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EFFECT OF D-GALACTOSE ON LIPOFUSCINOGENESIS IN THE BRAIN OF FEMALE ALBINO MICE

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Abstract: Lipofuscin granules accumulate in post mitotic cells during ageing and affect the normal cell functions. The accumulation of lipofuscin granules is directly correlated with oxidative stress. The purpose of this investigation was to study the effect of D-galactose on lipofuscinogenesis in various regions (cerebral cortex, hippocampus, corpora quadrigemina and cerebellum) of brain. The female albino mice were divided into two groups: 1) sham injected control group and 2) experimental group which were treated with subcutaneous injections of 5% D-galactose (0.5 ml per day) for fifteen days. Histochemical study of lipofuscin granules and biochemical estimation of fluorescence were carried out, which showed increased accumulation of lipofuscin granules and highly significant increase in fluorescence in various regions of brain, which indicates the Lipofuscinogenic effect D-galactose in the nerve cells.

Key words: D-galactose, Lipofuscin granules, Fluorescence, Ageing.

INTRODUCTION

Lipofuscin is a yellowish brown auto-fluorescent pigment that accumulates in the cytoplasm of varieties of post mitotic cells during ageing [1-8]. The accumulation of the lipofuscin granules is the result of deterioration caused due to oxidative stress. The increased production of free radicals results in formation of lipid peroxides [9-12], oxidation of proteins [13-16] and damage to DNA [17,18]. The damaged lipids and proteins are regularly sequestered to the lysosomes for degradation [19] but due to oxidative modification become indigestible, hence accumulate as lipofuscin granules [4,5,20,21]. These granules can not be removed from the post mitotic cells by exocytosis [4,5], therefore, accumulate in the cells that results the injury and affects the cellular metabolism.

There are reports that low dose of reducing sugar like D-galactose when injected subcutaneously result in the formation of advanced glycation end products which cause increased lipid peroxidation and decreased antioxidant enzymes [22-25], which are the hallmarks of oxidative stress. In the present investigation attempts were made to unravel the effect of D-galactose on accumulation of lipofuscin granules in various regions of brain of female albino mice.

MATERIALS AND METHODS

Female Swiss albino mice (*Mus musculus*) of age six months were used in the present investigation. The animals were reared in aluminium cages of dimensions 260 mm × 200 mm × 140 mm in groups of three animals/cage, in the Departmental animal house(Approved by CPCSEA) at a constant temperature of 29°C to 30°C with a light-dark cycle of 12/12 hours. They were supplied with pelleted mice food (Sagar Feed Center, Kolhapur) and drinking water *ad libitum*.

The animals were divided into two groups containing 6 animals in each group. The first group was considered as control and injected with 0.5ml sterile water/day in the neck for 15 days. The second group was injected with 5% aqueous solution of D-

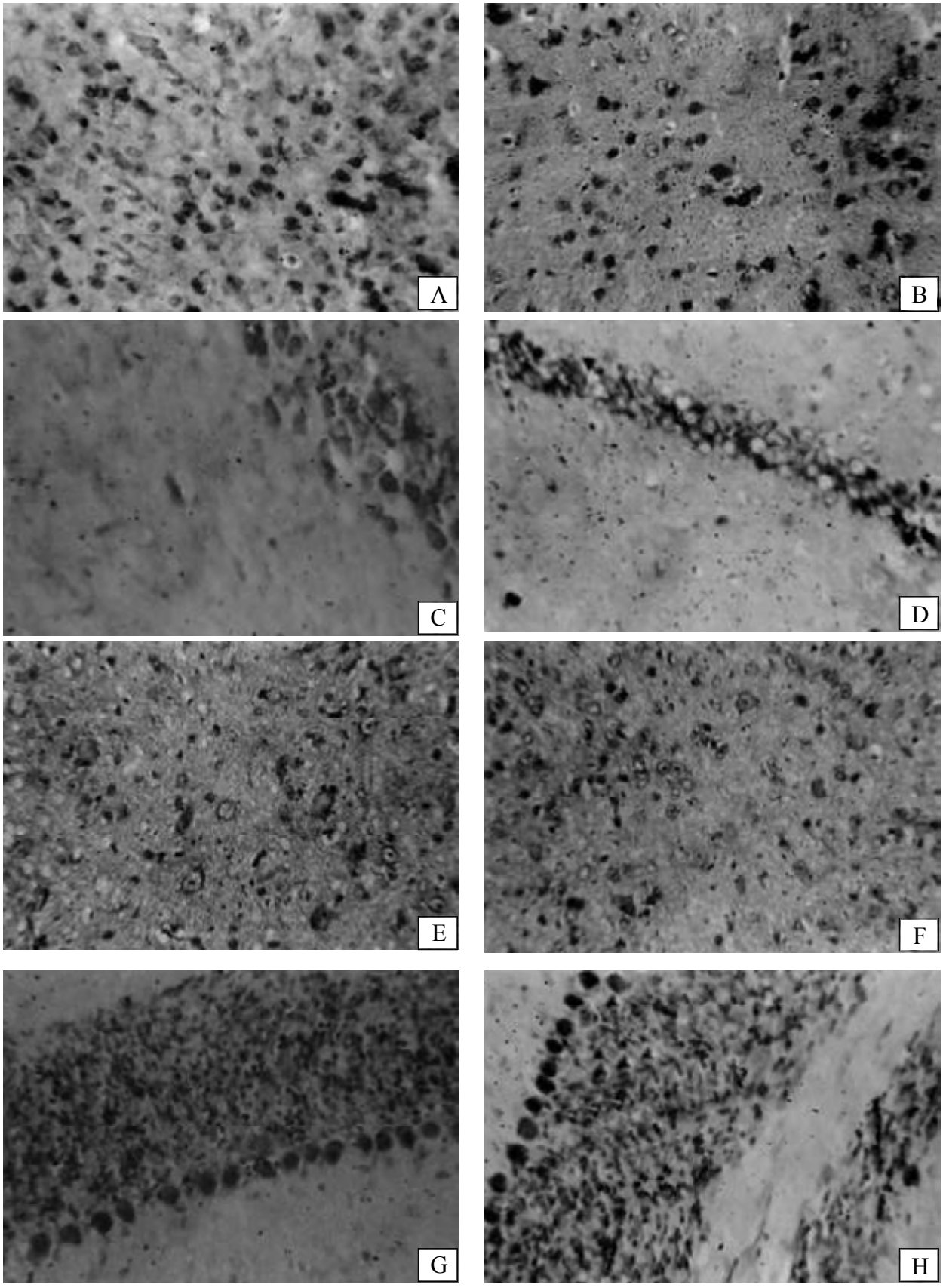

Fig. A Cerebal cortex control mice
Fig. B Cerebral cortex of D-galactose treated mice
Fig. C Hippocampus of control mice
Fig. D Hippocampus of D galactose treated mice
Fig. E Corpora quadrigemina of control mice
Fig. F Corpora quadrigemina of D galactose treated mice
Fig. G Cerebellum of Control mice
Fig. H Cerebellum of D galactose treated mice

Organ	Sham injected Control group	D galactose treated group	Statistical significance t value
Cerebral cortex	0.44 + 0.02	1.29 + 0.08	25.75 (p<0.001)
Hippocampus	0.32 ± 0.002	1.16 ± 0.11	18.09 (p<0.001)
Corpora quadrigemina	0.15 + 0.001	0.99 + 0.034	61.07 (p<0.001)
Cerebellum	0.32 + 0.002	1.55 + 0.17	17.71 (p<0.001)

Table 1: Effect of D-galactose on Fluorescent product (expressed in µg/mg protein) in various regions of brain of female albino mice (represented as Mean ± SD of six animals). P < 0.001 is highly significant

galactose 0.5ml/day in the neck for 15 days and considered as experimental group. All the injections were subcutaneous.

The animals were sacrificed after 24 hours of completion of the treatment by cervical dislocation. The schedule of the treatment was decided in such a way that on the day of sacrifice the animals must be in diestrous, since it is the most prolonged phase of the estrous cycle with less hormonal variations.

Histochemical study of lipofuscin granules: For histochemical study the brain was longitudinally cut into two equal halves and fixed in 10% neutral buffered formalin for 24 hours at 4°C. The tissue was washed under running tap water for 24 hours, dehydrated through alcohol grades, cleared in xylene and embedded in paraffin. 5 thick sections were cut on the rotary microtome and stained by Ziehl-Neelsen Carbol fuchsin method [26]

Biochemical extraction of lipofuscin and measurement of fluorescence : Fluorescence was measured as per Dillard and Tappel method [27]. The separated cerebral cortex, hippocampus, corpora quadrigemina and cerebellum were homogenized in a reaction mixture containing 75mM potassium phosphate buffer (pH 7.04), 1mM ascorbic acid, 1mm $FeCl_3$ and 0.001ml chlorotetracycline. The lipofuscin granules were extracted by adding 1ml of reaction mixture in 6 ml of chloroform: methanol mixture (2:1 v/v). The fluorescence was measured with photofluorometer calibrated with quinine sulfate. 1µg of quinine sulfate /ml of 0.1N H_2SO_4 were used as a standard and 0.1N H_2SO_4 was used as a blank.

RESULTS

The histochemical study shows an increased accumulation of lipofuscin granules in all regions of the brain of D-galactose treated group. The increased lipofuscin can clearly be seen in cerebral cortex (Fig. B), hippocampus (Fig. D), corpora quadrigemina (Fig. F) and cerebellum (Fig. H) as compared to control (compare figures A,C,E,G with figures B,D,F,H). The results of biochemical estimation, presented in table 1, are also supportive to that of histochemical observations. Study shows highly significant increase (P<0.001) in fluorescence in all the regions of brain of D-galactose treated mice as compared to control mice. Over all 3 to 6 fold increase of fluorescence is recorded in different regions of brain.

DISCUSSION

It is evident from the data that the observed increase in lipofuscin granules and the fluorescence is due to the effect of D-galactose. The later is a reducing sugar and irreversibly interacts with free amino groups of proteins, aldehyde and ketone to produce advanced glycation end products [28,29]. Glycated proteins produce fifty fold more free radicals than non glycated proteins [30]. The superoxide radicals, produced in the process, react with hydrogen peroxide to generate hydroxyl radicals that increase oxidative stress which is one of the causal factors in the accumulation of lipofuscin granules [2,3,31]. The glycated proteins are resistant to lysosomal degradation [29,32]. Thus glycation of macromolecules consequences in the increased production of free radicals and accumulation of indigested waste in the lysosomes that causes an in increase in lipofuscin granules in various regions of brain of D-galactose treated animals. Beside this, the cells have a limited capacity to produce lysosomal enzymes [33, 34]. A number of investigators have reported a reduction in lysosomal enzymes due to free radical attack causing the inefficiency of the lysosomes [3, 34-36]. The accumulation of damaged waste material autophagocytosed by the lysosomes on one hand, and reduced lysosomal efficiency on the other hand, consequence into the accumulation of lipofuscin granules in nerve cells leading to an increased oxidative stress in D-galactose treated mice.

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REFERENCES

[1] Nakano, M., Muzuno, T., Katoh, H. and Gotoh, S.: Mech Ageing Dev., 49(1): 41-48 (1989).

[2] Sohal, R.S. and Brunk, U.T.: Adv. Exp. Med. Biol. 266: 17-29 (1989).

[3] Marzabadi, M.R., Sohal, R.S. and Brunk, U.T.: APMIS, 99(5): 416-426 (1991).

[4] Terman, A. and Brunk, U.T.: APMIS, 106(2): 265-276 (1998).

[5] Terman, A. and Brunk, U.T.: Mech. Ageing Dev., 100: 145-156 (1998).

[6] Terman, A. and Brunk, U.T.: Mech. Ageing Dev., 104: 277-291 (1998).

[7] Pillai, M.M., Ashokan, K.V., Jadhav, S.J. and Pawar, B.K.: Indian J. Gerontol., 16(3-4): 199-210 (2002).

[8] Pillai, M.M., Pawar, S.S. and Vora, S.R.: Indian J. Comp. Animal Physiol., 21: 77-85 (2003).

[9] Tappel, A.L.: Lipid peroxidation and fluorescent molecular damage to membranes. In: *Pathobiology of Cell Membranes* (Trump B.F. and Arstila, A.U. eds), NY Acad. Press, pp 145-170 (1975).

[10] Hassan, M.: Ann. Nt. Acad. Sci. (India), 22: 69:91 (1985).

[11] Patro, I.K., Sharma, S.P. and Patro, N.: Proc. Nat. Acad. Sci. (India), 57(B): 105-108 (1987).

[12] Hammer, C. and Braum, E.: Comp. Biochem. Physiol., 90 (G): 7-17 (1988).

[13] Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S. and Stadtman, E.R.: J. Biol. Chem., 262: 5488-5491 (1987).

[14] Stadman, E.R. and Oliver, C.N. : J. Biol. Chem., 266: 2005-2008 (1991).

[15] Butler, R.N., Sprott, R., Warner, H., Bland, J., Feuers, R., Forster, M., Fillit, H., Harman, S.M., Hewitt, M., Hyman, M., Johnson, K., Kligman, E., McClearn, G, Nelson, J., Richardson, A., Sonntag, W., Weindruch, R. and Wolf, N. : J. Gerontol. A Biol. Sci. Med. Sci., 59(6): B560-B567 (2004).

[16] Poon, H.F., Calabrese, V., Scapagnini, G and Butterfield, D.A.: J. Gerontol. A Biol. Sci. Med. Sci., 59(5): M478-M493 (2004)

[17] Wang, Y.J., Ho, Y.S., Lo, M.J. and Lin, J.K.: Chem. Biol. Intreract., 94(2): 135-145 (1995).

[18] Beckman, K.B. and Ames, B.N.: Physiol. Rev., 78(2): 547-581 (1998).

[19] Ericsson, J.L.R.: Mechanisms of cellular autophagy. In : *Lysosomes in Biology and Pathology*, Vol.2 (Dingle J.T. and Fell H.B. eds.) , North Holland, Amsterdam pp 345-394 (1969)

[20] Brunk, U.T., Charles, J.B. and Sohal, R.S.: Mutation research, 275: 395-403 (1992).

[21] Zs-Nagy, I.: Arch. Geronto. Geriatr., 34: 329-341 (2002).

[22] Song, X., Bao, M., Li, D. and Li, Y.M.: Mech. Ageing Dev., 108 (3): 239-251 (1999).

[23] Ho, S.C., Liu, J.H. and Wu, R.Y.: Biogerontology, 4(1): 15-18 (2003).

[24] Deshmukh, A.A., Gajare, K.A. and Pillai M.M.: Abstract: Proc. Comp. Animal Physiol. Stress Physiol. Osmania university, Hyderabad, pp 28 (2005)

[25] Gajare, K.A., Deshmukh, A.A. and Pillai M.M.: Abstract: Proc. Comp. Animal Physiol. and Stress Physiol. Osmania University, Hyderabad, pp 16 (2005)

[26] Troyer, H.: Pigments and minerals. In: *Principles and Techniques of Histochemistry*. Little, Brown and Company, Boston, pp 163-171 (1980)

[27] Dillard, C.J. and Tappel, A.L.: Lipids 6(10): 715-721 (1971)

[28] Schmidt, A.M., Yan, S.D., Brett, J., Mora, R., Nowygrod, R. and Stern, D.: J. Clin. Invest., 91: 2155-2168 (1993)

[29] Munch, G, Simm, A., Double, K.L. and Riederer, P.: Alzheimer's Disease Rev., 1: 71-74 (1996)

[30] Mullarkey, C.J., Edelstein, D. and Brownlee, M.: Biochem. Biophys. Res. Commun. 173: 932-939 (1990)

[31] Kowald, A. and Kirkwood, T.B.: Mutat. Res., 316(5-6): 209-236 (1996)

[32] Vitek, M.P., Bhattacharya, K., Glendening, J.M., Stopa, E., Vlassara, H., Bucala, R., Manogue, K. and Cerami, A.: Proc. Natl. Acad. Sci. USA, 91: 4766-4770 (1994)

[33] Terman, A.: Redox Report, 6: 15-26 (2001)

[34] Brunk, U.T. and Terman, A.: Eur. J. Biochem., 269(8): 1996-2002 (2002).

[35] Ivy, G.O., Schottler, F., Wenzel, J., Baudry, M. and Linch, G: Science, 226 (4677): 985-987 (1984)

[36] Pillai, M.M., Ashokan, K.V. and Kurane, V.M.: J. Reprod. Biol. Comp. Endocrinol., 7(2): 58-71 (1995)