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# Honey as an Alternative to Stem Cells Therapy for Degenerated Rat Testis Due to Malnutrition

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# Honey as an Alternative to Stem Cells Therapy for Degenerated Rat Testis Due to Malnutrition

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**Abstract**

The purpose of the study was to determine the regeneration testis using honey as an alternative to stem cells therapy. 40 rats were divided into 4 treatments: normal male rats without honey (C-) and Three group of rats with degenerative testis due to malnutrition were treated without honey (C+), with 30%(v/v) honey (T1) and 50%(v/v) honey (T2) for 10 days. The results indicated the regenerative changes in testis and number of spermatogenic and Leydig cells, in C+ were significantly decreased ($p<0.05$) compared which to C-, T1, T2. In T2 hasn't showed significant decrease ($p>0.05$) compared with C-.

**Key words** : Testis, Regeneration, Honey, Rat.

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The stem cells therapy can help in various diseases including degeneration of testis due to malnutrition (Safitri *et al*., 2016a). Nevertheless, the complexity of the isolation, in vitro culture and transplant procedure are very expensive (Safitri and Hariadi, 2019). Honey is natural substance that can induce the stem cell regeneration (Prasetyo and Safitri, 2016; Hasib *et al*., 2017) for repaired degeneration of testis

### Materials and Methods

Twelve to fourteen weeks old male Wistar rats (n=40) weighing 300-350 g were divided into 4 groups, consisting of 10 rats each. Negative control (C-): normal male rats without honey. Positive control (C+): testis degeneration in malnourished rats without honey. Groups T1 and T2: testis degeneration malnourished rats

**Table I:** Number of testicular cells as affected by the treatments in wistar rats (mean ± SD)

| Treatments | Type Cells | | | |
|---|---|---|---|---|
| | Leydig Cells | Spermatogonia | Spermatocyte Pri-Sec | Spermatid |
| Negative control group | $37.66^{d} \pm 1.78$ | $54.25^{d} \pm 2.45$ | $75.25^{d} \pm 1.60$ | $115.50^{d} \pm 1.40$ |
| Positive control group | $5.25^{a} \pm 1,85$ | $15^{a} \pm 1,57$ | $30.44^{a} \pm 1.73$ | $13.15^{a} \pm 1.70$ |
| Treatment group T1 fed | $17.53^{b} \pm 1.60$ | $26.53^{b} \pm 1.34$ | $46.35^{b} \pm 1.40$ | $26.53^{b} \pm 1.34$ |
| Treatment group T2 fed | $26.75^{c} \pm 1.83$ | $49.75^{cd} \pm 1.35$ | $68.75^{cd} \pm 1.54$ | $79.65^{c} \pm 1.75$ |

$^{a,b,c,d}$ Different superscripts in the same column differ significantly *(P<0,005)*

were given 30% (v/v) and 50% (v/v) of honey for 10 days. Testis degeneration due to malnutrition was induced by fasting without food for 5 days (Safitri *et al.*, 2016b). Histopathological examination was done for assessing level testicular damage in the (Leydig and spermatogenic cells (spermatogonia, primary-secondary spermatocycte and spermatid) were counted in 40 slides from the treatment groups with the Hematoxylin Eosin staining.

**Results and Discussion**

Degeneration testicular tissue in rat due to malnutrition in positive control group (C+) has shown a seriously damaged seminiferous tubules compared to the negative control group (C-). In C+ was showed many fatty droplet and the location that is far separate between the tubules (Figure 1B). Malnutrition can be cause of a free radical trigger and can cause increased of oxidative stress. Oxidative stress can lead to the formation of reactive oxygen species (ROS) that attack of mitochondria in the cells, that is dangerous and can damage the cells. The fertility is dependent on mitochondria function.

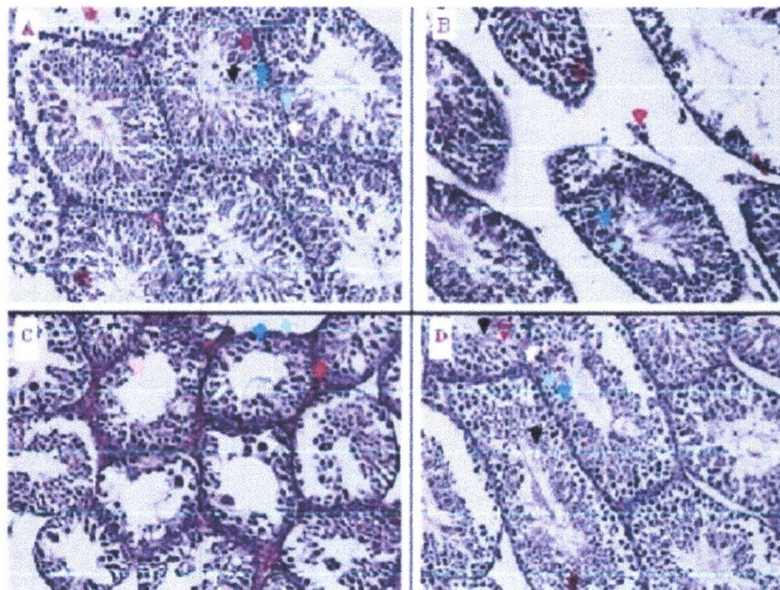


**Fig 1.** Histopathology of testicular cells. Yellow arrow ( ) = normal leydig cells, red arrow (↓) = nekrosis leydig cells, green arrow (↓) = spermatogonia cells, blue arrow (↓) = spermatocyte primary-secondary, black arrow (↓) = normal spermatid cells, orange arrow (↓) = nekrosed spermatid cells, purple arrow (↓) = sertoli cells (H+E 200X).

Whenever the speroninal mitochondria potential decreases their fertility potential will also decrease (Spalekova *et al.*, 2011). The increase in ROS was due to increase of malondialdehyde (MDA) level in the cells and their necrosis. (Samik and Safitri, 2017). The condition, in control positive group (C+) can lead to decreased number of Leydig and spermatogenic cells (spermatogonia, primary-secondary spermatocycte and spermatid) (Tabel I).

In the treatment group T1 fed with 30% (v/v) honey, the seminiferous tubule were damaged with many fatty droplets. The leydig cells, sertoli and spermatid cells were damaged with irregular shape (Fig 1C) and decreased in number (Table I).

In the treatment group T2 fed with 50% (v/v) honey, the seminiferous tubule has shown regeneration with fewer fatty droplets. The Leydig, sertoli, and spermatogenic cells have improved (Fig 1D) and increased in number (Table I). The regeneration of the seminiferous tubules and the leydig cells were also observed, in the positive control group (C+) there was a significant decrease in the number of these cells ($p<0,05$) when compared to C-, T1 and T2 (Table I). The 30% the honey treatment (T1) showed significant difference ($p<0,05$) when compared with the control negative group (C-) and (T2).

The 50% honey treatment has significantly ($p<0.05$) increased the regeneration of different types of tubular cells when compared to the C+ and T1 treatment groups, however its still lower than that of cells in the negative control groups (with normal testis). The reduction in the spermatogenic cells will lead to reduced testosterone. The Leydig cells are considered as the nourishing (mother) cells to the spermatozoa during their maturation. Hence any reduction in Leydig cells will reduce the corresponding number of spermatozoa (Ismudiono *et al.*, 2010; Hafez and Hafez, 2013) and morphological changes in spermatids.

## Summary

The results indicated that the effectiveness of the 50% honey therapy in the regeneration of seminiferous tubules but not the sum of number of leydig cells and spermatid cells.

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