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EFFECT OF *TOXOPLASMA GONDII* INFECTION IN MALE AND FEMALE RATS ON FETAL CHARACTERISTICS

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ABSTRACT
Toxoplasmosis in male and female rats decreased litter size and fetal weight and increased fetal resorption and early fetal death. Pyrimethamine and sulfadiazine were not free from reproductive adverse effects and they caused further deterioration of reproductive function which indicated that these drugs were not safe during pregnancy.

KEYWORDS: *Toxoplasma gondii*, male, female, rats, fertility, reproduction, fetal, characteristics.

INTRODUCTION
Toxoplasma gondii is a zoonotic, obligate intracellular protozoan parasite that has the capacity to infect all warmblooded animals. The infection does not cause clinical illness in the majority of animal species, in some it causes acute life-threatening disease and in others, particularly sheep, goats and pigs, it manifests itself as a disease of pregnancy by multiplying in the placenta and fetus. *Toxoplasma gondii* readily infects human beings. Transmission to the fetus occurs predominantly in women who acquire their primary infection during pregnancy. Infection is relatively common (approximately 30% of the population depending on age and environment). The infection can pose a serious threat to the fetus.[1-2] The overall risk of congenital infection from acute *T. gondii* infection during pregnancy ranges from 20% to 50% without treatment[3-4]. *Toxoplasma gondii* infection in pregnant women may cause poor obstetric outcomes such as spontaneous abortion, still-born and sterility. A survey of *Toxoplasma gondii* infection in 68 cases of sterility revealed a prevalence of 44.1%, which was significantly different from that in normal pregnant women, indicating that *Toxoplasma* infection could result in sterility.[5]

This study was carried out to investigate the effect of toxoplasmosis and its treatment on fetal characteristics of infected male and female rats.

MATERIAL AND METHODS
The study was carried out on 42 males and 42 females rats (*Rattus norvegicus*) ranging in weight from 250 to 300g, all rats were housed in an air-conditioned animal room at an ambient temperature of 23 ±2 C and in a 12h lightl / 12h dark cycle. Half of the males and females were infected intraperitoneally with 1 X 10^7 tachyzoites of *T. gondii* intraperitoneally.[6] Infected groups were examined for documentation of the infection with the using of real-time PCR. Infected groups (21 males and 21 females) and non infected group (21 males and 21 females) were divided into 3 subgroups (7 each) and treated with dimethyl sulphoxide (DMSO), sulphadiazine 12.5 mg/kg or pyrimethamine 200 mg/kg. Sulphadiazine and pyrimethamine were given in DMSO as a single oral daily dose for 60 days in males and 2 estrus cycles in females. At the end of the treatment period, infected and non infected males in each subgroup were matted with healthy females (with 2 regular estrus cycles) 1 male/1 females, while infected and non infected females were impregnated by healthy males during proestrus and for 24 hrs. Recovery of sperms in the vaginal smears was considered as day one of pregnancy. Females were killed at day 15 of gestation by cervical dislocation after light anesthesia. Fetuses of both groups were counted, weighted and examined for identification of resorption rate, early fetal death and malformations.[7-8]

RESULTS
Fetal characteristics
The litter size in healthy females fertilized by non infected males treated by DMSO was 11.29±1.20 fetus/dam, the litter size of females fertilized by non infected males treated by sulphadiazine was decreased to 5.00±0.82 ($P<0.001$) and pyrimethamine to 3.14±0.22 ($P<0.0001$). The litter size of the females fertilized by infected males treated by DMSO was 6.29±0.92, it was significantly less than litter size of females fertilized by non infected males treated by DMSO ($P<0.001$). Litter size of females fertilized by infected males treated by sulphadiazine was 4.57±0.83 and of those treated by

pyrimethamine was 3.14±0.12 in comparison with litter size of females fertilized by infected males treated by DMSO (P<0.05 and P<0.01 respectively).

The mean fetal weight of healthy female fertilized by non infected males treated by DMSO was 0.469±0.064 g, while the mean fetal weight of healthy female fertilized by infected male treated by DMSO was decreased significantly to 0.367±0.038g (P<0.05). Sulphadiazine treatment didn't change the mean fetal weight of healthy female either fertilized by infected or non infected male rats, But pyrimethamine decreased significantly (P<0.05) the mean weight of fetuses of healthy females fertilized by non infected, and (P<0.01) infected male rats.

The fetal resorption percent in healthy females fertilized by non infected males treated by DMSO was 0.00%, while, it was increased in females fertilized by non infected males treated by sulphadiazine to 2.778% (P<0.05) and pyrimethamine to 4.347% (P<0.05). The fetal resorption in females fertilized by infected males treated by DMSO was 2.222%, it was significantly more than that in female fertilized by non infected males treated by DMSO (P<0.05). However, the mean of fetal resorption percent in females fertilized by infected males treated by sulphadiazine and pyrimethamine was further increased significantly (P<0.01 and P<0.01 respectively) in comparison to females fertilized by infected males treated by DMSO.

The early fetal death percent in healthy females fertilized by non infected males treated by DMSO was 0.00%, while, it was significantly increased in females fertilized by non infected males treated by sulphadiazine to 7.33% (P<0.01) and pyrimethamine to 11.11% (P<0.001). The early fetal death in females fertilized by infected males treated by DMSO was 8.07%, it was significantly more than that in females fertilized by non infected males treated by DMSO (P<0.01). However, the early fetal death percent in females fertilized by infected males treated by sulphadiazine was significantly decreased, but pyrimethamine didn't show significantly changes in comparison to females fertilized by infected male treated by DMSO (table 1).

Table 1: Litter size, fetal weight/ g, fetal resorption ratio and early fetal lost ratio of healthy female rats fertilized by non infected and *Toxoplasma gondii* infected male rats treated with DMSO, sulphadiazine 200 mg/kg and pyrimethamine 12.5 mg/kg for 60 days.

Groups	Litter size Fetuses/dam	Fetal weight / g	Fetal resorption ratio	Early fetal lost ratio
Non infected treated with DMSO	11.29±1.20[a]	0.469±0.064[a]	0.00%[a]	0.00%a
Non infected treated with sulphadiazine	5.00±0.82[b]	0.433±0.058[a]	2.778%[b]	7.33%[b]
Non infected treated with pyrimethamine	3.14±0.22[c]	0.326±0.032[b]	4.347%[c]	11.11%[c]
Infected treated with DMSO	6.29±0.92[b]	0.367±0.038[b]	2.222%[b]	8.07%[b]
Infected treated with sulphadiazine	4.57±0.83[c]	0.350±0.036[b]	5.882%[c]	5.71%[d]
Infected treated with pyrimethamine	3.14±0.12[c]	0.280±0.024[c]	8.333%[d]	9.06%[b]

Vertically, similar letter means not significant

As shown in the table 2, the litter size in non infected females treated by DMSO was 11.29±1.08 fetus/dam, it was decreased to 6.14±0.063 fetus/dam in non infected females treated by sulphadiazine (P<0.01), and to 2.00±0.012 fetus/dam (P<0.001) in pyrimethamine treated non infected females. However, the litter size in infected females treated by DMSO was declined to 7.14±0.032 fetus/dam, it was significantly less (P<0.01) than that recorded in non infected females treated by DMSO. Treatment of infected females with sulphadiazine didn't exert further decreased in litter size, while it decreased to 2.00±0.012 (P<0.001) in pyrimethamine treated infected females in comparison with DMSO treated infected females.

The mean fetal weight in non infected female group treated by DMSO was 0.573±0.098g. It was significantly declined in non infected females treated by sulphadiazine 0.548±0.092g (P<0.05) and pyrimethamine 0.449±0.086g (P<0.01). The mean fetal weight was also significantly decreased in the infected female group treated with DMSO to 0.486±0.082g compared with non infected group treated by DMSO (P<0.05). The mean fetal weight was not changed when the infected female treated by sulphadiazine 0.439±0.088g, but it significantly decreased in pyrimethamine treated group 0.344±0.062g (P<0.01) compared with infected females treated by DMSO.

The fetal resorption was not recorded in non infected DMSO-treated female rats. It increased to 3.84% (P<0.05) in infected DMSO-treated females. In comparison with non infected DMSO-treated females, the non infected females treated by sulphadiazine showed 4.00% resorption ratio (P<0.05), and 12.50% (P<0.01) in pyrimethamine-treated non infected females. On the other hand, treatment of infected females with sulphadiazine significantly decreased the resorption rate to 1.82% (P<0.05), while pyrimethamine was significantly increased the resorption rate to 28.57% (P<0.0001) in comparison with that recorded in infected females treated by DMSO.

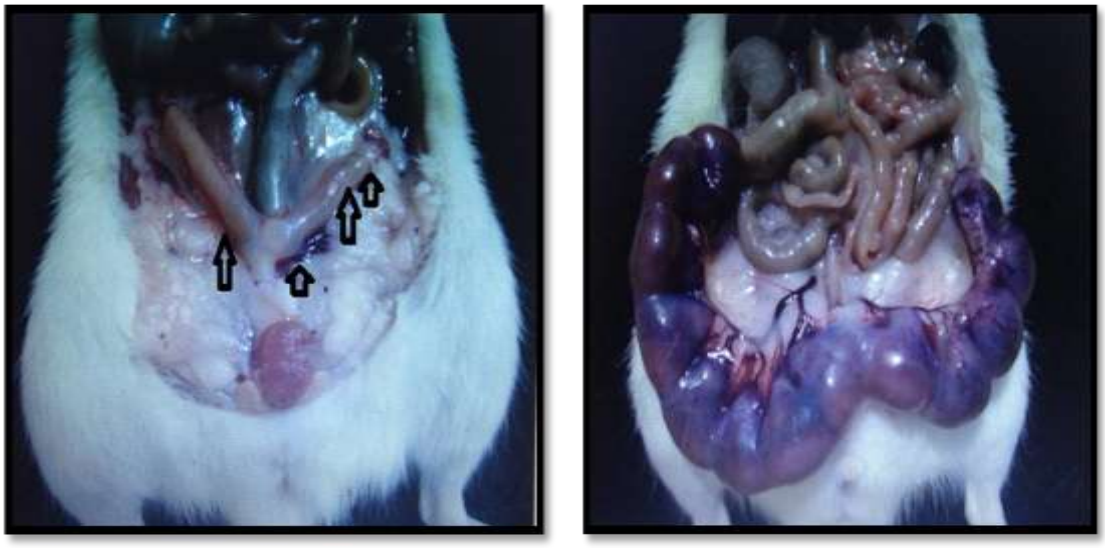
The early fetal death ratio was not recorded in non infected DMSO-treated female rats. It increased to 12.870% (P<0.01) in infected DMSO-treated females. In

comparison with non infected DMSO-treated females, the non infected sulphadiazine treated females showed 12.82% (P<0.01) early fetal death ratio, while pyrimethamine-treated non infected females showed 24.073% (P<0.001) early fetal death rate. On the other hand, treatment of infected females with sulphadiazine decreased the resorption rate to 2.772 %(P<0.01), while pyrimethamine increased the resorption rate to 39.286% (P<0.0001).

Table 2: Litter size, fetal weight/ g, fetal resorption ratio and early fetal lost ratio of non infected and *Toxoplasma gondii* infected female rats fertilized by healthy males and treated with DMSO, sulphadiazine 200 mg/kg and pyrimethamine 12.5 mg/kg for 60 days.

Groups	Litter size Fetuses/dam	Fetal weight/ g	Fetal resorption ratio	Early fetal lost ratio
Non infected treated with DMSO	11.29±1.08[a]	0.573±0.098[a]	0.00%[a]	0.00%[a]
Non infected treated with sulphadiazine	6.14±0.063[b]	0.548±0.092[b]	4.00%[b]	12.82%[b]
Non infected treated with pyrimethamine	2.00±0.012[c]	0.449±0.086[bc]	12.50%[c]	24.073%[c]
Infected treated with DMSO	7.14±0.032[b]	0.486±0.082[c]	3.84%[b]	12.870%[b]
Infected treated with sulphadiazine	6.43±0.030[b]	0.439±0.088[c]	1.82%[d]	2.772%a
Infected treated with pyrimethamine	0.71±0.006[d]	0.344±0.062[d]	28.57%[e]	39.286%[d]

Vertically, similar letter means not significant



A **B**

Figure1: fetuses of non infected female rat impregnated by healthy male at 15 day of pregnancy (A), and infected female treated by DMSO and impregnated by healthy male at 15 day of pregnancy showed resorption of all fetuses

Morph-metric measurements of the fetuses

Toxoplasmosis of male rates didn't induced significant changes in head antero-posterior diameter, head transverse diameter, chest antero-posterior diameter, chest transverse diameter, pelvis antero-posterior diameter, pelvis transverse diameter, fore limb length, hind limb length and tail length of the infected rats progeny, in comparison with non infected. However both sulfadiazine and pyrimethamine significantly decreased head antero-posterior diameter and head transverse diameter (P<0.05) of the progeny of the infected and non infected male rats (table 3).

Table 3: morph-metric measurements (mm) of the fetuses of non-infected and *Toxoplasma gondii* infected male rats treated by DMSO, sulfadiazine and pyrimethamine.

Groups / parameters	Females impregnated by non infected males treated by DMSO	Females impregnated by non infected males treated by sulfadiazine	Females impregnated by non infected males treated by pyrimethamine	Females impregnated by infected males treated by DMSO	Females impregnated by infected males treated by sulfadiazine	Females impregnated by infected males treated by pyrimethamine
Head antero-posterior diameter	7.08±0.98a	6.78±8.86b	6.29±1.02b	7.31±0.97a	6.81±0.93b	6.31±0.94b
Head	4.88±0.62a	4.28±0.71b	4.16±0.62b	4.51±0.67a	4.12±0.87b	4.10±0.84b

transverse diameter						
Chest antero-posterior diameter	5.15±0.83a	4.95±0.76a	4.93±0.70a	5.32±0.78a	5.52±0.93a	5.30±0.87a
Chest transverse diameter	3.52±0.58a	3.56±0.53a	3.30±0.52a	3.45±0.51a	4.34±0.73a	4.28±0.64a
Pelvis antero-posterior diameter	5.36±0.96a	5.34±0.96a	5.52±0.87a	5.33±0.88a	5.70±0.97a	5.52±0.79a
Pelvis transverse diameter	4.49±0.64a	4.42±0.66a	4.30±0.79a	4.56±0.80a	4.17±0.69a	4.18±0.68a
Fore limb length	4.66±0.62a	4.70±0.65a	4.70±0.86a	4.82±0.78a	4.53±0.73a	4.60±0.59a
Hind limb length	4.99±0.68a	4.78±0.64a	4.49±0.84a	4.72±0.74a	4.72±0.76a	4.72±0.82a
Tail length	5.46±0.92a	5.27±0.88a	5.39±0.96a	5.46±0.90a	5.22±0.97a	5.63±0.95a

Horizontally, different letter means statistically significant

On the other hand, toxoplasmosis of female rats also didn't induced significant changes in head antero-posterior diameter, head transverse diameter, chest antero-posterior diameter, chest transverse diameter, pelvis antero-posterior diameter, pelvis transverse diameter, fore limb length, hind limb length and tail length of the infected rats progeny, in comparison with non infected females. However both sulfadiazine and pyrimethamine significantly decreased head antero-posterior diameter and head transverse diameter ($P<0.05$) of the progeny of the infected and non infected female rats (table 4).

Table 4: morph-metric measurements (mm) of the fetuses of non-infected and *Toxoplasma gondii* infected female rats treated by DMSO, sulfadiazine and pyrimethamine.

Groups / Parameters	**Non infected females treated by DMSO impregnated by healthy male**	**Non infected females treated by sulfadiazine impregnated by healthy male**	**Non infected females treated by pyrimethamine impregnated by healthy male**	**Iinfected females treated by DMSO impregnated by healthy male**	**Infected females treated by sulfadiazine impregnated by healthy male**	**Infected females treated by pyrimethamine impregnated by healthy male**
Head antero-posterior diameter	7.83±0.92a	7.15±0.80b	6.92±0.78b	7.75±0.91a	6.81±0.72b	6.61±0.60b
Head transverse Diameter	5.40±0.52a	5.22±0.61b	5.15±0.58b	5.34±0.52a	4.85±0.48b	4.92±0.53b
Chest antero-posterior diameter	5.40±0.62a	5.47±0.72a	5.50±0.86a	5.49±0.62a	5.52±0.75a	5.30±0.64a
Chest transverse diameter	4.30±0.64a	4.16±0.86a	4.51±0.78a	4.40±0.73a	4.34±0.69a	4.30±0.82a
Pelvis antero-posterior diameter	5.57±0.83a	5.79±0.78a	5.55±0.81a	5.44±0.92a	5.70±0.98a	5.50±0.88a
Pelvis transverse diameter	4.18±0.72a	4.31±0.74a	4.14±0.83a	4.16±0.63a	4.17±0.75a	4.18±0.82a
Forelimb length	4.77±0.86a	4.78±0.78a	4.82±0.69a	4.62±0.76a	4.53±0.86a	4.60±0.62a
Hindlimb length	4.72±0.73a	4.85±0.83a	4.91±0.96a	4.68±0.78a	4.72±0.82a	4.70±0.82a
Tail length	5.30±0.92a	5.57±0.79a	5.24±0.87a	5.43±0.91a	5.22±0.84a	5.43±0.83a

Horizontally, different letter means statistically significant

Gross tteratogenic effects

No gross teratogenic effects were recorded except subdermal hemorrhagic patch in 4 of 32 fetuses (12.5%) in a group of healthy females impregnated by infected males treated by sulfadiazine (figure 2) and in 2 of 45 fetuses (4.44%) of infected females impregnated by healthy males and treated by sulfadiazine and in 2 of 50 fetuses of infected females impregnated by healthy males and treated by DMSO (figure 3).

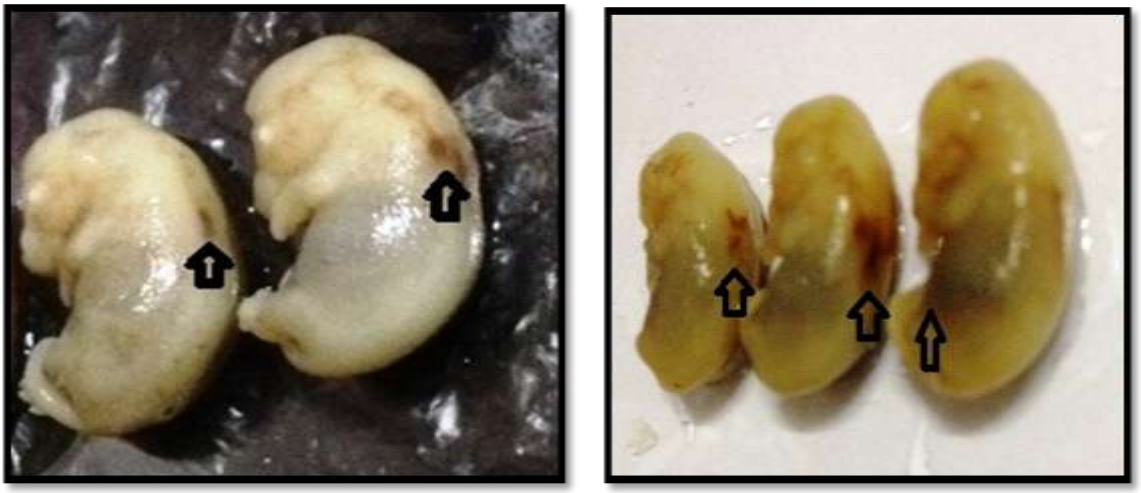
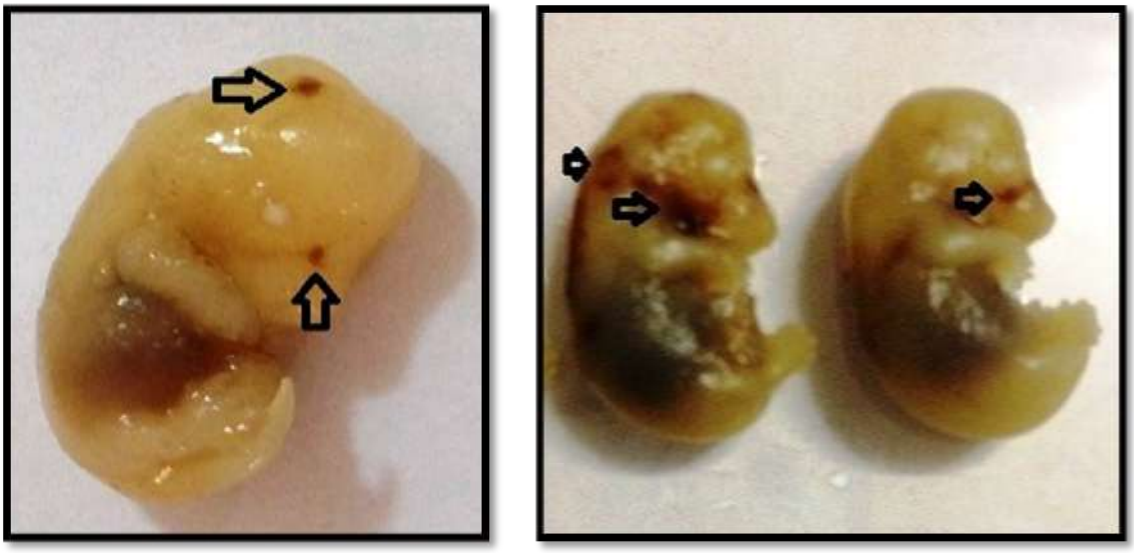


Figure 2: Subdermal hemorrhagic patch in fetuses of healthy females impregnated by infected males treated by sulfadiazine.



A **B**

Figure 3: Subdermal hemorrhagic patch in fetuses of infected females impregnated by healthy males and treated by sulfadiazine (A) and in fetuses of infected females impregnated by healthy males and treated by DMSO (B).

Fetal histological changes

No fetal histological changes were noted in the liver, kidney, lung and brain of the fetuses of infected and non infected male and female rats either treated by DMSO, sulfadiazine or pyrimethamine.

DISCUSSION

The decreasing of litter size, fetal weight, and increasing of fetal resorption and early fetal death in *Toxoplasma gondii* infected male and female rats in this study, could be attributed to endocrine effects of toxoplasma infection. Toxoplasmosis induced hypogonadotropic hypogonadism.[9-12] Toxoplasma infected mice showed that supraoptic and paraventricular hypothalamic nuclei were deformed and showed pyknotic neurons.[10] Furthermore, Interleukin-1b (IL-1b) levels were increased in toxoplasmosis. The levels of IL-1b correlated significantly in a negative manner with FSH, LH in toxplasmosis.[13] Interleukin-1b was known to suppress the hypothalamic- pituitary- gonadal (HPG) axis, directly or indirectly through increased corticotrophin-releasing hormone (CRH) and/or cortisol. It was also found that cytokines released peripherally in response to the parasite reached the hypothalamus and initiated a sequence of events that inhibited the pulsatile release of gonadotropin-releasing hormone (GnRH),

leading to the subsequent impairment of the pituitary-gonadal axis.[14] By these mechanisms, toxoplasmosis could interfere with pituitary and gonadal hormones secretion at hypothalamic level which subsequently decreased fertility.

Furthermore, the decreasing of litter size and fetal weight and increasing of fetal resorption and early fetal death associated with toxoplasmosis may resulted from the mutagenic effects in the germ cells in toxoplasma infected males and females, a pilot epigenetic study revealed that toxoplasmosis impaired the DNA methylation of selected genes.[15-16]

However, the decreasing of litter size, fetal weight and increasing of fetal resorption and early fetal death of healthy female impregnated by either non infected or toxoplasma infected male rats treated by either sulphadiazine or pyrimethamine and the decreasing of litter size, fetal weight and increasing of fetal resorption and early fetal death of infected and non infected females treated by healthy males receiving either sulphadiazine or pyrimethamine, could be attributed to the mutagenic characteristics of these drugs.[17-19] Pyrimethamine was found to produce a significant increase in structural chromosomal aberrations after acute treatment in bone marrow cells of mice ($P<0.001$). It also induced chromosome abnormalities in germ cells ($P <0.05$) at the highest dose.[20] Many sulphonamides were also mutagenic and cause fetal growth retardation.[21]

The mutagenic effects on germ cells usually affected litter size, fetal weight, fetal development and increased fetal resorption and early fetal death. According to the antifertility effects of pyrimethamine, some authors recommended using of this drug as a contraceptive.[22-23]

CONCLUSION

Toxoplasmosis deteriorated reproductive performance in both male and female rats. Pyrimethamine and sulfadiazine were not free from reproductive adverse effects and they caused further deterioration of reproductive function.

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