Pharmacodynamic effect of cannabis indica substances

L. KLABUSAY, J. LENFELD*

The aim of our work was to evaluate the pharmacological effect of the substances present in Cannabis indica. These substances were prepared at the Institute of Hygiene of prof. Kabelík (isolated extract from Cannabis indica) and the Institute of Chemistry of Prof. Šantavý (acid II., acetyl derivative of cannabidiolic acid).

Great difficulties in the application of these substances to experimental animals were caused by their very poor solubility in common physiological solvents. This fact certainly influenced our results to a large extent. As solvents, we had to use ether, alcohol, 0.5 n sodium carbonate, Michaelis apparatus with pH 8.13 and emulsions with gum arabic for oral administration. For this reason, and also for the relatively small number of animals, we consider the results of our experiments to be only indicative. When analyzing the substances contained in Cannabis indica, we found several distinct effects that we consider necessary to mention. These are analgesic, anticonvulsant and locally anaesthetic effects. Finally, the toxicity and local tolerability of these substances were determined.

The following were used: extractum spissum Cannabis indicae, isolated extract of Cannabis indica, acid II and acetylderivative of cannabidiolic acid.

I. Analgesic effect

The analgesic effect was observed on white rats by the method of mechanical stimulation according to Levyová and Buchelová in the modification according to Votava and on guinea pigs by the method according to Regniér.

Isolated extract from Cannabis indica has been studied on rats and guinea pigs.

We administered it orally in an emulsion with acacia gum from 10 to 500 mg/kg of weight, and subcutaneously and intraperitoneally in a 10% ethylene glycol solution up to doses of 500 m/kg of weight. To evaluate the analgesic effect using the Levy and Buchel method, we used 5 rats weighing 100 g for each dose and the same number of control animals that were given the same amount of solvent. The amount of ethylene glycol administered did not exceed 0.5 ml. When evaluating using the Regniér method, we used 5 guinea pigs weighing from 300 to 400 g and the same number of control animals. The analgesic effect of isolated extract from Cannabis indica was evaluated in guinea pigs only when administered orally.

In these experiments, it was found that isolated extract from Cannabis indica exhibits analgesic effects when administered orally from a dose of 100 mg/kg, when administered subcutaneously and intraperitoneally from a dose of 50 mg/kg. In these doses, analgesia lasts only a short time, 20 to 30 minutes. In higher doses (500 mg/kg), the isolated extract causes escalated analgesia lasting several hours, which is accompanied by overall depression of the central nervous system, possibly even sleep. The latency time for oral administration is 45 to 60 minutes and for intraperitoneal administration 15 to 20 minutes. Ethylene glycol did not have an analgesic effect in control animals in small doses.

Acid II and acetyl derivative of cannabidiolic acid were evaluated only on rats weighing 100 g according to the Buchel and Levy method. They were administered in ethylene glycol from 10 to 50 mg/kg intraperitoneally. The evaluation was conducted on 3 animals for each dose using the same number of control animals. Both substances were found to have no analgesic effect up to a dose of 50 mg/kg i.p. Higher doses and a larger number of animals could not be used because of the lack of these substances.

Extractum spissum Cannabis indicae, supplied from the pharmacy, was given per os to guinea pigs and rats. Even in large doses, the analgesic effect did not manifest itself.

II. Anticonvulsant effect

The evaluation was carried out only on isolated extract of Cannabis indica, on white mice and frogs. Convulsions were induced in white mice weighing 20 g with pentamethylene traamine (Pentazole) at a dose of 0.1 g per kg of body weight intraperitoneally, in frogs with strychnine at a dose of 40 mg/kg subcutaneously. Isolated extract from Cannabis indica was administered to mice 60 minutes before administration of Pentazole orally in a 10% emulsion from a dose of 0.3 to 1.0 g/kg body weight. For each dose (always 0.2 g per kg higher), we used 10 animals and the same number of mice served as a control.

An effective dose of isolated extract from Cannabis indica, which can prevent pentazole convulsions in 50% of mice, was found to be 0.61 g/kg per os.

In a similar way, a dose of isolated extract from Cannabis indica was found, which can prevent strychnine convulsions in 50% of animals. When administered subcutaneously and ethylene glycol is used as a solvent, this dose is 0.83 g/kg.

III. Local anaesthetic effect

We tested the locally anaesthetic effect for all substances. We evaluated surface anaesthesia according to the Lebduška and Vrba methods on the cornea of rabbits and guinea pigs and anaesthesia using the Bulbring and Wayda infiltration method on the skin of guinea pigs. We considered complete anaesthesia to be a condition when the animal did not respond to any of the six stimuli, performed every 3 seconds and every 5 minutes. Ethylene gyol was used as a solvent for all substances. The evaluation was always carried out on 5 animals using the same number of controls that were applied with ethylene glycol. Ethylene glycol itself is quite irritating, which is especially noticeable in superficial anaesthesia. We examined the substances in different concentrations, up to 1.5% concentration for acid II and acetyl derivative of cannabidiolic acid, up to 10% concentration for isolated extract from Cannabis indica, both in surface and infiltration anaesthesia.

Outcomes:

- Surface anaesthesia: acid II and acetyl derivative acid. Cannabidiols do not exhibit locally anaesthetic properties up to a concentration of 1.5%.

- Isolated extract from Cannabis indica has a complete local anaesthetic effect at a concentration of up to 10%. At lower concentrations, anaesthesia is not complete. When using a 10% solution of isolated extract from Cannabis indica, the locally anaesthetic effect lasts 45 - 90 minutes. Infiltration anaesthesia: acid II and acetyl derivative acid. Cannabidiols do not exhibit anaesthetic properties up to a concentration of 1.5%.

- Isolated extract from Cannabis indica is effective from a concentration of 5%.

IV. Toxicity

For the isolated extract from Cannabis indica, an LD of 50 was determined when administered orally in an emulsion with acacia gum. The mortality rate was subtracted within 48 hours. The LD 50 1.83 g/kg per os was calculated using the Burn method.

After small doses, the mice show no obvious symptoms. In doses of 1 g/kg, a slight suppression of motor functions with sluggishness and immobility occurs after a few hours. In even larger doses, there is a total depression and sleep, which ends with an exit within 24 hours.

For other substances for small amounts, LD 50 could not be determined.

V. Local tolerance

Local tolerability has been established for all substances. We evaluated it on the conjunctiva of the rabbit by instillation of solutions of 0.5 ml and in the subcutaneous tissue of the insertion of the auricle in the rabbit by applying 0.2 ml of solution according to the method of Lebdušek and Vrba. The reaction was evaluated according to the intensity of redness and its duration compared to the reaction after applying the solvent in the same amount. The disadvantage of the evaluation was that the solvent used - ethylene glycol - is also slightly irritating locally. We applied the substances from a concentration of 1 : 1000 to 1 : 20 to 3 animals for each dilution and the same number of animals were used as controls. We found that all substances even in a concentration of 1:1000 are locally highly irritating.

Two guinea pigs and two rabbits were also given an isolated extract of Cannabis indica into the muscle at a 3% concentration in 0.1 ml of athylene glycol. Ethylene glycol was also given to animals in the muscle of the other limb. In 48 hours in urethane anaesthesia, we revised the injection sites. In all animals, we found a large part of the substance not absorbed, a strongly inflammatory reaction with exudation of fluid, in rabbits up to 10 ml. After ethylene glycol alone, we found only a mild hyperemic reaction in the muscle.

VI. Other effects.

Acid II and acetyl derivative acid. Cannabidiol drugs were tested on isolated frog heart and blood pressure in rabbits in urethane anaesthesia. It was found that II acid slightly increases blood pressure in rabbits at a dose of 5 mg/kg IV, while an acetylderivative of cannabidiolic acid slightly decreases BP in rabbits at the same dose. On isolated frog heart, it was found that both substances increase tone and in larger doses induce typical cardiac arrest in systole. 0.5 N sodium carbonate solution was used as solvents. In a control experiment, sodium carbonate did not exhibit these effects.

DISCUSSION

We are aware that even though we have found certain distinct properties of Cannabis indica, the results of our work are only indicative and cannot be used to conclude and definitively decide the pharmacodynamic effects of these substances with regard to their use in practice. To a certain extent, our results were distorted by poor absorption of these substances in all methods of administration and also by unsuitable properties of the solvents used. It is worth noting that all pharmacological effects were found in the crude isolated extract of Cannabis indica, while both chemically pure substances isolated from cannabis proved to be ineffective. In our opinion, it is likely that the carriers of the effects caused by the cannabis extract are other substances that have not yet been isolated in their pure state, and it is also possible that the raw extract represents a balanced set of pharmacologically differently acting substances that can potentiate each other. These uncertainties will certainly be resolved by further chemical analysis and more detailed pharmacodynamic research.

SUMMARY

An indicative pharmacological analysis of cannabis substances was performed. Isolated cannabis extract has been found to have analgesic, anticonvulsant, and locally anaesthetic properties. Its toxicity (LD 50 (when administered orally in white mice) was determined to be 1.83 g/kg.

Acid II and acetyl derivative acid. Cannabidiol, pure substances isolated from cannabis, do not have these pharmacological properties. All substances are locally irritating.

In further work it will be necessary to explain this irritating effect due to the fact that the extract from the drug, deprived only of ballast substances, does not show an irritating effect in clinical use, but on the contrary, it clearly soothes pain, as was already known to ancient physicians (Extr. Cannabis in a tincture for corns) and as has now been newly confirmed by both dentists and otolaryngologists.