Antifertility studies of various chromatographic fractions of neem seed oil I female albino rabbits: A histological approach

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Abstract-
Background: Preparation of plant or parts of them were widely used in popular medicine since ancient times and till today the use of Phyto medicines is wide spread in most of the world's population. Among diverse herbal treasure *Azardichta indica* is a highly esteemed tree with several beneficial properties and applications. The aim of present study was to observe antifertility efficacy of Neem seed oil and its active Fractions on female albino rabbits with special reference to its histological aspect.
Methods: Animals were given neem seed oil alone and its fractions I, II, III, IV, V and VI to female albino rabbits between 10 to 500 mg/body weight orally for 28 days. The histology of ovary uterus, vagina and vital organ such as liver kidney and heart were done through standard method and histometric of ovary uterus and vagina was done through camera lucida.
Result: In present investigation no adverse effect reported on histoarchitecture of vital organs such as liver, kidney, heart t but in histological examination of ovary showed arrest of oogenesis at various stages of follicles development in Neem seed oil alone and its fraction IV and V treated group. The developing follicles undergo atresia with the derangement of the granulosa cells and the nuclear pykonsis in fraction IV and V. Similarly, they affect the uterus drastically. Histologically uterus showed a reduction in the height of glandular epithelial and endothelial wall showed smoothness and reduction. The thickness of vaginal epithelium reduces to a few cell layers.
Conclusion: The above result confirms the antiestrogenic nature of Neem seed oil and its Fraction IV and V. The vital organ histology confirms no toxic effect by plant products. The histometric of ovary follicle, thickness of vaginal epithelium and uterus epithelium reflects antiestrogenic nature of neem seed oil alone and its fraction IV and V. Thus, the above finding suggests the fraction IV and V and neem seed oil alone possesses some phytochemical which inhibits oogenesis and cause infertility in rabbits. Further experimentation is in progresses.

Key Words- **Antifertility, Antiestrogenic, Ovarian histoarchitecture.**

INTRODUCTION

Global population has severely disturbed the ecological balance and has forced mankind to develop new fertility regulation techniques[1]. Antifertility agents are drugs that control fertility and are also called oral contraceptive[2]. The use of oral contraceptive agent is as old as recorded history. Although a wide variety of synthetic contraceptive agents are available which cannot be used continuously due to their severe side effect, hence peoples are using traditional health care system[3-4] since ancient time peoples used plants to cure diseases and relieve physical suffering. Because of their acceptability, lesser side effect and effectiveness of many traditional medicines is now an acceptable fact[5]. Several plants product inhibits male and female fertility and may develop into contraception[6]. The Neem tree (*Azardrichta indica*) is one such medicinal plant and it is considered as "village pharmacy" in many parts of India and has played a key role in ayurvedic medicine and agriculture[7-9]. Every part of tree such as fruit, leaf and bark are used as traditional medicine for house hold remedy against various human aliments from antiquity[10-11]
Keeping this view in mind, the current study was under taken to evaluate the Antifertility effect of hexane extracted Neem seed oil and its various chromatographic Fraction in female albino rabbits with special reference to its histological aspect.
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MATERIAL AND METHOD

PREPRATION OF PLANT EXTRACT

The ripe fruit of Neem were collected in an around new campus of jai narayan vyas university, Jodhpur. The plant material was identify by botany department of jai narayan university, jodhpur. After cleaning and depulping the seed were dried in bright sunlight. The Neem kernels is the richest source of Neem seed oil so seed were decorticated mechanically removing the husk . The crushed kernels were initially blended with the hexane. The crushed material was packed into cellulose thimble and placed in extractor. Hexane was recover by distillation and the residue left after distillation was collected and to prevent oxidation Neem seed oil was stored under refrigeration.

FRACTIONATION

50gm of Neem seed oil was chromatographed over deactivated silica gel in glass Colum and six major Fractions were eluted with EtoH/hexane in six different proportions.
These Fractions were eluted separately and dried under reduced pressure. They were diluted as per required doses in olive oil and used for treatment.

INFRARED SPECTROSCOPY

To characterize and identify the chemical groups present in the fractions, the fractions were subjected to infrared absorption spectroscopy.FT-IR of all the six fractions ware recorded on FTIR Instrument FT/IR-410, JASCO Corporation, Japan. The samples were scanned with resolution of 4 cm^{-1}. 180 accumulations were taken for all the samples.

Comparative Presentation of IR Spectra:

SAMPLE	C-H STRETCHING	C=O STRETCHING	C-H BENDING	SOME OTHER PEAKS
Fraction I	2948, 2917, 2850	1737, 1702	1463	719, 688 (V.low intensity)
Fraction II	2993,2919,2850,2675	1735, 1712	1463	721, 688 (v.low intensity)
Fraction III	3006,2921, 2858, 2674	1747, 1714	1463	721, 688 (v.low intensity)
Fraction IV	3004, 2919, 2850	1735, 1712	1463	721, 688 (v.low intensity)
Fraction V	3006, 2954, 2923,2852, 2674	1745, 1714	1463	721, 688 (v.low intensity)
Fraction VI	3006,2958,2921, 2852	1741, 1714, 1697, 1683	1463	721, 688 (v.low intensity)
Pure from Fraction IV	3012, 2925, 2854,2674	1708	1463	765
Pure comp. Fr. 4 subtracted from Fr IV.	3019, 2931, 2856	1712	_	757 (v.low intensity)

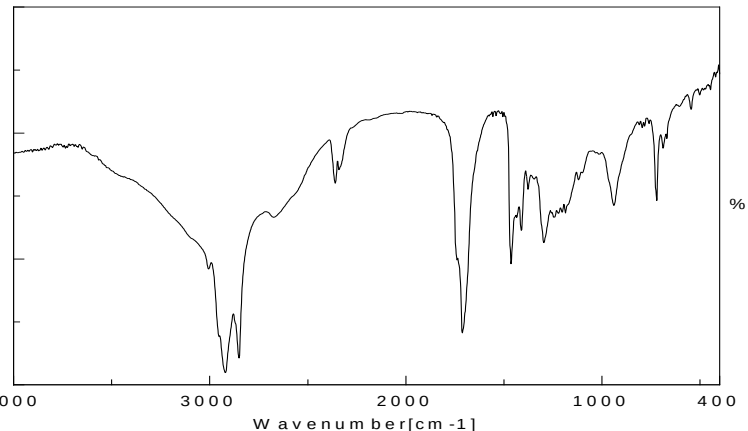


Fig1.. I.R. Spectroscopy of Fraction IV

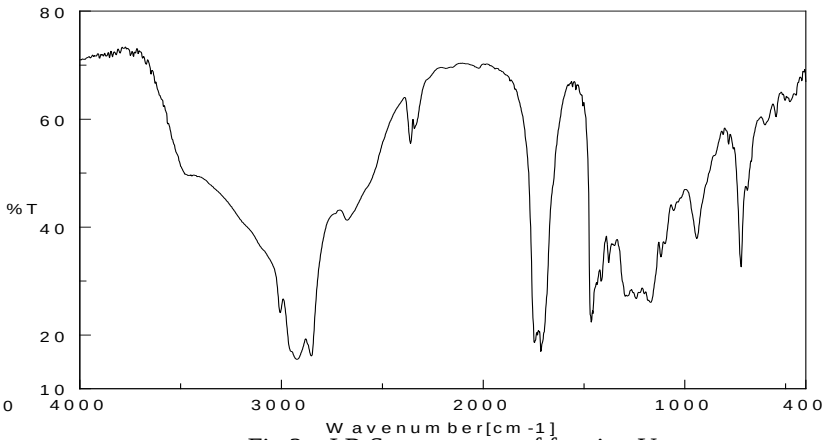


Fig.2 I.R.Spectroscopy of fraction V

ANIMALS AND TREATMENT

Healthy adult female Albino rabbits were brought from forest department of Jodhpur (Rajasthan). Weight and age of animals were1.25 -1.75 kg and 10 to 12 months respectively and were housed in well lighted air conditioned room in metallic wire gauge

cages under controlled environment with 24hrs light/dark cycle. All the rabbits were fed on standard rabbit chow supplied by Hindustan liver ltd. India. The food was supplemented with green leafy and seasonal vegetables and water ad libitum. The main experiment was divided into eight groups. The control and experimental groups usually consisted of eight animals each. The Chromatographic fractions of Neem seed oil were dissolved in sterile olive oil and administered orally (experimental protocol was approved by departmental council).

Group 1 Vehicle treated control or Intact: The group received drug vehicle only i.e. Olive oil (1 ml / kg body Wt./ day) for 30 days 0rally.

Group 2 The female rabbits of this group were fed with Neem seed oil (500 mg /kg body Wt /day.) for 30 days orally.

Group 3 Each of the animal of this group was treated with chromatographic fraction I of Neem seed oil. The dose level given was 9 mg / kg body Wt. / day orally the duration of treatment was 30 days.

Group 4 The female rabbits of this group were treated with chromatographic fraction II of Neem seed oil. The dose level was 10 mg /kg body Wt. / day, oral. The duration of treatment was 30 days.

Group 5 Each of the animals of this group was treated with chromatographic fraction III of Neem seed oil. The dose level given was 19 mg / kg body Wt. / day orally. The duration of treatment was 30 days.

Group 6 This group received the chromatographic fraction IV of Neem seed oil. The dose was 28 mg / kg Wt. /day, for 30 days. The route of drug administration was orally.

Group 7 Each of the animals of this group was treated with chromatographic fraction V of Neem seed oil. The dose level given was 10 mg / kg body Wt. / day orally. The duration of treatment was 30 days

Group 8 The animals of this group were treated with chromatographic fraction VI The dose level was 7 mg / kg body Wt. / day, orally for 30 days.

After 24 hours of last dose, all animals of the group were sacrificed under prolonged ether anaesthesia. Blood was collected through cardiac puncture using clean, dry syringe for haematological studies. The reproductive track was taken out and trimmed free of fat and cleaned of adherent tissue and weighed. The reproductive organs considered in the present study were ovary, uterus and vagina. For histological observation Bouin's fixed reproductive organs along with vital organs were cut in to small pieces and processed through ethanol xylene series. The paraffin embedding was followed by section cutting (5µm thickness) and staining (Harris haematoxylin and eosin) the stained sections were examined for histopathological changes. Follicular types such as primary, secondary and mature were calculated by observing stained sections of ovary.

RESULT

BODY AND ORGAN WEIGHT

Non-significant changes occurred in body weight of experimental animals of all treatment groups. Administration of Neem seed oil (Gr.2) and its chromatographic Fraction IV and V (Gr. 6-7) brought about significant reduction in the weight of ovary and uterus in relation to control.

HAEMATOLOGY

The haematological parameters such as Blood sugar, RBC, WBC, Haemoglobin concentration of vehicle treated control(Gr.1), Neem seed oil(Gr.2) Fraction I(Gr.3), Fraction 2(Gr.4),Fraction III(Gr.5), Fraction IV(Gr.6), Fraction V(Gr.7) and Fraction VI(Gr.8) values were all found in normal range in all treatment groups.

HISTOLOGICAL OBSERVATION

Histological picture of ovary revealed that the maximum severe impact of test substance was on Neem seed oil (Gr.2), Fraction IV (Gr.6) and Fraction V (Gr.7) treated experimental groups and showed degenerative changes in the germinal epithelium. The atretic follicles and growing follicles were observed but the normal graffian follicle was not seen. Microphotograph of ovary of control showing follicles of various stages of development. X 100 HE. (fig.3). Microphotograph of Neem seed oil treated ovary showing degenerative changes in mature follicle without oocyte. X 100 HE.(Fig.4) Microphotograph of fraction IV treated ovary showing the absence of mature & healthy follicles and presence of degenerative follicle without oocyte X 100 HE (Fig.5) and Microphotograph of fraction V treated ovary showing degenerative matured follicle without oocyte. X 100 HE(Fig.6) While as in fraction I (Gr.3), Fraction II (Gr.4), Fraction III (Gr.5), and Fraction VI (Gr.8) showed normal histoarchitecture of ovary. Similarly Microphotograph of the uterus of control showing normal columnar epithelial cells of lamina propria and the uterine glands. X 200 HE (fig.7) whereas microphotograph of Neem seed oil (Gr.2)(Fig.8), Fraction IV (Gr.6) (Fig.9) and Fraction V (Gr.7) (Fig.10) treated group showed severe degeneration in the endometrium wall of the uterus. It shows the shrinkage of columnar epithelial cells of lamina propria. The uterine gland cells showed degenerative changes and the number of glands was also reduced Same as Microphotograph of the vagina of control showing the normal stratification of the epithelium, well developed lamina propria and the lumen filled with mucus, leukocytes & epithelial cells. X 200 HE (Fig 11). Whereas the Neem seed oil (Gr.2)(Fig 12), fraction IV (Gr.6)(Fig13) and fraction V (Gr.7)(Fig.14) treated rabbits vagina showed severe degenerative changes. The thickness of the epithelium was highly reduced. The glandular cyst and the lamina propria were compact and the cervical canal was empty.

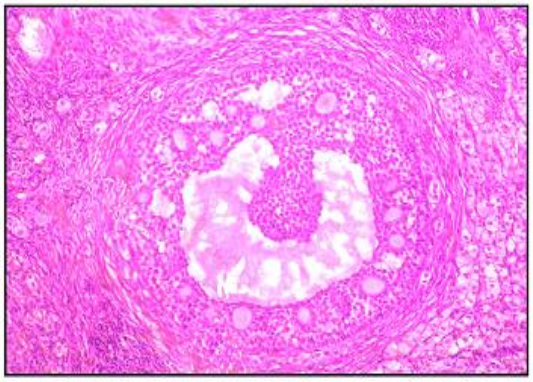


Fig.3 **Ovary (control)**

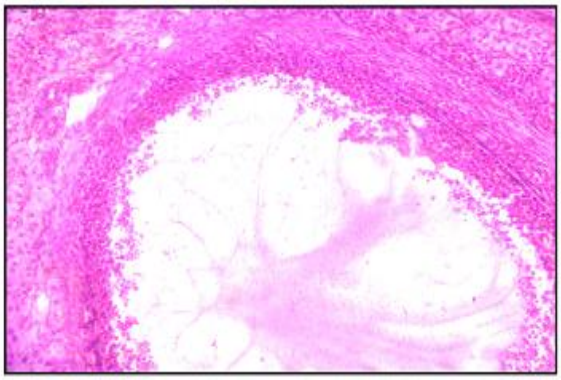


Fig 4. **Ovary (neem seed oil)**

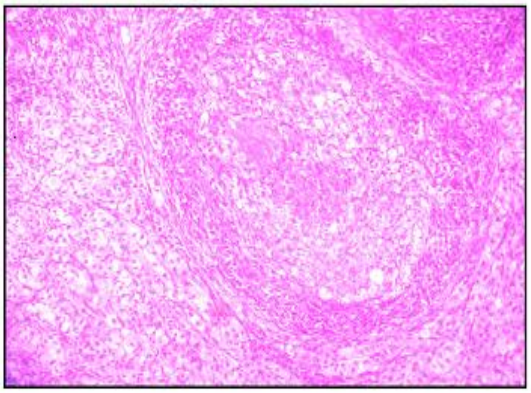


Fig 5. Ovary (Fraction IV)

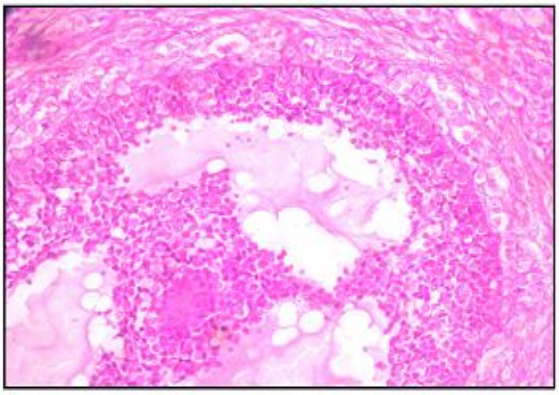


Fig 6. Ovary (Fraction V)

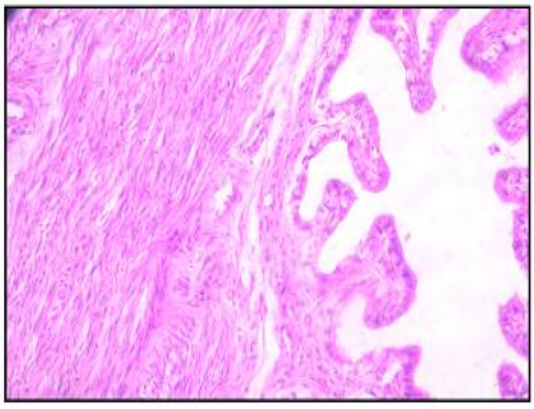


Fig 7. Uterus (control)

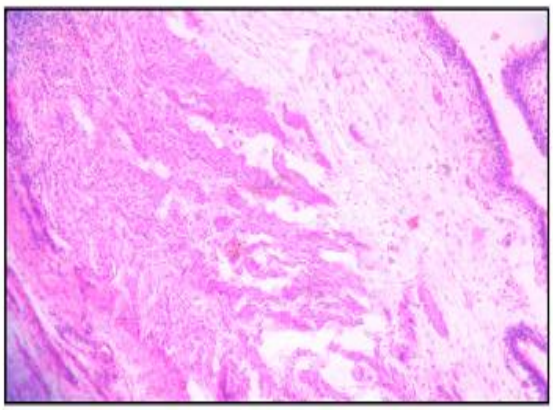
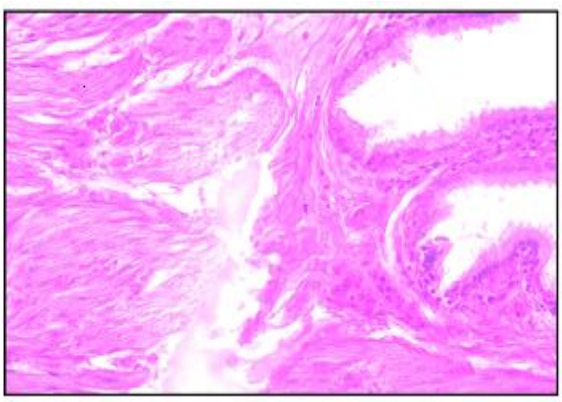


Fig 8. Uterus (Neem seed oil)



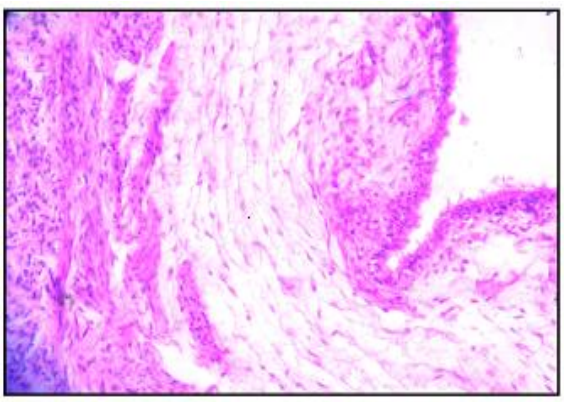


Fig 9. Uterus (Fraction IV)

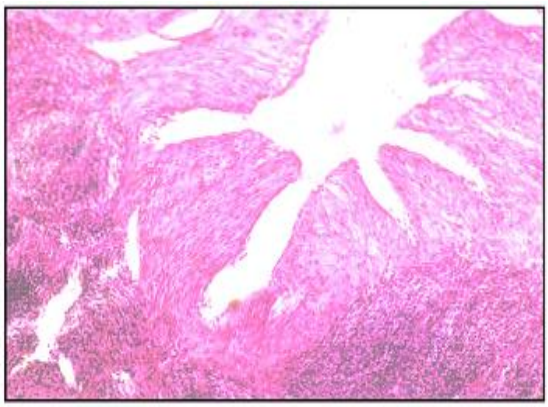


Fig 10. Uterus (Fraction V)

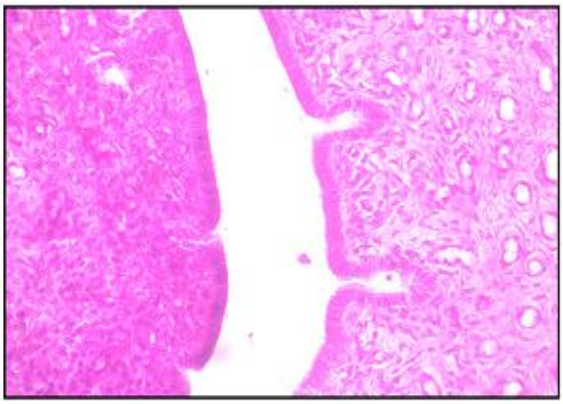


Fig 11. Vagina (control)

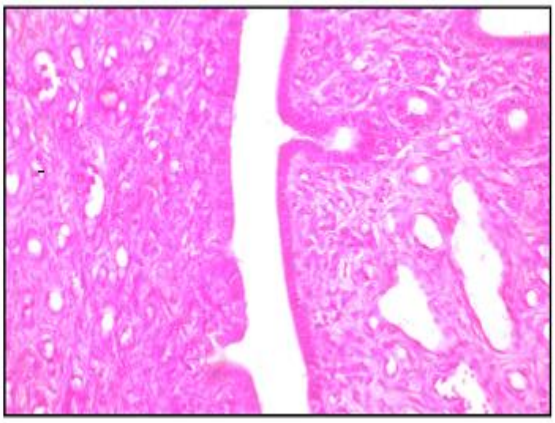


Fig 12. Vagina (Neem seed oil)

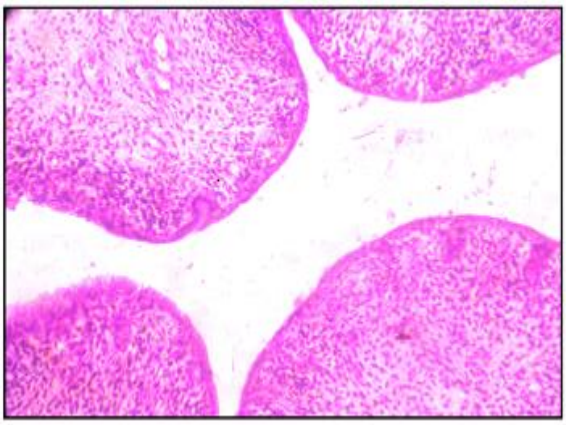


Fig. 13. Vagina (Fraction IV)

Fig 14. Vagina (Fraction V)

DISCUSSION

In the present investigation it has been observed that the treatment with Neem seed oil (Gr.2) and Fraction IV (Gr.6) and V (Gr.7) cause degenerative changes in the ovary. Histological examination of follicles of various classes of ovaries of Neem seed oil (Gr.2) alone or its Fractions IV (Gr.6) and V (Gr.7) showed that their clear arrest in the developmental follicles. The graffin follicles undergo atresia with the derangement of the granulosa cells and the nuclear pyknosis in Fraction V (Gr.7), the oocyte also degenerate and corpra lutea shows the sign of regression. The degenerative changes in the ovary along with the slight decline in the weight indicate the suppression of ovarian activity[13-15] . This may be due to the inhibition of the synthesis and or release of the gonadotropins from the pituitary or to the direct inhibitory effects on the ovaries[16-17] . In other fraction such as I, (Gr.3) II (Gr.4), III (Gr.5) and VI (Gr.8), the histology shows no effect on ovary. Histologically the uterus wall is very significant for attachment of embryo. The major structural balance of uterine wall is mainly depending on estrogen[18-21] . The histologically uterus showed a reduction in the heights of lumenal and the grandular epithelial in Neem seed oil alone and its fraction IV and V treated groups. Similarly the endothelial walls showed smoothness & reduction of glands indicating the inhibition of secretory activity[22-23] and expressed antiestrogenic nature of this plant products similarly The thickness of vaginal epithelium reduce to a few cell layer this confirm the antiestrogenic nature of Neem seed oil and its Fraction IV and V because The vaginal function is to give passage to the reproductive organs and its functional status is dependent on varying levels of the circulating hormones that is estrogen[24-27] . In the present investigation no adverse effect was reported on histoarchitecture of vital organs such as liver, kidney, heart and also the haematological parameters were in normal range in all treated group. It confirms the non toxicity of Neem Seed Oil and its various functions.

From above discussion it is clear that the Neem seed oil (Gr.2) alone and in Fraction IV (Gr.6) and V (Gr.7) possesses antiestrogenic property; it may directly affecting the main reproductive organs such as ovary and uterus or indirectly through pituitary gonadal system.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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