no obvious adverse toxicological effects and is therefore of high pharmaceutical quality.

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### REFERENCES

Bos CE, Vari Doorme H, Lerk CF (1989). Microbiological stability of drugs stored under tropical conditions. Int. J. Pharm. 55: 175–183.
British Pharmacopoeia (1980). Her Majesty's Stationery Office, London. p. A55.

Dacie JV, Lewis SM (1991). Practical haematology, 7th edition. Churchill Livingstone, Edinburgh p.13.
Eastwood MA, Brydon WG, Anderson DM (1984). The effects of dietary gum tragacanth in man. Toxicol. Lett. 21: 73 – 81.
Eastwood MA, Brydon WG, Anderson DM (1990). The effects of dietary methylcellulose in man. Food Addit. Contam. 7: 9 – 19.
Herrman JL, Younes M (1999). Background to the ADI/TDI/PTWI. Regul. Toxicol. Pharmacol. 30: S109 – S113.
Hu Y, Martin J, Le Leu R, Young GP (2002) The colonic response to genotoxic carcinogens in the rat: Regulation by dietary fibre. Carcinogenesis 23: 1131–1137.
Ismail-Beigi F, Faradji B, Reinhold JG (1977). Binding of zinc and iron to wheat bread, wheat bran, and their components. Am. J. Clin. Nutr. 30: 1721–1725.
Iwuagwu MA, Eba YI, Omokhafe AA, Anozie JD (1996). Locally sourced celluloses as compressed tablet excipients. J. West Afr. Pharm. 10: 18 – 24.
Klaassen CD, Eaton DL (1991). Principles of toxicology. In: Amdur MO, Doull JD, Klaassen CD (Eds). Casarett and Doull's Toxicology: The Basic Science of Poison. Pergamon Press, New York, pp.32 – 33.
Larsen JC, Richold M (1999). Report of workshop on the significance of excursions of intake above the ADI. Regul. Toxicol. Pharmacol. 30: S2 – S12.
Martindale: The Extra Pharmacopoeia 28th edition, 1982. The Pharmaceutical Press, London, p. 952.
Okhamafe AO, Azubuike CPC (1994). Direct Compression Studies on a low-cost cellulose derived from maize cob. J. Pharm. Sci. Pharm. Pract. 2: 26 – 29.
Okhamafe AO, Ejike EN, Akirinola FF, Ubuane-Inedegbo A (1995). Aspects of the tablet disintegrant properties of bagasse and maize cob cellulose. J. West Afr. Pharm. 9: 8–13.
Okhamafe AO, Igboechi AC, Obaseki TO (1991). Celluloses extracted from groundnut shell and rice husk. 1: Preliminary physicochemical characterisation. Pharm. World J. 8: 120–123.
Okhamafe AO, Igboechi AC, Ubrufih CE, Akinyemi BO, Ighalo MO (1992). Celluloses extracted from groundnut shell and rice husk. 2: Disintegrant properties. Pharm. World J. 9: 11 – 16.
Okor RS, Iwu-Anyanwu U, Okhamafe AO (1992). Swellability of acrylate methacrylate cellulose matrix systems and the effects of solute diffusion rates. J. Appl. Poly. Sci. 44: 749 – 750.
Omogbai EKI, Ozolua RI, Idaewor PE, Isah AO (1999). Some studies on the rodenticidal action of indomethacin. Drug Chem. Toxicol. 22: 629 – 642.
Ozolua RI, Pogoson EE, Ideh HI, Obianwu HO, Omogbai EKI (1996). The effect of SKF 525A on chronic amphetamine-induced behavioural sensitisation in rats. J. West Afr. Pharm. 10: 25 – 29.
Pasman WJ, Saris WH, Wauters MA, Westerterp-Plantega MS (1997). Effect of one week of fibre supplementation on hunger and satiety rating and energy intake. Appetite 29: 77–87.
Piggott KAD, Antonopoulos DA, Kocherginskaya SA, White BA (2003). Proteomics and genomics of cellulolytic ruminal microorganism *Ruminococcus flavefaciens* FD-1. Hughes Undergraduate Research Fellow Seminar, Speeman College.
Reinhold JG, Faradji B, Abadi P, Ismail-Beigi F (1991). Decreased absorption of calcium, magnesium, zinc and phosphorous by humans due to increased fibre and phosphorous consumption as wheat bread. Nutr. Rev. 49: 204–206.
Smith C, Hellebusch SJ, Mandel KG (2003). Patient and physician evaluation of a new bulk fibre laxative tablet. Gastroenterol. Nurs. 26: 31 – 37.
Uesu NY, Pineda EAG, Heichenleitner AAW (2000). Microcrystalline cellulose from soybean husk: Effects of solvent treatments on its properties as acetylsalicylic acid carrier. Int. J. Pharm. 206: 85–96.
Whitely LO, Higgins JM, Purdon MP, Ridder GM, Bertram TA (1996). Evaluation in rats of the dose-response relationship among colonic mucosal growth, colonic fermentation, and dietary fibre. Dig. Dis. Sci. 41: 1458 – 1467.