**Experimental Researches**

# Long-term acetaminophen (paracetamol) treatment causes liver and kidney ultra-structural changes during rat pregnancy

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**Summary**

Acetaminophen (paracetamol) is an analgesic-antipyretic drug virtually devoid of typical anti-inflammatory activity and hence free of some of the side-effects of aspirin and related agents (e.g. gastric erosion and bleeding complications). The worldwide use of paracetamol as a household analgesic, including during pregnancy, prompted us to investigate its potentially deleterious effects in that setting. Pregnant rats were treated with paracetamol (150, 500 or 1,500 mg/kg, once a day by gavage) from the first day up to term pregnancy. In the group treated with the lowest doses, no histological changes were noticed in maternal and fetal livers or kidneys when examined under light or electron microscopy. With the higher doses, however, various dose-dependent effects of paracetamol were observed, namely necrotic areas of the liver seen with light microscope and further confirmed by electron microscopy. The kidneys revealed degeneration and necrotic foci under light microscopy with ultrastructural derangements. Electronmicrographs of the liver revealed hepatocytes bearing translucent bodies as a consequence of a dilated smooth endoplasmic reticulum. There were signs of necrosis both in the hepatocytes (lysis of mitochondria and presence of lipid droplets) and renal tissue (mitochondrial cytolysis in convoluted tubules). Our data point out the fact that both maternal and fetal tissues can be adversely affected by paracetamol.

*Key words:* Acetaminophen; Kidney; Liver; Rat pregnancy.

## Introduction

Acetaminophen (paracetamol) shares with acetylsalicylic acid, dipyrone and nonsteroidal anti-inflammatory agents, efficacy for similar problems such as pain of musculoskeletal origin, fever, and mild to moderate headache. The main reason for prescribing acetominophen to pregnant women is probably to obtain effective analgesia with minimal erosive effects on the gastric mucosa. However, the potential effects of its hepatotoxicity during pregnancy remain unknown.

Though the clinical effects of acetaminophen are subject to the "ceiling effect" (i.e., doses above the usual ones do not show proportionally higher therapeutic effects), laymen frequently take more than the needed doses in an attempt to achieve pain control. This fact is a matter of concern since ingestion over 10-15 g/day may lead to hepatic injury [1, 2]. The most serious adverse effect of acute overdosage of acetaminophen is a dose-dependent, potentially fatal hepatic necrosis [3]; renal tubular necrosis and hypoglycemic coma also may occur. In addition, acetaminophen is now the second leading cause of toxic drug ingestions in the United States [4].

Due to its relative safety and efficacy, acetaminophen is one of the most largely used analgesic-antipyretic drugs during pregnancy [5, 6]. The abuse of this drug is presumably due to the over-the-counter availability and the marketing publicity, which has at times failed to take into account some less obvious, but yet potentially severe, side-effects [7, 8]. The concern relates to the ability of acetaminophen to cross the placenta and hence to potentially act on the fetus at every phase of its development [9, 10].

The relative overall general safety of acetaminophen in adults is overshadowed by the possible subtle but potentially pervasive effects during pregnancy. Some studies involving over 10,000 observations during pregnancy did not detect drug-induced dysmorphosis [11, 12]. However, a few reports reveal conflicting data, either confirming its safety [13-15] or showing severe side-effects of this drug associated to dysmorphosis and hepatotoxycity [16-18].

Because the liver and kidneys are the main target organs of acetaminophen toxicity, we examined the effects of this drug during the entire period of pregnancy in the rat.

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## Materials and Methods

Female adult virgin, EPM-1 Wistar rats weighing about 200 g, under routine laboratory care, were mated in the proportion of two females for every male for 12 h. Pregnancy was determined by the finding of spermatozoa in the vagina according to Hamilton and Wolfe [19]. Forty pregnant rats were then randomly divided into four groups and treated from the 1st up to the 20th day of gestation as follows: C, control group, treated with dis-tilled water (acetaminophen vehicle); A125, A500 and A1500, groups of pregnant rats treated respectively with 125, 500 or 1,500 mg acetaminophen/kg. Drugs and drug vehicle were given once a day in a final volume of 1 ml. This study was approved by the Institutional Committee of Ethics on Animal Care and Use of the Federal University of São Paulo, Brazil.

At term (20th day of pregnancy) the animals were sacrificed by deep ether anesthesia. Maternal and fetal liver and kidney samples were taken by laparotomy. Part of the material was immersed in Bouin's solution for further hematoxylin-eosin staining and light microscopy study. The remaining samples were immersed in a 2% glutaraldehyde solution and processed for electron transmission microscopy examination [20] with an EM-900 model Zeiss electron microscope at 80 kV.

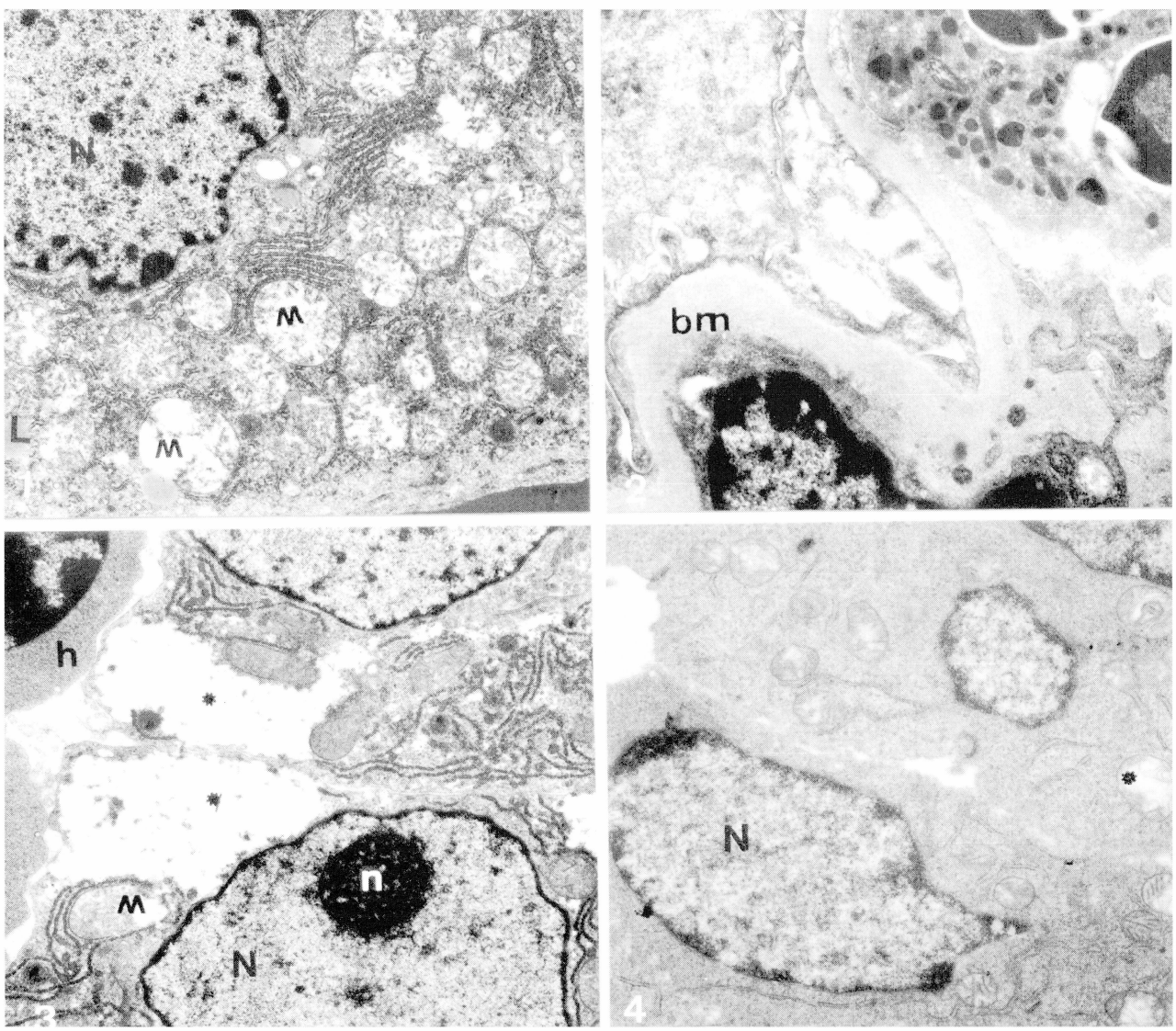


Figure 1. — Electronmicrograph showing part of a hepatocyte of a term-pregnant rat that had been treated with 500 mg/kg of acetaminophen during the entire period of pregnancy (group A500). The nucleus (N) is irregularly shaped, contains euchromatin and is heterochromatic in some regions. The cytoplasm is somewhat disorganized, bearing a retracted rough endoplasmic reticulum (*). It is apparent that a great number of lipid droplets are present (L). Many mitochondria (m) appear faded (X 42,500).
Figure 2. — Electronmicrograph showing part of a glomerulus of a pregnant rat in group A1500. There is a thickened basal membrane containing disorganized podocytes in the glomerulus (BM) (X 42,500).
Figure 3. — Electronmicrographic aspect of a fetal hepatocyte from a rat in group A500. Erythrocytes (h) can be seen surrounding the hepatocyte. The nucleus (N), bearing a nucleolus (n), appears predominantly euchromatic. Several mitochondria with few cristae, most of them crystallized (mc), are seen; many translucent areas (*) are also apparent (X 42,500).
Figure 4 — Electronmicrographic aspect of a fetal proximal convoluted tubule from the A500 group showing several cells with euchromatic nuclei (N) and a few crystallized mitochondrial cristae (*) (X 42,500).

## Results

The animals treated with daily doses of 125 mg/kg during the entire pregnancy (group A125) did not show hepatic or renal morphological alterations. However, pregnant rats of the A500 and A1500 groups showed necrotic areas in the liver on light microscopy examination, further confirmed by electron microscopy (translucent cytoplasmic areas, lipid droplets, mitochondrial crystolysis and irregular nuclei) (Figure 1). Similarly, in the kidneys we observed degeneration and necrotic foci under light microscopy, with ultrastructural derangements (thickened basal membrane containing disorganized podocytes in the glomeruli, and electrondense bodies with mitochondrial crystolysis in the convoluted tubules) (Figure 2). All these alterations were somewhat dose-dependent, as they appeared to be more frequent and severe in the A1500 than in the A500 group.

Acetaminophen crosses the placental barrier and can interfere with rat pregnancy. In fact, the drug was demonstrated to cause reductions of the number and the weights of litters and placentae, and a 3-7 times higher incidence of reabsorptions than those seen in control animals [21]. Thus, the livers and kidneys of term concepts were also examined. Electronmicrographs of livers revealed hepatocytes bearing translucent bodies as a consequence of a dilated smooth endoplasmic reticulum. There were signs of necrosis, both in the hepatocytes (lysis of mitochondria and presence of lipid droplets; Figure 3) and in the renal tissue (mitochondrial cytolysis in convoluted tubules; Figure 4). The lesions were more evident and abundant in the concepts from groups treated with the higher doses of acetaminophen (A500 and A1500).

## Discussion

The binding of acetaminophen to plasma proteins is variable; at therapeutic doses approximately 10-30% of the drug is bound, and hence it is widely distributed in most body fluids. Elimination is predominantly renal, after hepatic conjugation with glucuronic acid (ca. 60%), sulfuric acid (ca. 30%) or cysteine (ca. 3%), in addition to small amounts of hy-droxylated and deacetylated metabolites [22]. A small proportion of acetaminophen is N-hydroxylated by CYP2E and CYP3A to form N-acetyl-benzoquinoneimine [23], a toxic in-termediate which reacts with sulphydryl groups in glutathione and is finally excreted as mercapturic acid into the urine [24, 25]. Acetaminophen metabolism occurs in the endo-plasmatic reticulum and the hepatic toxic damage may be related to this organelle in ex-cess of P450 activity [26]. This idea is supported by our electronmicrographic results which showed signs of hepatic damage with smooth endoplasmatic reticulum changes.

Acetaminophen is well tolerated with doses below the toxic threshold (i.e., below 150 mg/kg in humans). In fact, the animals treated with daily doses of 125 mg/kg during the entire pregnancy (A125 group) did not show hepatic or renal morphological alterations. However, acute hepatotoxicity (using 250 mg/kg or more) may ensue, essentially because the toxic intermediate N-acetyl-benzoquinoneimine is formed in amounts sufficient to deplete hepatic glutathione. This leads to an increased susceptibility of hepatocytes to oxidant injury and damage of cell macromolecules, causing dysfunction of enzymatic systems [22]. Hepatocyte necrosis may follow the intracellular accumulation of $Ca^{2+}$, activation of $Ca^{2+}$–dependent endonucleases and DNA fragmentation [27, 28].

In conclusion, both maternal and fetal tissues can be affected by acetaminophen at the ultrastructural level in this rat model. Our results suggest that acetaminophen in high doses for long-term use may be dangerous during pregnancy.

## References


[1] Davis M., Harrison N.G., Ideo G., Portmann B., Labadarios D., William R.: "Paracetamol metabolism in rats relationship to covalent binding and hepatic dam-age". *Xenobiotica,* 1976, *6,* 249.

[2] Zieve L., Anderson W.R., Dozeman R., Draves K., Lyftogt C.: "Acetaminophen liver injury: sequential changes in two biochemical indices of regeneration and their relationship to histologic alterations". *J. Lab. Clin. Med.*, 1985, *105,* 619.

[3] Thomas S.H.: "Paracetamol (acetaminophen) poisoning". *Pharmacol. Ther.,* 1993, *60*, 91.

[4] Litowitz T.L., Holm K.C., Clancy C., Schmitz B.F., Clark L.R., Oderda G.M.: "Annual report of the american association of the poison control centers toxic exposure surveillance system". *Am. J. Emer. Med.,* 1999, *17*, 435.

[5] Niederhoff H, Zahradnik H.: "Analgesics during pregnancy". *Am. J. Med.,* 1983, *14,* 117.

[6] Kozer E., Koren G.: "Management of paracetamol overdose: current controversies". *Drug Safety*, 2001, *24*, 503.

[7] Heymann M.A.: "Non-narcotic analgesics. Use in pregnancy and fetal and perinatal effects". *Drugs,* 1986, *32* (suppl. 4), 164.

[8] Butters L., Howie C.A.: "Awareness among pregnant women of the effect on the fe-tus of commonly used drugs". *Midwifery,* 1990, *6,* 46.

[9] Levy G., Garrettson L.K., Soda D.M.: "Evidence of placental transfer of acetamino-phen". *Pediatrics,* 1975, *55*, 895.

[10] Collins E.: "Maternal and fetal effects of acetaminophen and salicylates in pregnancy". *Obst. Gynecol.,* 1981, *58* (suppl. 5), 57.

[11] Heinonen O.P., Slone D., Shapiro S.: "Birth Defects and Drugs in Pregnancy". Littleton, MA, Publish Sci Group, 1977.

[12] Thulstrup A.M., Sorensen H.T., Nielsen G.L., Andersen L., Barret D., Vilstrup H., Olsen J.: "Fetal growth and adverse birth outcomes in women receiving prescriptions for acetaminophen during pregnancy". *Eur. Stud. Group. Amer. J. Perinatol.,* 1999, *16*, 321.

[13] Byer A.J., Traylor T.R., Semmer J.: "Acetaminophen overdose in the third trimester of pregnancy". *JAMA,* 1982, *247,* 3114.

[14] Ludmir J., Main D.M., Landon M.B., Gabbe S.G.: "Maternal acetaminophen over-dose at 15 weeks of gestation". *Obst. Gynecol.,* 1986, *67,* 750.

[15] Rosevear S.K., Hope P.L.: "Favourable neonatal outcome following maternal paracetamol overdose and severe fetal distress: case report". *Br. J. Obst. Gyn.*, 1989, *96,* 491.

[16] Haibach H., Akhter J.E., Muscato M.S., Cary P.L., Hoffmann M.F.: "Acetaminophen overdose with fetal demise". *Am. J. Clin. Pathol.*, 1984, *82*, 240.

[17] Wang P.H., Yang M.J., Lee W.L., Chao H.T., Yang M.L., Hung J.H.: "Acetaminophen poisoning in late pregnancy. A case report". *J. Reprod. Med.*, 1997, *42*, 367.

[18] Pastore L.M., Hertz-Picciotto I., Beaumont J.J.: "Risk of stillbirth from medications, illnesses and procedure medications". *Perinatol. Epidemiol.,* 1999, *13,* 421.

[19] Hamilton J.B., Wolfe J.M.: "The effect of male hormone substance upon birth and prenatal development in the rat". *Anat. Rec.*, 1983, *70,* 433.

[20] Mamede J.A.V., Oliveira-Filho R.M., Simões, M.J., Mora O A., Espiridião S., Kulay Jr. L.: "Hepatic and renal effects of azidothymidine and acyclovir on pregnant rats". *Clin. Exp. Obstet. Gynecol.*, 2000, *27,* 227.
[21] Andalaft Neto J., Mamede J.A.V., Simões M.J., Oliveira-Filho R.M., Kulay Jr L.: "Ef-fects of paracetamol on rat gestation". *Acta. Obstet. Gynecol. Scand.*, 1997, *76,* 78.
[22] Roberts II L.J., Morrow J.D.: "Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout". In: Hardman J.G., L.E. Limbird (eds.): Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed., New York, McGraw-Hill, 2001, 703.
[23] Sinclair J., Jeffery E., Wrighton S., Kostrubsky V., Szakacs J., Wood S, Sinclair P.: "Alcohol-mediated increases in acetaminophen hepatotoxicity: role of CYP2E and CYP3A". *Biochem. Pharmacol.*, 1998, *55,* 1557.
[24] Mitchell J.R., Jollow D.J., Potter W.J., Gillette J.R., Brodie B.B.: "Acetaminophen-induced hepatic necrosis. IV. Protective role of gluthatione". *J. Pharmacol. Exp. Ther.*, 1973, *187,* 211.
[25] Jollow D.J., Thorgeisson S.S., Potter W.Z., Hashimoto H., Mitchell J.R.: "Acetamino-phen-induced hepatic necrosis". *Pharmacol.*, 1974, *12,* 251.
[26] Fisher M.B., Campanale K., Ackermann B.L., VandenBranden M., Wrighton S.A.: "In vitro glucuronidation using human liver microsomes and the pore-forming peptide alamethicin". *Drug. Metabolism.*, 2000, *28,* 560.
[27] Hessel G., De-Santi-Neto, D., Collares E.F.: "Correlation between the severity of acute hepatic necrosis induced by acetaminophen and serum aminotransferase levels in fasted and sucrose-fed rats". *Braz. J. Med. Biol. Res.*, 1996, *29,* 793.
[28] Cohen S.D., Khairallah, E.A.: "Selective protein arylation and acetaminophen-induced hepatotoxicity". *Drug. Metabol.*, 1997, *29,* 59.

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