SUMMARY
The present investigation reports effect of an alkylating and a non-alkylating chemosterilant, apholate (2,2,4,4,6,6 hexakis (1-aziridinyl) - 2,2,4,4,6,6 hexahydro, 1,3,5,2,4,6 tri-azatriphosphorine) and hempa (hexamethyl phosphoramid e or tris (dimethyl amino) phosphine oxide) on some tissues viz., brain, corpus cardiacum, fat body and testes, of an orthopteran insect Poekilocerus pictus Fabr. (acrididae, orthoptera). Chemosterilants are chemicals that have sterilising effects on insects. They are thus reproduction inhibiting agents which can be used to control insect pests rather than killing the later by chemical pesticides. Most of the work done on chemosterilants are on the sterility and reproduction (egg viability, egg hatching etc.) and longevity of insects, although they can also cause other physiological effects. Moreover, histopathological studies as induced by these chemicals have only been done on gonads, mainly the ovaries. There is not much report on the pathological effects on the neuroendocrine system or the fat body, neither on histochemical effects on the later. There is no work on the morphometric changes caused by these chemicals in any insect tissues.

The present investigation was undertaken with all the above into consideration. Poekilocerus pictus Fabr. was selected as the test insect because it is a secondary pest of tomato, brinjal, papaya etc. and easily available around Sagar. It can be reared in the laboratory and has been studied in this department for various aspects of its growth, development and reproduction. Brain, corpus cardiacum, fat body and testes were chosen because it is known from previous work on the same and other insects that
a feed back mechanism exists between the neuroendocrine system, fat body and gonads of insects.

The changes in the above organs have been studied from morphometric aspects measuring the cell and nuclear size by okular micrometer and analysing them statistically to know the significance of the differences between the control and treated organs, histopathological aspects, making observations on the damage induced in the organs by the chemosterilants and from the aspects of the changes in the chemical constituents in the neurosecretory cells and fat body, particularly proteins, glycogen and lipid using histochemical methods, like paraldehyde fuschin (PF), mercuric Bromophenol blue (HgEBB), periodic acid Schiff's PAS) and Sudan Black-B staining. Two different doses viz. 0.062 ml and 0.125 ml were injected to one day old adult males by a 1 ml microsyringe and the tissues dissected out for observations after 3, 7, 15, 20 and 30 days as the case may be.

Interesting results as a consequence of the various observations by the methods mentioned above, have been obtained in the present study. It has been found that both apholate and hempa cause reduction (shrinkage) in the cell size in most of the organs studied. The nuclear size is also decreased in many cases. In case of the mature germ cells of testes i.e. spermatids and spermatozoa, the same finding was met with, that is, in most cases they were reduced in size/length or their size appeared to be bigger where they were hypertrophied or distended.
However, 'hyperprophied' spermatozoa, in this work has been named to those which were very small in length like the atrophied ones, but the former were very thick in comparison to the thin and under-developed later type of abnormal spermatozoa.

The statistical analysis by student's 't' test, showed very highly significant changes (well below a probability of 5%) in the germ cells of testes, in comparison to those in the fat body and neurosecretory cells of the brain, in that order. However, the morphometric changes were independent of the pathological changes in the tissues or even the histochemical changes in the fat body. That is to say, that even if the morphometric changes seemed to be negligible or not significant, there were still clear pathological damage in the tissues in the form of vacuolated cytoplasm, condensed or contracted chromatin in the nuclei, indistinguishable peripheral globules and central globules in fat cells and their abnormal histochemical picture etc.

Histopathological changes as an impact of pholate and hempa treatment included in general, appearance of vacuoles in the cytoplasm of neurosecretory cells, corpus cardiacum, cells of fat body and in testes follicles; breaking up of cell walls, specially in the fat body; breaking of germinal epithelium and loosening of germ cells; very intense reaction of the neurosecretory material in neurosecretory cells and corpus cardiacum and of the chemical constituents (protein, glycogen and lipids) in fat cells which apparently meant that they were not released, or in some
cases their feeble reaction showing that the materials were not incorporated/synthesised in their respective organs. In the former case where intense staining reaction was shown, the respective material (neurosecretory material or metabolites in the fat body) were not granular and uniformly distributed as in normal (control) cells. Other histopathological changes included indistinguishable peripheral and sometimes control globules in fat cells. The pathological effects discernible from the nuclei of all the organs studied was mostly their condensed chromatin (i.e. pycnotic nuclei) in contrast to granular uniformly distributed chromatin of nuclei in the control cells, and in many cases contracted chromatin resulting in vacocules also in the nucleus.

In some cases, particularly with apholate treatment, binucleate neurosecretory cells and fat cells were seen as a result of broken cell walls which could be either due to a failure in spindle formation in some cases or to that of karyolysis in others. The present investigation shows that both the chemosterilant cause inhibition of release or checked synthesis of neurosecretory material in the neurosecretory cells of brain. This effect, in general, was more with increased dose and longer post treatment period and was stronger with apholate treatment.

The present investigations, showed a changed the histological structure of the testes follicles with chemosterilants treatment. This was discernible with each dose of both the chemicals at every post treatment period with less damage at
3 days and moderate to severe damage between 7-20 days. With the lower dose of hempa, after 30 days, the damage to the germinal epithelium and younger germ cells seemed recovered but the mature germ cells were still affected. The pathological picture of testes follicles was obvious by a broken germinal epithelium, loosened germ cells, shrunken younger germ cells (spermatogonia, primary and secondary spermatocytes), pycnotic germ cells, mostly atrophic spermatids and either shrunken (atrophied) or small and thick (hypertrophied) spermatozoa, both of which failed to agglutinate and form sperm bundles. Loosening of the germ cells gave a vacuolated look to the testis follicles.

The present histochemical findings for the chemosterilants treated fat body of P. pictus shows that the chemical constituent of the fat body, just like the neurosecretory material in the brain and corpus cardiacum, were adversely affected by treatment with apholate and hempa. The necrotic fat cells retained the proteins without releasing them which was shown by their intense staining reaction in the cytoplasm with very slight difference with each dose and every post treatment period of both the chemicals. Glycogen on the other hand, was seen to be very much depleted from the fat cells after chemosterilant treatment irrespective of its type, dose or post treatment period. Glycogen is particularly needed for testicular development and has an important role in normal growth of spermatogenic cells as established by the previous work. Lipids were scarcely present in fat cells treated with the lower dose (0.062 ml) of apholate and both the doses (0.062 and
0.125 ml) of hempa. With the high (0.125) dose of apholate, the fat cells were filled with lipids showing an inhibited release. Presumably a scarce distribution of glycogen and in some cases, lipids is due to their hampered synthesis or/and incorporation in fat cells.

The development and proper functioning of gonads in *Poekilocerus pictus* depends upon a proper metabolic functioning of the fat body. A normal activity of the fat body in turn, is regulated by the neuroendocrine system, specially the neurosecretory cells of the brain. The results of the present work clearly shows that both the chemosterilants apholate and hempa cause inactivity of the brain cells and the neurohaemal organ corpus cardiacum. This is due to general necrosis of the neurosecretory cells and either failure of release of the neurosecretory material in some cases or failure of its incorporation itself in neurosecretory cells, in the other. Both the doses of apholate were more damaging than hempa. The fat body is the main organ for storage of energy reserves like glycogen and lipids. Which are transported to their required target organs via the haemolymph. The same is true for proteins which are synthesised in the fat body and then transported for various metabolic functions. The male *P. pictus* requires more glycogen, than females, for its testicular development as shown by previous authors in this laboratory. Glycogen in testicular membrane has an important role in normal growth of spermatogonia cells. Presumably, the malfunctioning of the chemosterilant treated brain, hence an impaired synthesis and/or release of neurosecretory material looses control of proper synthetic
function of the fat body which in turn fails to supply the damaged germinal epithelium in the testes as well as loosened germ cells also hamper with the proper nourishment of the later.

It is thus inferred that the affected spermatogonia by chemosterilant treatment cause interruption of spermatogenesis at the spermatogonial level. This seems significant because discontinuity of spermatogenesis will be the result when all or most of the spermatogonia are damaged, and this will lead to permanent sterility. Even when these primary germ cells seem to be slightly recovered, e.g. with 0.062 ml hempa after 30 days, the mature germ cells are still affected leading again, to infertility. Hypertrophied as well as atrophied sperms (in some cases) fail to form sperm bundles like in control (or normal) males showing disturbed physiological state of the gonad and adds to infertility.

Insect chemosterilant can act either by attaching the gamete directly or through interference with the tissues supporting or nourishing the gametes, which may be taken as an indirect action. It has been found in the present work that the germinal epithelium in the testis of chemosterilised male is also damaged, the damage being slight to severe depending upon the dose of the chemical and the post treatment period. This hamperes with the proper nourishment of the germ cells. Moreover, the main organ of intermediary metabolism the fat body is already inhibited from its normal function and transporting nourishment to the target organs, specially gonads. There may be a direct interaction of the
chemosterilant with gametes excluding cytotoxicity like interaction with gonadal tissues leading to production of abnormal gametes, interaction with regulatory control centers (i.e. brain and associated glands) leading to malfunctioning of gonads, which has been shown in the present work; induction of nutritional deficiencies leading to malfunctioning of gonads and selective cytotoxic action killing the gametes or the cells in gonadal tissues. However, this investigation was an effort to include observations also on effects of chemosterilants on the main organ of intermediary metabolism in insect i.e. the fat body and it has rendered positive results as describe above. Out of the two chemosterilant used, apholate showed stronger effects than hempa. Insects also survived longer, without being sluggish and inactive, with hempa, specially with its lower dose. Hempa has been proved to be a weaker (mild) chemosterilant for P. pictus, although its qualitative effects are the same as its alkylation counterpart apholate. The reason for this and the mechanism, of chemosterilant action has been discussed.

The present study has shown a very slight recovery, in case of the neurosecretory cells only in terms of distribution of neurosecretory material and in the younger germ cells (spermatogonia and primary spermatocytes) in P. pictus, and only with the lower dose (0.062 ml) of hempa after 30 days. This means that in male P. pictus a complete recovery from the effects of chemosterilants used, has not been found i.e. whatever minute amount is retained/bound in the body is enough to trigger and maintain
the changes leading to sterility of the males.

Thus, it was significant to observe in the present investigation, degenerated germ cells and absence of sperm bundles (aspermia) as a result of treatment with apholate and hempa in males *P. pictus*. Thus a sufficient population of males in a sexually reproducing population can be rendered sterile by inducing aspermic conditions. Chemosterilised males are less effective in sperm transfer, the probable cause being a reduction in their mating drive. Failure of proper metabolic functioning of the fat body because of loss of control by the damaged neurosecretory system seems to be the main reason of disturbed testicular function. For a direct application in the field however, these chemosterilants would need to have all common safely requirements expected from an approved insecticides. Nevertheless, the present laboratory experiments add not only to the knowledge of physiological investigations on the neuroendocrine, metabolic and reproductive tissue, but on the morphometric changes in these tissue and histochemical effects in the fat body as well, the observations which have not been reported well so far as the author is aware. Further more, chemosterilants just as ovicides, larvicides and adulticides may have other physiological effects and only such combination of activities can make a chemical highly effective insect control agent. The inclusion of sterilising, or more broadly, reproduction inhibiting properties among the important and desirable characteristics of insect control agents have mainly led to the researches devoted to chemosterilants and their application. The ability to manipulate the regulatory
processes controlled by endocrine hormones, which is the subject of intensive efforts of numerous industrial and academic laboratories today, augurs well for the future of studies on effects of chemosterilants.

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